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Genetic population structure of red snappers (*Lutjanus malabaricus* Bloch & Schneider, 1801 and *Lutjanus erythropterus* Bloch, 1790) in central and eastern Indonesia and northern Australia

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The genetic population structure of red snapper *Lutjanus malabaricus* and *Lutjanus erythropterus* in eastern Indonesia and northern Australia was investigated by allozyme electrophoresis and sequence variation in the control region of mtDNA. Samples were collected from eight sites in Indonesia and four sites in northern Australia for both species. A total of 13 allozyme loci were scored. More variable loci were observed in *L. malabaricus* than in *L. erythropterus*. Sequence variation in the control region (left domain) of the mitochondrial genome was assessed by RFLP and direct sequencing. MtDNA haplotype diversity was high (*L. erythropterus*, 0·95 and *L. malabaricus*, 0·97), as was intraspecific sequence divergence, (*L. erythropterus*, 0·0–12·5% and *L. malabaricus*, 0·0–9·5%). The pattern of mtDNA haplotype frequencies grouped both species into two broad fisheries stocks with a genetic boundary either between Kupang and Sape (*L. malabaricus*) or between Kupang and Australian Timor Sea (*L. erythropertus*). The allozyme analyses revealed similar boundaries for *L. erythropterus*. Seven allozymes stocks compared to two mtDNA stocks of *L. malabaricus* including Ambon, which was not sampled with mtDNA, however, were reported. Possible reasons for differences in discrimination between the methods include: i) increased power of multiple allozyme loci over the single mtDNA locus, ii) insufficient gene sampling in the mtDNA control region and iii) relative evolutionary dynamics of nuclear (allozyme loci) and mitochondrial DNA in these taxa. Allozyme and haplotype data did not distinguish separate stocks among the four Australian locations nor the central Indonesian (Bali and Sape locations) for both *L. malabaricus* and *L. erythropterus*.

Key words: allozymes; Australia; fisheries stocks; genetic structure; Indonesia; mtDNA.

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INTRODUCTION

Two red snappers, *Lutjanus erythropterus* Bloch and *Lutjanus malabaricus* (Bloch & Schneider) are fished commercially in northern Australian, Papua New Guinean and Indonesian waters by commercial, artisanal and recreational fishers. The red snapper fishery in Australian waters is a trawl and trap demersal fishery. In the north-west of Australia, the fishery targets red emperor *Lutjanus sebae* (Cuvier) and goldband snapper *Pristipomoides multidens* (Day), but also takes both *L. erythropterus* and *L. malabaricus* using traps and droplines. A small trawl fishery operates in northern Arafura Sea and is directed towards *L. erythropterus* and *L. malabaricus*. In Australia, the total catch of all red snappers is estimated to be <3000 t (Blaber *et al.*, 2005).

The Indonesian red snapper fishery is more diverse in fishing gears and boat size with the fishery covering most of the archipelago. Accurate catch statistics are not available with the exception of the number of trawlers, bottom longline and trap vessels (both local and foreign-owned) operating in Indonesian waters. The commercial catch is probably an order of magnitude greater than the Australian catch. This estimate does not include artisanal boats that land their catches to local markets for consumption. Fishing pressure is intense as they are premium food fishes in northern hemisphere export markets.

Biologically, the two species are similar (Froese & Pauly, 2001). They are distributed widely in the tropical Indo-Pacific, have the same prey species and have similar maximum sizes and ages. They are highly fecund, are open water, serial spawners between October and February and have pelagic larvae of unspecified duration. *Lutjanus* species are reef associated as adults and can be found on trawl grounds as juveniles; they prefer depths of 5–100 m (Kailola *et al.*, 1993). There are concerns about the sustainability of these fishes based on their biological characteristics and their exploitation by both Australian and Indonesian fishers.

Limited information is available on the genetic structure of *L. malabaricus* and nothing is known on *L. erythropterus*. In a study of *L. malabaricus* from four sites across northern Australia, Elliott (1996) was able to discriminate between the north-west shelf sample and the Gulf of Carpentaria samples, using allozymes. These locations are >2000 km apart. The allele frequencies from the east coast of Australia were not significantly different from either the Gulf or north-west shelf samples. Mitochondrial DNA data, however, discriminated the east coast samples from the rest, suggesting the Torres Straits could be a barrier to gene flow (Elliott, 1996).

The lack of population structure uncovered in northern hemisphere red snappers (Gold *et al.*, 1997; Heist & Gold, 2000), and the large geographic scale of genetic differences reported for Australasian red snapper (Elliott, 1996; Ovenden *et al.*, 2002) is consistent with the perceived high dispersal capacity of marine fish species. Eggs, larvae, juveniles and adults are all capable of dispersal by advection or active swimming in the extensive marine environment that counters the accumulation of genetic differences through local evolutionary forces. A recent study of goldband snapper (Ovenden *et al.*, 2004) showed fine scale genetic structure in this region. The presence of deep water between the islands of Bali, Lombok, Sumbawa (Sape), Timor (Kupang) and the Australian sampling

sites, could create a barrier to fish movements between the Indonesian and Australian fisheries [Fig. 1(a), (b)]. In the shallower continental shelf that spreads over most of the Arafura Sea, the main barrier to movement of fishes (and hence gene flow) may be distance combined with deep water barriers to dispersal.

One of the questions that initiated this study was whether the Indonesian and Australian fishers were utilizing the same stock of red snappers (*L. erythropterus* and *L. malabaricus*). This genetic structure analysis of two species of red snapper in south-east Asian waters tests for presence of concordant genetic structure among sympatric species that have similar life-history strategies, biology and distribution. In addition, it provides input to the stock assessment process for tropical red snappers in Australia and Indonesia by defining stock boundaries that may be shared between the nations. The inferred affect of biogeographic features such as deep water trenches and reef-associated islands on red snapper genetic population structure may be duplicated among species, which would validate their importance in shaping marine biodiversity in the region.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PRESERVATION

To test the extent of genetic structure both within and between eastern Indonesia and northern Australia, samples of locally caught *L. erythropterus* and *L. malabaricus* were collected in markets and from throughout the Sunda Shelf and Arafura Sea. Samples were obtained from eight sites within Indonesia and four sites within Australia. Because some samples could not be frozen in the field for subsequent allozyme analyses, locations such as Tanimbar Island and Indonesian Timor Sea were analysed with mtDNA only. Samples from Indonesia were either dissected in the field or whole fish returned to the Gondol Laboratory (Bali) on ice for dissection. Australian samples were processed at the Cleveland Laboratory (Queensland).

For mtDNA analysis, *c.* 10–20 mm^2 of fin tissue or 5 mm^3 of muscle tissue was stored in DMSO (20% dimethyl sulphoxide in 5 M sodium chloride) at room temperature in the field and $-80°$C in the laboratory. In some cases where only frozen tissues were available, allozymes tissues (Australian Arafura and Timor Sea samples) were used to extract mtDNA. The target was 50 fish genotyped from each location. With this sample size, haplotype frequencies $>0{\cdot}03$ can be detected at a 95% CL according to binomial theory (Bartley *et al.*, 1995).

ELECTROPHORESIS

After dissection, all allozyme tissues were stored at $-20°$C until processed. In the laboratory, *c.* 1 g each of muscle and liver was placed into 1·5 ml micro-centrifuge tubes and 3–5 drops of homogenizing buffer (0·1 M Tris, 0·1 M EDTA, 5 μM NADP+, pH 7·0) were added to the samples. The vials were kept at $-20°$C until electrophoresis. Gels were made with 10% potato starch (Smithville, StarchArt, TX, U.S.A.) in a specific buffer solution. Before each gel run, tissues were partially thawed, then centrifuged at 10 000 *g* for 3 min at 4°C. Supernatant was soaked onto paper wicks of *c.* 2–3 mm width, allowing 45 samples (including controls) per gel. The rest of the electrophoretic protocol and histochemical staining used in this study followed Shaklee & Keenan (1986), Aebersold *et al.* (1987) and Shaklee *et al.* (1990). The histidine-citrate buffer used by Elliott (1996) was unsuccessfully tried while trying to improve resolution of some loci.

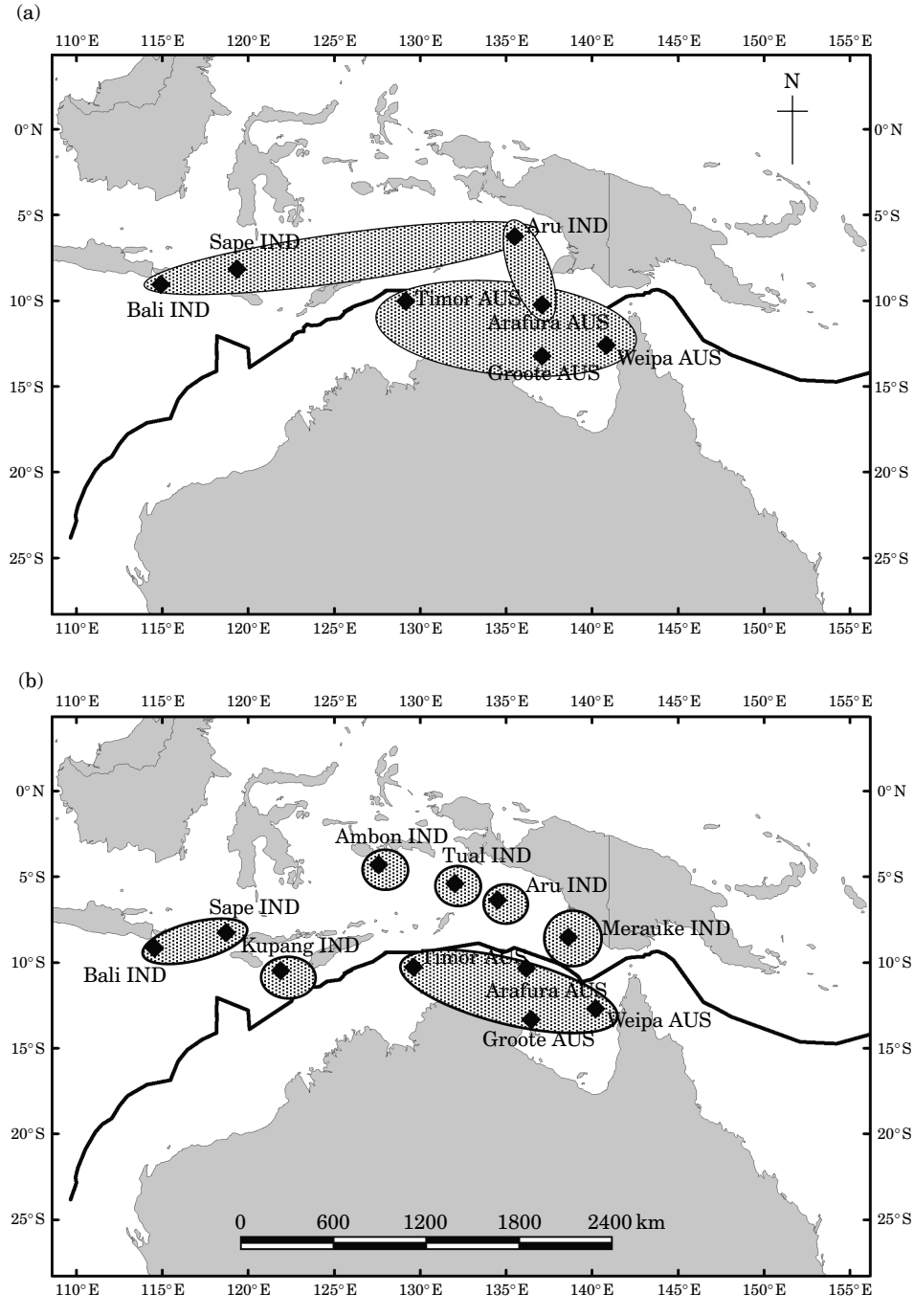

FIG. 1. The location of genetic stocks of (a) *Lutjanus erythropterus* and (b) *Lutjanus malabaricus* in eastern Indonesia and northern Australia. The origin of each sample is indicated: IND, Indonesia and AUS, Australia; —, the division between Australian and Indonesian fishing zone.

A total of 21 specific enzymes were used during the initial survey to identify allelic variation. Seven buffer systems were used to obtain optimum resolution per enzyme stain. Four enzymes, *Xdh*, *PEP*(phe-leu), *Mpi* and *Fdh* had insufficient activity to be scored reliably. Other loci, (*AH*, *AAT-1*, *AAT-2*, *Est-D*, *ME-1* and *Mdh-2*) appeared variable and produced good activity but were either too labile (*AH*) or could not be resolved well enough to be scored unambiguously in the majority of samples. The remaining 14 enzymes yielded information on 19 loci. Six of these loci were monomorphic while the remaining 13 loci exhibited at least one heterozygous individual over all samples. Loci, and tissues and buffers used to resolve the 13 variable loci are listed in Table I and samples sizes for each collection are listed in Table II. The most common allele at each locus was assigned a relative mobility of 100 and the mobility of all other alleles was calculated relative to this allele.

MITOCHONDRIAL DNA

For mtDNA haplotype determination a rapid method (Chelex) was used to prepare genomic DNA as PCR template. Approximately 375 bp of the 5′-end of the control region (D-loop) was amplified as described (Ovenden *et al*., 2002), with primers Pro889U20 (CCW CTA ACT CCC AAA GCT AG) and TDKD1291L21 (CCT GAA ATA GGA ACC AAA TGC). These primers were designed to have a high degree of homology based on the sequences of other lutjanids from Genbank. Red snapper RFLP haplotypes were determined with four (*L. erythropterus*; *EcoRI G'AATTC, EcoRV GAT'ATC, Hinf I G'ANTC* and *NlaIII CATG*') or two (*L. malabaricus*; *Alu I AG'CT, NlaIII CATG*') restriction enzymes.

STATISTICAL ANALYSIS

Allozymes

All genetic analyses of allozymes data were undertaken with the programme 'Genes in populations' version 2 (May *et al*., 1995) with allele pooling following the procedures suggested by the software. The results were compared for consistency to those obtained using the genic differentiation routine in GENEPOP, v3.1d programme (updated in March 1999 from v1.2 in Raymond & Rousset, 1995). Observed genotypic proportions were tested for agreement with expected Hardy–Weinberg equilibrium proportions using a log-likelihood ratio *G*-test and χ^2 probabilities (Sokal & Rohlf, 1981). Coefficients of

TABLE I. Allozyme loci scored in *Lutjanus erythropterus* and *Lutjanus malabaricus*

Loci	Pattern	Tissue	Buffer	Species
ADH	dimer	Liver	CAAPM	*L. erythropterus, L. malabaricus*
EST-2	monomer	Liver	CAAPM	*L. erythropterus, L. malabaricus*
G3PDH	dimer	Muscle	CAAPM/TC8	*L. malabaricus*
GP-3	monomer	Muscle	TC8	*L. malabaricus*
GPI-1	dimer	Muscle	TC8/CAAPM	*L. erythropterus, L. malabaricus*
GPI-2	dimer	Muscle	TC8/CAAPM	*L. malabaricus*
IDH-1	dimer	Liver	TC8	*L. erythropterus, L. malabaricus*
IDH-2	dimer	Muscle	TC8	*L. erythropterus, L. malabaricus*
LDH	tetramer	Muscle	TC8	*L. erythropterus, L. malabaricus*
MDH-1	dimer	Liver	TC8	*L. malabaricus*
ME-2	tetramer	Muscle	TC8/CAAPM	*L. erythropterus*, *L. malabaricus*
PEPlgg	dimer	Liver	CAAPM/TC8	*L. erythropterus*, *L. malabaricus*
PGM-2	monomer	Muscle	TC8	*L. erythropterus, L. malabaricus*

TABLE II. Sample sizes from all sites in Australia and Indonesia for both *Lutjanus malabaricus* and *Lutjanus erythropterus* (see Fig. 1 for a map of the collection sites). The numbers of fishes whose mtDNA control region was analysed with restriction enzymes or fully sequenced is given in parentheses

Location	N_{allo}	N_{mtDNA}	Location	N_{allo}	N_{mtDNA}
L. malabaricus			*L. erythropterus*		
Indonesian samples			Indonesian samples		
Ambon	117	–	Ambon	12[1]	–
Arafura Sea	–	46	Arafura Sea	–	41
Aru Sea	–	33 (3)	Aru Sea	–	16 (3)
Bali, Jimbaran	56	36 (7)	Bali, Jimbaran	87	27 (3)
Kupang	50	60 (3)	Kupang	9[1]	22 (3)
Merauke	99	49 (3)	Merauke	–	0 (3)
Probolinggo/Aru Island	47	–	Probolinggo/Aru Island	42	16 (3)
Sape	82[2]	82 (3)	Sape	65	19 (3)
Tanimbar	–	33 (2)	Tanimbar	–	–
Tual	114	–	Tual	13[1]	–
Timor Sea	–	–	Timor Sea	–	26
Australian samples			Australian samples		
Arafura Sea	57	43	Arafura Sea	51	42 (2)
Darwin	–	–	Darwin	136	29
Groote GoC	60	24	Groote GoC	59	13 (2)
Timor Sea	129	43	Timor Sea	132	45
Weipa GoC	43	31	Weipa GoC	20	13
Malaysian samples	46	–	Malaysian samples	56	–

[1]Not analysed due to reduced allozyme activity.
[2]Includes Tanjung Luar.

inbreeding values, F_{st} (Wright, 1965) and G statistics (Sokal & Rohlf, 1981) calculated by 'Genes in populations' (May *et al*., 1995) were tested for significant deviations from zero and from expected values. Initially, data from all sites were compared to see if there was significant genetic heterogeneity among sites. To determine where significant differences existed between sites, a series of adjacent pair-wise comparisons were made for each sample within each country and all test probabilities were adjusted for multiple tests. Differences between samples were considered to be significant when the overall comparison was significant across all variable loci and not a single locus. In some cases, one or more loci out of the nine to 13 tested per comparison were significantly different, while the overall calculation was not significant. In this case, the comparison was not considered significant.

A comparison between six common loci, *ADH, GPI-A, GPI-B, ME-2, PEP-lgg* and *PGM-2*, from Elliott (1996) and the present study was made for *L. malabaricus* by backcalculating allele numbers from allele frequencies and sample sizes from Elliott's (1996) published tables and applying a simple χ^2 test of observed (present data) *v.* expected (Elliott's data) alleles. This method allowed the similarity between Elliott's Timor Sea (North-West Shelf) samples and Kupang data from this study to be tested. In addition, it gave us the opportunity to compare samples from the same geographic areas over a temporal scale of at least 5 years. Temporal tests of allele frequency stability were made for the Groote site from both studies and Elliott's (1996) Eastern Gulf of Carpentaria and Weipa in the present study. Elliott's (1996) samples were from 1991 to 1994 compared to collections in 1999 and 2000 for the present study. To avoid

inconsistencies in allele designation between both studies, alleles other than the common '100' form, were pooled as the alternate allele for all six loci.

Mitochondrial DNA

Average gene diversity (h) per population was calculated according to Nei & Kumar (2000) where x_i is the population frequency of the ith haplotype. The probability of genetic non-differentiation between populations was assessed from mtDNA RFLP haplotype frequencies using an analog of Fisher's exact test (Raymond & Rousett, 1995). For exact tests the probability of observing a contingency table less likely than the sample configuration was assessed by a random walk between the states of a Markov chain. The number of Markov chain steps was 100 000 with intervals of 3000 dememorisation steps before comparing the alternative table to the observed table. A standard Bonferroni correction was applied to determine the level of significance for multiple tests. The pattern of intraspecific genetic variation among populations of *L. erythropterus* and *L. malabaricus* was further examined using *F*-statistics calculated from haplotype frequencies. The matrix of pair-wise *F*-statistics was plotted using MDS implemented in the software R (R Development Core Team, 2004). For the MDS analyses, negative *F*-statistics were set to zero. Exact tests and *F*-statistics were calculated in Arlequin v2.0 (Schneider *et al.*, 2000).

RESULTS

ALLOZYMES

Variation was found in three loci in the Indonesian populations, [*General Protein-3, LDH* and *GAPD* (*G3pdh*)], that were not reported as variable in Australian *L. malabaricus* samples by Elliott (1996). No evidence, however, was found of allozyme variation in the samples at the *EST-D* locus despite strong activity and high allelic diversity in liver tissues as reported by Elliott (1996). Elliott (1996) also reported variation at a second peptidase locus, *PEPphe-leu* (*PEP-C*), which could not be duplicated when *phe-leu* was used as substrate. The *phe-leu* bands appeared the same as for the *PEPleu-gly-gly* (*PEP-B*) locus. Loci with difficult to interpret variation or inconsistent banding patterns were discarded (*AH, Xdh, AAT-1, AAT-2*).

For *L. malabaricus* comparisons within Indonesia and within Australia, only 10 and 11 (of the 13) variable loci respectively were observed. Similarly for *L. erythropterus* samples, of a total of 11 variable loci, only five and 10 loci were variable in Indonesian and Australian samples respectively.

All loci for *L. malabaricus* were in Hardy–Weinberg equilibrium. Comparisons of genetic discreteness between populations within Indonesia (Table III) revealed significant population differentiation among the seven samples. Progressive, adjacent pair-wise comparisons showed there were six genetically discrete populations, (Jimbaran + Sape), Kupang, Ambon, Tual, Aru Island., Merauke [Table III and Fig. 1(b)].

Australian samples (Timor Sea, Arafura Sea, Groote and Weipa) were genetically homogeneous. All comparisons of nearest sites with Indonesia, *e.g.* Kupang *v.* Timor Sea, Merauke *v.* Arafura Sea, Aru Island *v.* Arafura Sea, Tual *v.* Arafura Sea and Ambon *v.* Arafura Sea, revealed significant differentiation between Australian and Indonesian populations (Table III). The exact collection location of the Merauke sample, however, was unknown and could easily have

TABLE III. Allozyme genetic differentiation as measured by indices of fixation (F_{ST}) used for *Lutjanus malabaricus* population discrimination. The F_{ST} deviation from zero is tested with the *G* statistic, which is treated as a χ^2 value. *N* (variable loci) refers to the number of loci used in the comparison and, where significant, the number of significant loci/number of variable loci analysed. H_T is the total heterozygosity. Only significant *P* values are displayed (after Bonferroni adjustment)

L. malabaricus	F_{st}	*G*(d.f.)	*P*	*N* (variable loci)
Comparison (within countries)				
All Australian sites	0·019	$74{\cdot}0_{63}$	NS	11
Mean ± S.E. H_T = 0·073 ± 0·033				
All Indonesia sites	0·028	$212{\cdot}4_{105}$	<0·001	6/10
Mean ± S.E. H_T = 0·023 ± 0·007				
Comparison (Within Indonesia)				
Bali *v.* Sape	0·007	$18{\cdot}0_{15}$	NS	8
Kupang *v.* Ambon	0·021	$66{\cdot}7_{16}$	<0·001	5/9
Ambon *v.* Merauke	0·02	$88{\cdot}7_{16}$	<<0·001	6/9
After progressive pooling				
(Bali + Sape) *v.* Kupang	0·017	$44{\cdot}3_{20}$	0·005	3/9
Bali + Sape *v.* Ambon	0·004	$48{\cdot}6_{21}$	<0·001	2/10
Bali + Sape *v.* Merauke	0·015	$61{\cdot}1_{18}$	<0·001	2/8
Comparison (Australia *v.* Indonesian waters)				
Merauke *v.* Arafura Sea	0·043	$100{\cdot}4_{15}$	<<0·001	4/9
Merauke *v.* Timor Sea	0·054	$172{\cdot}1_{19}$	<<0·001	5/12
Ambon *v.* Arafura Sea	0·03	$82{\cdot}4_{19}$	<<0·001	3/11
Ambon *v.* Timor Sea	0·033	$108{\cdot}6_{20}$	<<0·001	5/12
Control comparisons				
Malaysia *v.* Ambon	0·075	$71{\cdot}1_{14}$	<<0·001	2/8 loci
Malaysia *v.* Bali + Sape	0·077	$86{\cdot}7_{20}$	<<0·001	1/4 loci
Malaysia *v.* Australia	0·096	$117{\cdot}8_{8}$	<<0·001	1/9 loci

originated from a wide region, including Aru Island or Tual fisheries. It was less likely to have originated from Australian waters because of the declared fishing zone boundaries.

The temporal comparison of *L. malabaricus* allele distributions in *ADH*, *GPIA*, *GPIB*, *ME2*, *PEPlgg* and *PGM2*, with Elliott (1996) revealed significant differences between studies (Table IV). Elliott's (1996) North-West Shelf sample is roughly analogous to the present study Timor Sea site, which is >500 km to the north-east. This is comparable to the geographic separation between the other Australian sites in the present study. There was a significant difference between East Gulf of Carpentaria (Elliott, 1996) compared to Weipa (this study) after Bonferroni corrections ($P < 0{\cdot}005$, Table IV), although the significant χ^2 probability may have been influenced by small sampler sizes in this study (Table II). West Gulf of Carpentaria (Groote in Elliott, 1996) *v.* Groote (this study) was not significantly different.

All loci for *L. erythropterus* were in Hardy–Weinberg equilibrium except for *PGM-2* in the Groote sample ($P < 0{\cdot}05$) where there was a heterozygote deficiency. Seven variable loci were available for a global comparison of genic

TABLE IV. Comparison with previous *Lutjanus malabaricus* allozyme studies in northern Australia (Elliott, 1996) for six loci: *Adh*, *GPI-1*, *GPI-2*, *ME-2*, *PEP-lgg* and *PGM-2*

Site comparison	χ^2	d.f.	*P*	*N* loci
NWS *v.* Kupang	268·9	10	<<0·001	5 significant out of 6
NWS *v.* AustTimor Sea	77·1	10	<0·001	3/6
Groote 1996 *v.* Groote present	3·9	6	ns	0/6
EgoC *v.* Weipa present	20·4	6	<0·005	2/6

NWS, North-West Shelf 1996; EgoC, East Gulf of Carpentaria 1996. Only significant *P* values are displayed (after Bonferroni adjustment)

differentiation. There were no significant differences between all three Indonesian sites [Fig. 1(a)]. The five Australian sites were also not significantly different from each other, although the pooled Australian samples were significantly different from the pooled Indonesian samples (Table V). Curiously, the Aru Island sample was not significantly different from the Arafura Sea sample, yet the other Indonesian sites (Jimbaran and Sape) were each significantly different from Australian samples [Fig. 1(a) and Table V]. In addition, the Malaysian juveniles were not significantly different from Indonesia, although it was a limited sample (Table V, muscle loci only). This supports the notion of a genetically homogeneous Arafura basin population that includes Aru Islands and northern Australia.

TABLE V. Allozyme genetic differentiation as measured by indices of fixation (F_{ST}) used for *Lutjanus erythropterus* population discrimination. The F_{ST} deviation from zero is tested with the *G* statistic, which is treated as a χ^2 value. *N* (variable loci) refers to the number of loci used in the comparison and, where significant, the number of significant loci/number of variable loci analysed. H_T is total heterozygosity. Only significant *P* values are displayed (after Bonferroni adjustment)

L. erythropterus	F_{st}	*G*(d.f.)	*P*	*N* (variable loci)
Comparison (within countries)				
AUSTRALIA Mean ± S.E. H_T = 0·058 ± 0·027				
Within Arafura Sea (two samples)	0·020	$7{\cdot}8_6$	NS	3
Within Timor Sea (five samples)	0·032	$86{\cdot}3_{88}$	NS	11
Arafura *v.* Groote *v.* Timor *v.* Weipa	0·007	$53{\cdot}4_{63}$	NS	10
INDONESIA mean ± S.E. H_T = 0·029 ± 0·007				
Jimbaran *v.* Sape	0·005	$8{\cdot}8_8$	NS	5
Comparison (between countries)				
Arafura *v.* Indonesia	0·036	$47{\cdot}7_{13}$	<0·001	2/6
Timor *v.* Indonesia	0·036	$85{\cdot}1_{20}$	<0·001	2/10
Australia *v.* Indonesia	0·036	$89{\cdot}8_{19}$	<0·001	2/8
Aru *v.* Arafura	0·005	$7{\cdot}4_6$	NS	5 loci
Aru *v.* Timor	0·009	$22{\cdot}9_{19}$	NS	9 loci
Aru *v.* Darvin juveniles (<150 mm L_S)	0·006	$10{\cdot}4_7$	NS	3 loci
Malaysia *v.* Indonesia	0·033	$14{\cdot}0_6$	NS	4 loci

MITOCHONDRIAL DNA

For *L. erythropterus*, restriction enzymes *Eco*R1, *Eco*RV and *Nla*III produced one, two or three fragments that were used to assign a morph (A or B) to each fish. Haplotypes for *L. malabaricus* were constructed using restriction enzymes *NlaIII* and *XbaI*, which both had three morphs (A, B and C). Reference sequences (data not shown) for each species have been deposited in Genebank (*L. erythropterus*, AY205238/9; *L malabaricus* AY205240/1) Haplotype frequencies are summarized in Table VI.

Spatial variation in haplotype frequencies between adjacent populations for both red snapper species revealed the presence of separate fisheries stocks in the study area. In *L. erythropterus*, the populations from Bali and Sape were from the same genetic stock, possibly including the population from Kupang ($P > 0{\cdot}05$ Table VII), The majority of the populations in northern Australia and central and northern Indonesia were not significantly differentiated defining a genetic stock that occupies several hundred kilometres from west to east. There were two additional populations in northern Australian waters that were adjacent to this geographically widespread stock, but which may have been distinct. The haplotype frequencies of the population from Weipa in the eastern Gulf of Carpentaria was significantly different to all remaining populations ($P < 0{\cdot}005$) except the population from Groote in the west of the Gulf ($P = 0{\cdot}1880$).

The mtDNA genetic stock structure of *L. malabaricus* was similar to *L. erythropterus* (Table VII) with two major stocks; one in central Indonesia and the other to the north of Australia. The central *L. malabaricus* stock consisted of populations from Bali and Sape that were largely distinct from the remaining populations (Table VII). Unlike *L. erythropterus*, however, the Kupang population of *L. malabaricus* was not part of the central stock. The haplotype frequencies of the Kupang population were indistinguishable from populations in eastern Indonesia and northern Australia that formed a widespread and homogeneous stock from there to the eastern Gulf of Carpentaria and north to Tanimbar Island. The pattern of haplotype frequency similarity among genetic stocks of *L. erythropterus* and *L. malabaricus* was reflected in the three dimensional placement of populations in the MDS plot (Fig. 2).

DISCUSSION

Allozyme genetic variation for *L. erythropterus* and *L. malabaricus* was low in this study in comparison to freshwater and anadromous fish species, but similar to many marine teleosts (Ward *et al.*, 1994). Indices of fixation (F_{ST}) reflected the low genetic variation (Tables III and V) although this did not preclude statistically significant genetic differences between Australian and Indonesian samples and within Indonesia. Hartl (1980) suggests that for a variety of organisms, including humans, values of F_{ST} between 0·05 and 0·15 indicate moderate genetic differentiation between subpopulations, which places the observed genetic differences between Australian and Indonesian *L. malabaricus* populations in this category. Most of the other significant comparisons within Indonesia and for all *L. erythropterus* samples fall in the range of little genetic differentiation ($F_{ST} < 0{\cdot}05$) suggested by Hartl (1980). Ward *et al.* (1994)

TABLE VI. Mitochondrial DNA control region RFLP haplotype frequencies of Australian, Papuan New Guinea and Indonesian populations of red snappers, *Lutjanus erythropterus* and *Lutjanus malabaricus*

	Australia				Indonesia					
Haplotype	Timor	Arafura	Weipa	Groote	Sape	Kupang	Arafura	Aru	Timor	Bali
L. erythropterus										
N	45	42	13	13	19	22	41	16	26	27
AAA	0·33	0·24	0·15	0·39	–	0·18	0·49	0·50	0·27	0·04
BBA	0·27	0·38	0·15	0·31	0·21	0·14	0·24	0·25	0·39	0·15
BAA	0·31	0·29	0·08	0·15	0·79	0·64	0·22	0·25	0·23	0·82
ABA	0·04	–	–	–	–	0·05	0·02	–	0·08	–
BBB	0·04	0·10	0·46	0·15	–	–	0·02	–	0·04	–
AAB	–	–	0·15	–	–	–	–	–	–	–
BAB	–	–	–	–	–	–	–	–	–	–

	Australia				Indonesia						
Haplotype	Timor	Arafura	Groote	Weipa	Merauke	Kupang	Sape	Bali	Arafura	Tanimbar	Aru
L. malabaricus											
N	43	43	24	31	49	60	82	36	46	33	33
AB	0·35	0·26	0·42	0·36	0·22	0·32	0·67	0·67	0·20	0·24	0·24
BB	0·23	0·30	0·29	0·10	0·33	0·15	0·09	0·03	0·35	0·36	0·24
CA	0·19	0·26	0·13	0·32	0·20	0·32	0·12	0·03	0·24	0·27	0·33
AA	0·07	0·02	–	–	–	–	–	0·06	0·02	–	–
CB	0·09	0·09	0·17	0·23	0·20	0·18	0·09	0·19	0·20	0·12	0·18
AC	0·07	0·02	–	–	0·04	0·02	0·02	0·03	–	–	–
BC	–	0·02	–	–	–	–	0·01	–	–	–	–
CC	–	0·02	–	–	–	0·02	–	–	–	–	–

TABLE VII. Significant *P*-values of non-differentiation between *L. erythropterus* and *L. malabaricus* populations using an analog of Fisher's exact test

Timor, Australia	Timor	Arafura	Weipa	Groote	Sape	Kupang	Arafura	Aru	Timor
L. erythropterus									
Arafura, Australia	NS								
Weipa, Australia	0·0001	NS							
Groote, Australia	NS	NS	NS						
Sape, Indonesia	0·0009	0·0015	0·0001	0·0001					
Kupang, Indonesia	NS	NS	0·0002	NS	NS				
Arafura, Indonesia	NS	NS	0·0004	NS	0·0001	NS			
Aru, Indonesia	NS	NS	0·0029	NS	0·0003	NS	NS		
Timor, Indonesia	NS	NS	0·0041	NS	0·0007	NS	NS	NS	
Bali, Indonesia	0·0001	0·0001	0·0001	0·0001	NS	NS	0·0001	0·0001	0·0001

Timor, Australia	Timor	Arafura	Groote	Weipa	Merauke	Kupang	Sape	Bali	Arafura	Tanimbar
L. malabaricus										
Arafura, Australia	NS									
Groote, Australia	NS	NS								
Weipa, Australia	NS	NS	NS							
Merauke, Indonesia	NS	NS	NS	NS						
Kupang, Indonesia	NS	NS	NS	NS	NS					
Sape, Indonesia	0·0030	0·0011	NS	0·0095	0·0000	0·0005				
Bali, Indonesia	0·0025	0·0000	0·0099	0·0019	0·0000	0·0000	NS			
Arafura, Indonesia	NS	NS	NS	NS	NS	NS	0·0000	0·0000		
Tanimbar, Indonesia	NS	NS	NS	NS	NS	NS	0·0001	0·0000	NS	
Aru, Indonesia	NS	NS	NS	NS	NS	NS	0·0003	0·0000	NS	NS

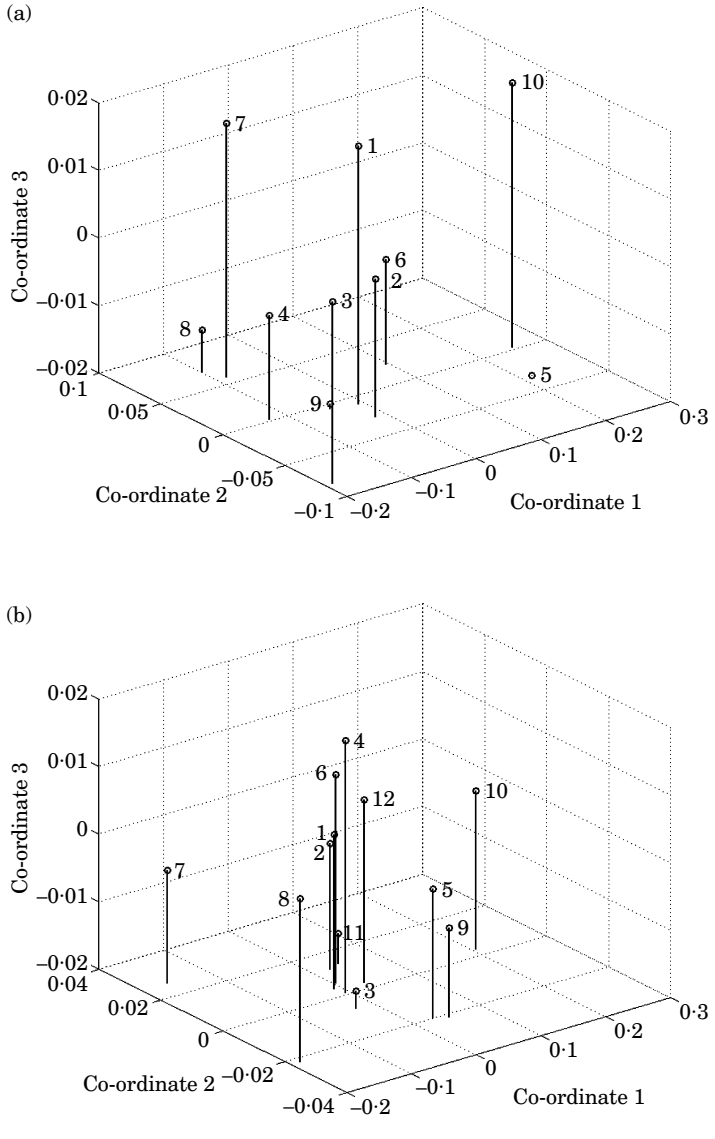

FIG. 2. MDS plot of similarity between (a) *Lutjanus erythropterus* and (b) *Lutjanus malabaricus* populations based on pair-wise F_{ST} calculated from mtDNA haplotype frequencies. The populations were from (a) Australia [Timor (1) and Arafura (2) Seas, Weipa (3) and Groote Eylandt (4)] and Indonesia [Sape (5), Kupang (6), Arafura (7), Aru (8) and Timor (9) Seas and Bali (10)] and (b) Australia [Timor (1) and Arafura (2) Seas, Groote Eylandt (3) and Weipa (4)], Indonesia [Merauke (5)] and Indonesia [Kupang (6), Sape (7), Bali (8), the Arafura (9) and Timor (10) Seas, Tanimbar (11) and the Aru Sea (12)].

calculated an average G_{ST} (analogous to F_{ST}) of 0·062 from literature values for 57 species of marine fishes, which suggests the F_{ST} for *L. malabaricus* was representative of many other studies. Within Australia, however, *L. ertythropterus* showed less genetic differentiation (F_{ST}) than *L. malabaricus* (Tables III and V), although H_{TS} were comparable between species.

Substantial evidence is presented for the presence of multiple fisheries stocks for two red snapper species in northern Australian and central and eastern Indonesian waters. Stock boundaries presented here are not definitive, as they may be widened with future sampling effort, or new stocks may be revealed. A minimum of two fisheries stocks are proposed for *L. erythropterus* and seven for *L. malabaricus*, excluding the reference stock from Malaysia. The two methods, allozymes and mtDNA, differ in the number of stocks identified for *L. malabaricus*. The allozyme data suggests seven discrete populations: Bali (Bali, Lombok and Sumbawa), Kupang, Ambon, Tual, Aru, Merauke and all Australian samples forming a single stock. mtDNA, however, only discriminates two populations with the boundary between the islands of Sumbawa (Sape) and Timor (Kupang, Table VII)

Each species has a widespread stock in the shallow, continental shelf waters of northern Australia. In the case of *L. erythropterus*, this crosses the outer limit of the Australian fishing zone, but does not cross the Australian fishing zone for *L. malabaricus* based on the allozyme analyses. For *L. erythropterus* and *L. malabaricus* this stock includes populations in the shallow waters around Aru Island and within the Arafura Sea. In Indonesian waters the opposite was found for *L. malabaricus*; that is, the allozyme analysis suggests genetically discrete populations at all sites sampled. Although the seven allozyme stocks include two sites not sampled for mtDNA, Ambon and Merauke, there are three other genetically discrete stocks not identified from mtDNA haplotype frequencies. Gardner & Ward (1998) found more discrete stocks of gummy shark *Mustelus antarcticus* Günther around Australia using allozymes than with mtDNA haplotypes. Allozymes successfully distinguished close populations of Angel shark *Squatina californica* Ayres along the California coast (Gaida, 1997). Smith *et al*. (1997) also reported mtDNA haplotype analyses revealed less genetic subdivision than allozymes in a study of orange roughy *Hoplostethus atlanticus* Collett off New Zealand. There is precedence for the discovery of multiple fisheries stocks or genetically discrete populations in lutjanids in Indonesia. Ovenden *et al*. (2004) reported five discrete populations of *P. multidens* from mtDNA haplotype frequencies from the same geographic area as this study.

A possible explanation for observed differences in population discrimination by allozymes and mtDNA may be that more rigorous analyses of mtDNA by targeting more restriction sites could have uncovered more differences that were correlated with the allozymes patterns. The haplotype diversity revealed by restriction enzyme analysis for both species, however, was high suggesting that sequence variation in mtDNA was adequately revealed by the methods used. The present approach also avoided the statistical problems associated with the analysis of single-observation data that are commonly generated when a number of restriction enzymes are used and consequently many samples have unique haplotypes. The relative usefulness of nuclear (allozyme loci) and mitochondrial genomes for genetic stock discrimination may be governed by the evolutionary

dynamics of these lutjanid species. Elliott (1996) also found that allozyme loci revealed different aspects of the population genetic structure of *L. malabaricus* compared to mtDNA.

The Ambon site (tissues for mtDNA were not available), Tual and Aru Island sites have relatively deep bodies of water separating them that are >1000 m in depth. This could act as a barrier to direct population mixing during larval, juvenile or adult stages.

The degree of population structure reported here is greater than for northern hemisphere populations of red snapper species analysed to date with similar methods (Shaklee and Samollow, 1984, Camper *et al.*, 1993, Bagley *et al.*, 1999). Multiple genetic stocks, however, have been found for other north Australian and south-east Asian marine fish species with similar distributions. The shallow Torres Strait region between Cape York in northern Queensland and southern Papua New Guinea appears to have played a role in shaping the population structure of *L. malabaricus* (Elliott, 1996) and *Scomberomorus commerson* (Lacepède) (Shaklee *et al.*, 1990). Genetic separation between northern Australian and central and western Indonesian populations of *P. multidens* (Ovenden *et al.*, 2002) has been reported. Combined with the data reported here for two species of red snappers (*L. erythropterus* and *L. malabaricus*), the presence of deep water appears to be important in genetic population cohesion. Stocks appear to be homogeneous in shallow water to Australia's north, but generally genetically distinct across deep straits, such as the Timor Trench and in the Banda, Savu and Flores Seas. This hypothesis appears to apply equally well to bottom-dwelling (*L. erythropterus* and *L. malabaricus*) demersal (*P. multidens*) and epipelagic (*S. commerson*) species and presumably acts as a barrier to dispersal for all life-stages of these species, from egg to adult. This is not surprising for stock differences on broad spatial scales where isolation by distance is a possibility, but the geographic proximity of *L. erythropterus* stocks on Timor (Kupang) and Australian Timor Sea and *L. malabaricus* stocks on Sape and Timor, suggests that the dispersal capacity of these snappers is low. This generalization cannot be applied to all lutjanids, nor perhaps to all red snappers, as Ovenden & Street (2003) found little population genetic structure in another co-occurring red snapper, *Lutjanus argentimaculatus* (Forsskål) with microsatellite and mitochondrial loci.

The allozyme results presented are the first published for *L. erythropterus* and they extend the range of samples previously reported for *L. malabaricus* in northern Australia (Elliott, 1996). Although not all variable loci in *L. malabaricus* were repeatable in the samples, other loci were found to be variable (Elliott, 1996). The allozyme results support Elliott's (1996) findings of no genetic discrimination within the Gulf of Carpentaria. When the Weipa sample was compared against Elliott's (1996) Eastern Gulf of Carpentaria sample for six loci common to both studies, there was a weak significant difference. This may be a consequence of sampling differences or laboratory differences in detection of alleles. Similarly, his North-West Shelf sample was significantly different from both the Kupang and Australian Timor Sea samples from this study, supporting the present results that show there is a genetic boundary between Kupang (Kupang, Sape and Jimbaran combined) and Australian coastal *L. malabaricus* populations.

Levels of detected allozyme variation were higher in Australia than in Indonesia with mean ± S.E. heterozygosities (H_T) of 0·073 ± 0·033 and 0·023 ± 0·007 respectively for *L. malabaricus* and 0·059 ± 0·027 and

0·029 ± 0·007 respectively for *L. erythropterus*. *Lutjanus malabaricus* samples in Australia were not genetically differentiated across the present sampling range, although Elliott (1996), on a larger geographic scale, distinguished north-west coast *L. malabaricus* from Gulf of Carpentaria and east coast samples. He calculated H_T = 0·136 for 10 polymorphic loci. This is almost double the Australian samples H_T (0·073 see Table III). His high H_T is a result of high levels of variation in the *PGM-1* locus, which was unscorable in the present liver samples. When the six reliably scored loci common to both studies were analysed, the present H_T = 0·131 ± 0·059. Obviously, this calculation of H_T based on the six common loci ignored the less variable loci in the study and produced a biased, but higher value of total heterozygosity.

Lutjanus malabaricus genetic heterogeneity was evident between the Australian Timor Sea samples and both Kupang and Bali + Sape, between Kupang and both Ambon and Merauke [Fig. 1(b)] and between Ambon and Merauke (Table III) over a wider geographic range. The genetic discreteness of the Ambon, Tual and Aru Islands suggests that deep-water existing between these sites may provide barriers to gene flow. The Arafura Sea basin consists of at least four stocks, Aru Islands, Merauke (although these fish probably were captured well off-shore in the Arafura basin), Australian Arafura and Timor Seas. The discrimination between the north Australian sites and Aru Islands and Merauke may indicate an isolation by distance mechanism separating these populations. In contrast, *L. erythropterus* showed genetic homogeneity from Bali to Aru Islands (*c.* 1500 km). Johnson *et al.* (1993) made similar conclusions about genetic homogeneity from an allozyme study of *L. sebae* over 2080 km of north-west Australian coastline. They found no evidence of genetic subdivision and an average F_{ST} of 0·003.

This genetic analysis of the population structure of the red snapper in both Australia and Indonesia suggests that consideration must be given to a multiple stock fishery model for sustainable management decisions relating to these fisheries. Allozyme electrophoresis in this study has identified a minimum number of discrete stocks, five in Indonesia and a single stock in Australian waters for *L. malabaricus* as well as the Malaysian stock. Elliott (1996), however, found that Australian populations of *L. malabaricus* consist of at least three genetically discrete populations sampled across >3000 km of coastline from west to east. Given the relatively low F_{ST} values, the genetic stock boundaries identified so far must be considered approximate and subject to some gene flow. For the Exclusive Economic Zone (EEZ) boundary, the reality of the fishery is that the same stock is fished by both countries and hence allowable catch as well as effort, must account for depletion of a common resource as well as recognizing the genetically separate stocks within both Australia (Elliott, 1996) and Indonesia (this study).

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