Fisheries Research 100 (2009) 148–155

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

A comparison of the population genetics of *Lethrinus miniatus* and *Lutjanus sebae* from the east and west coasts of Australia: Evidence for panmixia and isolation

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ARTICLE INFO

Article history:
Received 10 March 2009
Received in revised form 26 June 2009
Accepted 2 July 2009

Keywords:
Lethrinus miniatus
Lutjanus sebae
mtDNA
Genetic diversity
Neutrality tests and selection
Resilience to perturbations

ABSTRACT

Lethrinus miniatus and *Lutjanus sebae* are important commercial and recreational species of reef fish. Within Australian waters the former species is less widespread than the latter and has a discontinuous distribution, whilst the latter is continuously distributed in tropical Australian waters. The demographic attributes of these species (e.g. long life span, low rates of natural mortality) make them vulnerable to over-exploitation. Consequently, conservative harvest strategies including no-take zones for these species have been adopted by fisheries management agencies to control exploitation. Information on the genetic stock structure of these species is important for developing specific management strategies. However, little is known about genetic stock structures within and between east and west Australian populations of these species. The current study used the mitochondrial genome hypervariable region 1 (HVR1) of the control region to examine variation between two sites from both the east and west Australian coasts for each species. HVR1 for *L. sebae* did not differ genetically either within or between coasts ($F_{st} < 0.018$, $p > 0.15$) at the sites studied, suggesting a panmictic population structure. Similarly, *L. miniatus* did not differ significantly between sites sampled within coast. However, the west coast HVR1 for *L. miniatus* east and west coast populations, were discrete (F_{st} of at least 0.92, $p < 0.0001$). The degree of genetic sub-division between east and west coast populations indicates that they should be managed as discrete stocks. Further, when considering both species, the lower genetic (both haplotype and nucleotide) diversity in three of the four sites on the west coast of Australia, indicates that this region is genetically impoverished and neutrality tests suggest that selection is responsible. Consequently, west Australian populations will be less resilient to perturbations (e.g. fishing, climate change) than east Australian populations, which have higher genetic diversity.

1. Introduction

Worldwide fish stocks have experienced increasing fishing pressure since the latter half of the past century, which has lead to fisheries collapses in many cases (Myers and Worm, 2003; Sale et al., 2005; Pauly et al., 2002). Coral reef fisheries are no exception, and this has serious implications for the health of coral reefs (Bellwood et al., 2004; Jackson et al., 2001). *Lethrinus miniatus* (Family: Lethrinidae, subfamily: Lethrininae; red throat emperor) and *Lutjanus sebae* (Family: Lutjanidae, subfamily: Lutjaninae; red emperor snapper) are both highly targeted by commercial and recreational fishers on the tropical east and west coasts of Australia (Newman and Dunk, 2002; Williams et al., 2006; Newman et al., in press). As with many targeted reef fish species, high levels of exploitation have resulted in diminished population sizes of *L. miniatus* and *L. sebae* throughout their range around the Australian coast. Targeted fishing activities in association with the low production potential of these species as a result of their life history attributes (Newman and Dunk, 2002; Newman et al., in press) have been recognised by fisheries management agencies in Queensland and Western Australia through the implementation of rigorous harvest and conservation strategies (Newman et al., 2008). Significant no-take and restricted zones, as well as catch size and number restrictions are now in place for many reef fish species on the GBR and in Western Australian waters. Current management and conservation strategies are generally based on the belief that there is

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0165-7836/$ – see front matter.
doi:10.1016/j.fishres.2009.07.003

one interbreeding genetic population of each species of reef fish throughout a major region such as the GBR, and perhaps Australia-wide. A detailed understanding of the genetic stock structure and associated linkages among populations of these important reef fish species is required in order to improve and enhance management arrangements.

The range of *L. miniatus* in the Indo-Pacific is restricted to the Ryukyu Islands, eastern Philippines, northern Australia and New Caledonia (Carpenter and Niem, 2001), whereas, *L. sebae* is found throughout the Indo-West Pacific region (Froese and Pauly, 2006). The Australian distribution of *L. miniatus* is understood to be discontinuous around the top of Australia. The northern-most range in Western Australia for *L. miniatus* is the Montebello Islands (20°15′S), whereas in eastern Australia the northern-most range along the Great Barrier Reef (GBR) is around Cairns (16°35′S), though large numbers have been reported seasonally near Cooktown (15°28′S) and small numbers are occasionally caught in the Gulf of Carpentaria. In contrast, the iconic reef fish *L. sebae* is more widespread and continuously distributed around northern Australia and in the Indo-West Pacific (Kailola et al., 1993).

Here, we aim to determine the population genetic stock structure of Australian populations of both species at both inter- and intra-regional scales, using the HVR1 containing region of the mtDNA. Given the contrasting distributions of these species between east and west Australia, we test hypotheses for both species that between and/or within regional populations are genetically differentiated.

2. Materials and methods

2.1. Sample collection

Fin clips or tissue samples were collected during Effects of Line Fishing Experiment (ELF) surveys undertaken by the Reef Cooperative Research Centre (CRC) in 1998 from adult *L. miniatus* from the GBR near Gladstone—Sandshoe Reef (SR, $n = 17$) and Townsville—Dip Reef (DR, $n = 20$). Fin clips or tissue samples were collected from adult *L. miniatus* from fishers in the Kalbari area, Western Australia (KB, $n = 19$; collected 1998) and Montebello Islands, Western Australia (MB, $n = 18$, collected 2000). GBR *L. miniatus* samples were initially preserved in a brine ice slurry for up to 3 h before they were transferred to liquid nitrogen, followed by storage at −70 °C. When later thawed for DNA extraction, samples were then placed into 80% ethanol for further storage at −20 °C. Western Australian *L. miniatus* samples were initially preserved in 20% DMSO, 100 mM EDTA, and saturated NaCl solution in the field for transportation purposes. Samples were then washed in TE buffer pH 8.0 and placed into 80% ethanol and stored at −20 °C.

Fin clips or tissue samples for *L. sebae* were all collected in 2005 from adult fish. Samples were from a reef shoal approximately 40 km east of Yeppoon on the Central Queensland coast (locally called Catfish Shoal, CF, $n = 20$); further north of Yeppoon at High Peak Island (HP, $n = 14$); in the vicinity of Browse Island in the Kimberley region of Western Australia (BI, $n = 20$); and at Montebello Islands, Western Australia (MI, $n = 18$). Sites of sample collection for both species are shown in Fig. 1. Samples of *L. sebae* were initially preserved in 20% DMSO, 100 mM EDTA, and saturated NaCl solution, washed in TE and placed into 80% ethanol for storage as above.

2.2. DNA extraction

After washing ethanol from samples using TE buffer, total DNA was extracted from approximately 10 mg of fin or tissue using a standard Proteinase K digest of tissue in CTAB buffer/NaCl, followed by NaCl–chloroform purification and ethanol precipitation (Sambrook et al., 1989). Concentrations and purity of DNA were determined from absorbance measurements at 260 and 280 nm, and 260/280 nm ratios were above 1.5.

2.3. Primer development and validation

Species-specific primers were previously developed, validated and reported for *L. sebae* (Aspden et al., 2006). These species-specific primers used to amplify the marker for *L. sebae* were:

RECQUF (forward): 5′ATTATAAGCTAAACTACTCTTTGCATATAC3′
RECQUR (reverse): 5′CGATTATTGTCCCTCACCC3′.

DNA extracted from fin clips from the 17 *L. miniatus* samples from SR were first used to develop and test species-specific primers, both for further use in identification of tissues, larvae, and eggs, but more immediately to have a useful set of working primers for amplification of DNA in the current study. PCR was initially undertaken on 3 of the SR DNA samples using universal primers L15995 and H16498 (Kocher et al., 1989), previously reported to generate the HVR1 fragment in *Chlorurus sordidus* (Bay et al., 2004), and *L. sebae* (Aspden et al., 2006). PCR reactions (25 μl) were used containing 100 μM each dNTP, 7.5 pmol of each primer, 1.0 mM $MgCl_2$, 1.0 unit Taq polymerase (Promega) and 2.5 μl of 10× PCR buffer (Promega) and approximately 25 ng of DNA template. The touchdown PCR protocol involved an initial 2 min at 94 °C, followed by 5 cycles at 94 °C for 30 s, 45 °C for 30 s and 72 °C for 2 min. Five cycles followed with the annealing temperature reduced to 43 °C, followed by 25 cycles with the annealing temperature at 41 °C. PCR was completed with 10 min at 72 °C.

PCR products of each sample were run using horizontal gel electrophoresis and a 1% agarose, Sybr Green (Astral Scientific Pty. Ltd., NSW, Australia) stained gel. PCR products were visualized at approximately 400 bp using the Gel Doc system (BIO-RAD Pty. Ltd., NSW, Australia) and with reference to Hyperladder II (BioLine (Aust) Pty. Ltd., NSW, Australia). Bands were excised and purified using a Gel Cleanup System (Promega Corporation). Approximately 3 pg of DNA from the purified band was added to a sequencing reaction using the Big Dye Terminator v3.1 Cycle Sequencing Kit according to manufacturer's directions (Applied Biosystems). Initially, 3 samples were sequenced in both the forward and reverse direction on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) producing a 396 bp sequence. A GenBank BLAST search revealed that the *L. miniatus* sequences aligned most closely to mtDNA d-loop data for the Gray weakfish, *Cynoscion regalis* (accession: DQ179650). A broad search for other gene sequence information for *L. miniatus* revealed only microsatellite and cytochrome *b* genetic data (van Herwerden et al., 2003).

The regions at the start and end of the HVR1 sequences for the 3 *L. miniatus* samples were found to be highly conserved enabling species-specific primers to be designed using Primer Express® Software v2.0 (Applied Biosystems, 2001), as follows: RTECQUDLF: 5′TTCTCATTAAACTACCTTTTGTTCG3′; and RTECQUDLR: 5′GCACTATGTGAAACCCCCA3′ which were prepared by GeneWorks (Adelaide, Australia).

To verify the efficacy of the RTECQU primers for this study they were next used to amplify the 17 SR *L. miniatus* samples in the study, using the PCR protocol indicated below, to determine segregating sites. PCR products were electrophoresed through a 1% agarose gel and the HVR1 product bands were purified from the gel and the DNA sequenced.

To determine species selectivity of the RTECQU primers, a PCR panel of DNA from various fish species was screened including: *L. miniatus*; *Cromileptes altivelis*; *Choerodon schoenleinii*; *Choerodon cephalotes*; *Diagramma labiosum*; *Lutjanus carponatus*;

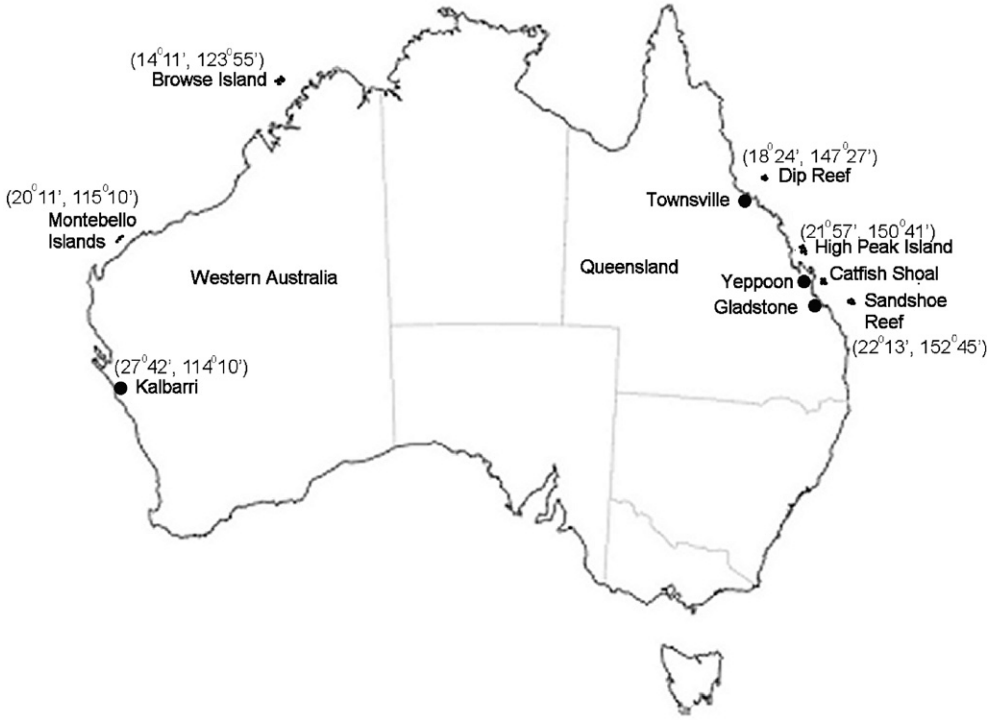

Fig. 1. Map of Australia showing the collection sites for *L. miniatus* and *L. sebae* samples and related reference towns/cities, as indicated in the text. Numbers in parentheses represent latitude and longitude respectively.

Lethrinus laticaudis; *Choerodon venustus*; *L. sebae*; *Lethrinus nebulosus*; *Epinephelus quoyanus*; *Lutjanus adetii*.

2.4. PCR conditions and sequencing

For *L. miniatus* in the study, and for the above panel of species, 25 μl PCR reactions were used containing 100 μM each dNTP, 7.5 pmol of each RTECQUDL species-specific primer, 1.0 mM $MgCl_2$, 1.0 unit Taq polymerase (Promega) and 2.5 μl of 10× PCR buffer (Promega) and approximately 25 ng of DNA template. The PCR protocol was as follows: 2 min at 94 °C; followed by 30 cycles of 94 °C for 30 s; 50 °C for 30 s; 72 °C for 1 min 30 s; then 72 °C for 10 min.

For *L. sebae*, PCR protocol for the population genetic studies was as follows: 2 min at 94 °C; followed by 30 cycles of 94 °C for 30 s; 49 °C for 30 s; 72 °C for 1 min 30 s; then 72 °C for 10 min. Concentrations of reagents and primers (RECQU) used were as described above for PCR for *L. miniatus*. All electrophoresis and sequencing in the study were undertaken as described above for primer development and validation.

2.5. Statistical analyses

Initial sequence alignments with comparison of forward and reverse strand sequences were done manually using BioEdit v7.05 (Tom Hall, Ibis Therapeutics, Carlsbad, CA). Multiple alignment of sequences including outgroup samples was done using ClustalW (Higgins et al., 1994). Gaps were not included in further analyses. Using Modeltest v3.7 (Posada and Crandal, 1998) the best substitution model and gamma distribution shape parameter were determined as HKY + G, gamma = 0.313 for *L. miniatus* and HKY + G, gamma = 0.268 for *L. sebae*.

Pairwise F_{st}, AMOVA and genetic diversity indices were computed using Arlequin v2.001 (Schneider et al., 2000) with best model and gamma values specified. Minimum Spanning Trees (MST) obtained from the population genetic analysis using Arlequin (Schneider et al., 2000), were constructed for each species using Sneato (Wooding, 2004, http://www.xmission.com/~wooding/Sneato/index.html) in order to determine if populations appear to be expanding, as evidenced by "starburst" MSTs, also noted for other reef fish populations, including parrot fishes (Dudgeon et al., 2000), and wrasses (Chen et al., 2004). Population expansion scenarios were then formally tested using a combination of mismatch distribution analyses and measures of neutrality, Tajima's *D* and Fu's *Fs* statistics, using Arlequin v2.001 (Schneider et al., 2000). Together this allows for alternative explanations to be invoked, in the event that the null hypothesis of population stasis assuming selective neutrality is rejected.

3. Results

3.1. Primer validation for L. miniatus

The PCR test panel results using the developed RTECQU primers on the range of species, upon electrophoresis revealed bands only for the *L. miniatus* test samples, indicating a high level of primer specificity for this species. The 17 *L. miniatus* samples from SR revealed 335 bp sequences with 32 segregating sites, 16 parsimony informative sites and 15 HVR1 haplotypes, and therefore were deemed suitable for this population genetics study. The sequence data segregation by parsimony informative sites for SR samples is shown in Table 1.

3.2. Sequencing results

The HVR1 amplicon length was determined as 335 bp for all east coast *L. miniatus* in the study, however, all west Australian *L. miniatus* had 23 bases missing (indels) compared to the east coast *L. miniatus* as determined by sequencing at 312 bp. These missing bases were from a single indel event at the same site within the HVR1 amplicon from each individual. The amplicon length was determined as 301 bp for all *L. sebae* samples assayed from the east and west coasts of Australia, with no indels present

Table 1
All haplotypes identified by parsimony informative sites of HVR1, *L. miniatus* from Sandshoe Reef.

	Sequence data separation by parsimony informative sites															
	6	69	81	102	113	133	145	156	157	187	203	204	221	239	267	337
	A	G	A	G	C	G	C	G	A	C*	T	A	C	T	C	T
1		A	–	–	–	–	T	–	–	–	C	–	–	C	–	–
2		–	–	A	T	A	–	–	–	–	–	–	–	–	–	C
3		–	–	–	–	–	–	A	–	–	–	–	–	–	–	–
4		–	G	A	–	–	–	–	–	–	–	–	–	–	–	–
5		–	–	A	T	A	–	–	–	–	–	–	–	–	–	C
6		–	–	A	–	A	–	–	–	–	–	–	–	–	–	C
7		–	G	A	–	–	–	–	–	A	–	–	–	–	–	–
8		–	–	–	–	–	–	A	–	–	–	–	–	–	–	–
9		–	–	–	T	A	–	–	–	–	–	G	T	–	T	–
10		–	–	–	–	–	–	–	G	–	–	–	–	–	–	–
11		–	–	–	–	A	–	–	–	–	–	–	–	C	–	–
12		–	G	A	–	–	–	–	G	–	–	–	–	–	–	–
13		A	–	–	–	–	T	–	–	–	C	–	–	C	–	–
14		–	–	A	–	A	–	–	–	–	–	–	–	–	–	–
15		–	–	–	–	A	–	–	–	–	–	G	T	–	T	–
16	–	–	–	–	–	–	–	A	–	A	–	–	–	–	–	–
17	–	–	G	A	–	–	–	–	–	–	–	–	–	–	–	–

* The only transversion identified for *L miniatus*.

Table 2
Genetic diversity indices and mean within group genetic distances for *L. miniatus* in different east E and west W Australian coast populations results ± SEM.

Site	Number of individuals n	Number of haplotypes	Haplotypic diversity, *h*	Nucleotide diversity, %π
Sandshoe Reef E	17	14	0.98 ± 0.03	2.0 ± 1.1
DipReef E	20	15	0.95 ± 0.03	1.7 ± 1.0
Total GBR	37	29	0.96 ± 0.02	1.7 ± 0.9
Kalbari fishers W	19	7	0.61 ± 0.13	0.4 ± 0.3
Montebello Island W	18	8	0.75 ± 0.10	0.4 ± 0.3
Total WA	37	15	0.68 ± 0.09	0.4 ± 0.3

in these samples. GenBank Accession numbers for *L. miniatus* are EU835243–EU835317, including an outgroup sequence. GenBank Accession numbers for *L. sebae* are EU835318–EU835390, including an outgroup sequence.

3.3. *Lethrinus miniatus*

The genetic diversity was shown by all parameters to be higher for the east Australian coast fish compared to the west Australian coast fish, with almost double the number of haplotypes among the east coast samples (Table 2). Diversity indices at sites within the same coast appeared very similar.

There was no difference between the two east coast or between the two west coast groups ($p > 0.15$, Table 3), but pairwise F_{st} between east and west coast samples for *L. miniatus* were different ($p < 0.001$). The more statistically rigorous AMOVA results of samples structured by region, confirmed genetic partitioning between regions, with a highly significant among region partition, $\varphi_{ct} = 0.934$ ($p < 0.001$). More than 93% of the genetic variation existed among the regions.

Table 3
Pairwise F_{st}s between groups for *L. miniatus* in different east E and west W Australian coast populations.

	Sandshoe Reef	Dip Reef	Kalbari
Sandshoe Reef E			
Dip Reef E	−0.003		
Kalbari W	0.92*	0.93*	
Montebello Island W	0.92*	0.93*	0.007

* $p < 0.0001$.

The MST identified that there are 26 synapomorphic substitutions that unite the WA stock as a distinct clade from the GBR stock. This was confirmed with rigorous phylogenetic analyses including Maximum Parsimony, Neighbour Joining and Bayesian Inference (not shown). It also suggested that both east and west coast stocks independently, were expansions from an already well inter-mixed ancestral haplotype stock in each region, as the most common haplotypes in each region were central in the MST (Fig. 2). Additionally, when the samples within each genetically distinct population were combined and formally tested for evidence of population stasis under an assumption of selective neutrality, both Tajima's test for selective neutrality, *D* (Tajima, 1983) and Fu's *Fs* parameter, *Fs* (Fu, 1997) rejected the hypothesis of population stasis and selective neutrality in the west Australian population ($D = -2.33117$, $p < 0.002$ and $Fs = -11.090$, $p < 0.0001$). For the east Australian population selective neutrality of the mtDNA was accepted ($D = -1.37671$, $p < 0.082$), whilst population stasis was rejected ($Fs = -11.972$, $p < 0.0001$).

3.4. *Lutjanus sebae*

The genetic diversity was shown by all parameters to be similar for CS, HP and MI (Table 4), however the samples from BI showed apparent reduced diversity by comparison, with almost half the number of haplotypes as the other three groups. Also, the genetic diversity of the east coast *L. sebae* appeared lower than that for the *L. miniatus* east coast samples analysed.

There were no significant pairwise F_{st} differences between any of the intra- or inter-coastal groups ($p > 0.15$, Table 5). AMOVA results, when samples were structured by region, confirmed the lack of genetic structure, $\varphi_{st} = -0.006$ ($p < 0.594$), $\varphi_{sc} = -0.008$ ($p = 0.598$)

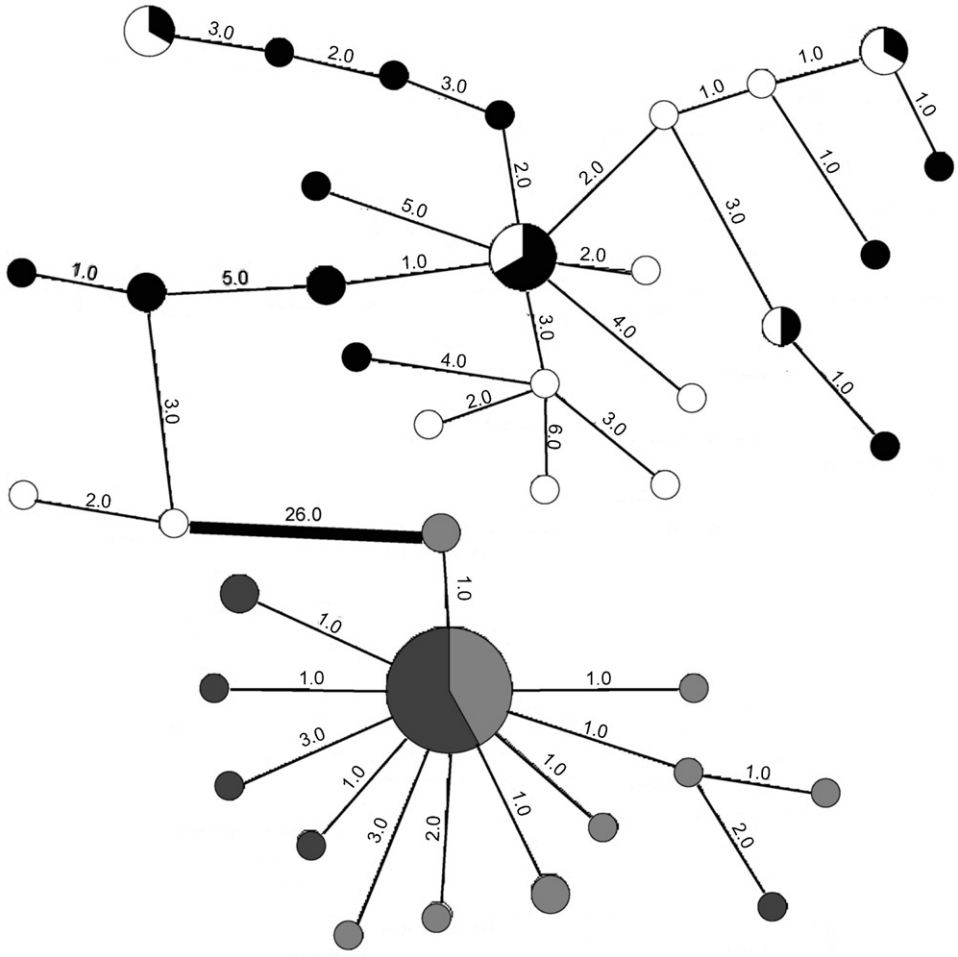

Fig. 2. Minimum spanning tree MST with number of substitutions between haplotypes indicated on connectors. Locations sampled are represented by different coloured fills, as shown on the key to the figure. SR = Sandshoe Reef, east coast; DR = Dip Reef, east coast; KB = Kalbari fishers, west coast; MB = Montebello Islands, west coast.

Table 4
Genetic diversity indices and mean within group genetic distances for *L. sebae* in different east E and west W Australian coast populations ± SEM.

Site	Number of individuals n	Number of haplotypes	Haplotypic diversity, *h*	Nucleotide diversity, %π
Catfish Shoal E	20	9	0.84 ± 0.06	0.61 ± 0.41
High Peak Island E	14	8	0.89 ± 0.06	0.76 ± 0.50
Browse Island W	20	5	0.69 ± 0.08	0.32 ± 0.25
Montebello Island W	18	12	0.92 ± 0.05	0.74 ± 0.48
Total	72	34	0.82 ± 0.03	0.55 ± 0.36

and $\varphi_{ct} = 0.002$ ($p = 0.335$) values. All of the genetic variation existed within populations and none of it among regions.

The MST identifies that there are no synapomorphic substitutions that unite the WA or GBR stocks into distinct regional clades. This is consistent with phylogenetic analyses including Maximum Parsimony, Neighbour Joining and Bayesian Inference (not shown). It also suggests that the inter-mixed east and west coast stock is an expansion from a couple of already well inter-mixed ancestral haplotypes, as the most common haplotype in both regions are shared among regions and are central in the MST (Fig. 3). Mismatch distribution analysis and both Tajima's test for selective neutrality, *D* (Tajima, 1983) and Fu's *Fs* parameter, *Fs* (Fu, 1997) rejected the hypothesis of a static population under an assumption of selective neutrality when all samples were combined due to the lack of genetic partitioning between and within regions ($D = -2.006$, $p < 0.011$ and $Fs = -22.149$, $p < 0.0001$).

Table 5
Pairwise F_{st}s between groups for *L. sebae* in different east E and west W Australian coast populations[¶].

	Catfish Shoal	High Peak Island	Browse Island
Catfish Shoal E			
High Peak Island E	−0.001		
Browse Island W	−0.006	0.018	
Montebello Island W	−0.03	−0.003	−0.021

[¶] $p > 0.15$ for all pairwise comparisons.

4. Discussion

This study showed significant differences between east and west Australian coast *L. miniatus* populations, indicating distinct genetic stocks. In contrast, *L. sebae* did not differ between east and west coasts, indicating a single Australian genetic stock for this species. Genetic diversity of *L. miniatus* was relatively high on the GBR compared with Western Australian *L. miniatus*, and approximately twice

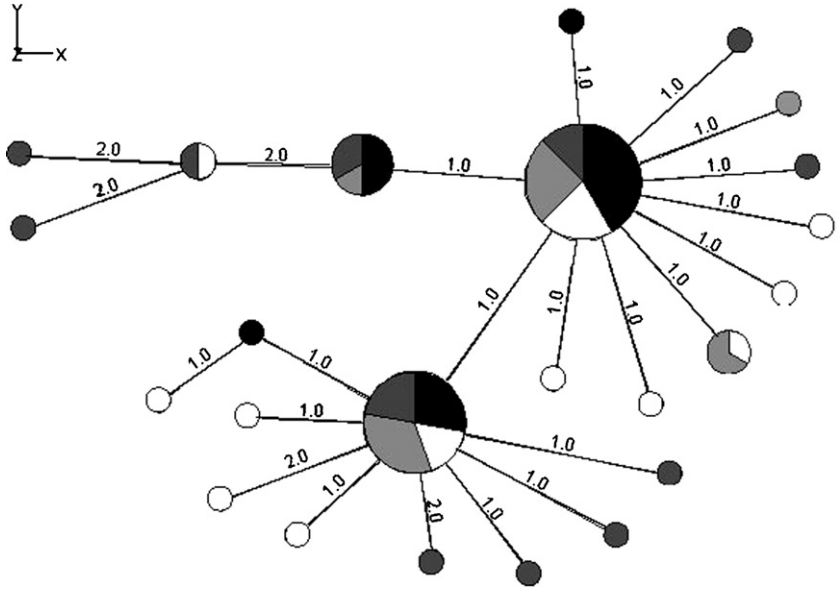

Fig. 3. Minimum spanning tree MST with number of substitutions between haplotypes indicated on connectors. Locations sampled are represented by different coloured fills, as shown on the key to the figure. CF = Catfish Shoal, east coast; HP = High Peak Island, east coast; BI = Browse Island, west coast; MI = Montebello Islands, west coast.

the diversity exhibited by *L. sebae*. Populations within coasts did not differ genetically for either species, but genetic diversity was lower in west than east coast locations, except for *L. sebae* at Montebello Island. Population expansions were confirmed for all *L. miniatus* and *Lutjanus sebae* stocks, but selective neutrality was only confirmed for the east Australian *L. miniatus* stock. This suggests that *L. sebae* and west Australian *L. miniatus* may have experienced selective sweeps.

4.1. *Lethrinus miniatus*

At the inter-regional spatial scale these HVR I results suggest that there are at least two genetic stocks in Australia for management purposes. A previous study, using six microsatellites also detected significant genetic partitioning between WA and the GBR (Scott and van Herwerden, unpublished). Specifically, pairwise F_{st} values ranged from 0.044 to 0.07, $p < 0.001$ (Scott and van Herwerden, unpublished) using the same *L. miniatus* specific microsatellite markers as van Herwerden et al. (2003). It seems likely that, as *L. miniatus* has not been reported in significant numbers north of the Montebello Islands in Western Australia, the pelagic larvae of east and west coast stocks of *L. miniatus* do not intersect, as has also been reported for *P. leopardus* from these coasts (van Herwerden et al., 2006).

At the regional scale there was no evidence of distinct genetic stocks on the east coast of Australia, represented by two GBR reefs, approximately 750 km apart, based on mtDNA. This view was supported at the regional level for *L. miniatus* on the GBR by a population genetics study using eight microsatellite markers in which no discrimination between fish stocks were detected on GBR reefs from Gladstone, Mackay and Townsville (van Herwerden et al., 2003). Collectively these studies suggest that *L. miniatus* on the GBR may be managed as a single interbreeding stock. These findings may be due to the nature of the chain of reefs and islands forming the GBR and the pelagic and migratory nature of *L. miniatus* larvae and the consistent regular southward flow of the East Australia Current, even though the adults are understood to be largely sedentary (van Herwerden et al., 2003).

In Western Australia the situation is more complex, as the lack of genetic structure found in the present mtDNA study is inconsistent with findings by Scott and van Herwerden (unpublished), which showed that genetic partitioning existed between Kalbarri and North Island of the Houtman Abrolhos Islands in WA (in addition to the WA-GBR partitioning previously noted). This apparent discrepancy of population genetic structure in WA between the mtDNA and the nuclear marker studies suggests that the Abrolhos Islands population of *L. miniatus* is genetically partitioned from the Kalbarri population, whilst Kalbarri and Montebello Island populations are not genetically partitioned. Based on geographic distance, this is unexpected, as Kalbarri is only 100 km north of the Abrolhos Islands, whilst Kalbarri is nearly 10 times further from Montebello Island to the north. Furthermore, these three populations are most likely connected by the Leeuwin Current, which flows southward along the WA coast (Lenanton et al., 1991). However, the partitioning at the Abrolhos Islands based on microsatellite data (and for which there is not mtDNA data) may be due to ecological factors, because there is a relatively small upwelling at the Abrolhos Islands, evident from the occurrence of temperate macroalgae, seagrasses, fishes, western rock lobster (*Panulirus cygnus*), molluscs, echinoderms, sponges and Australian sea lions (*Neophoca cinerea*), which coexist with tropical species (Collins, 1991). Upwelling alters environmental conditions substantially, which in turn may impact on the fishes residing at the Abrolhos Islands. Of relevance here is the finding that the mtDNA of the west Australian *L. miniatus* population in the present study has undergone a selective sweep.

Ecological partitioning of this nature and at this spatial scale was also found for labrid reef fishes off the coast of Brazil (Rocha et al., 2005). This warrants further investigation of WA *L. miniatus* populations at a finer spatial scale and with both nuclear and mitochondrial markers.

4.2. *Lutjanus sebae*

At the inter-regional scale HVR1 sequences were similar for east and west coast *L. sebae*, with a continuous distribution around the north of Australia. Thus, east and west Australian populations likely form a single inter-breeding genetic stock. As adult populations of *L. sebae* are largely sedentary (Stephenson et al., 2001), this study indicates that there is widespread dispersal of *L. sebae* larvae around the Australian coastline, resulting in high levels of gene flow, as has also been shown for several other reef organisms, including other coral reef fishes and sea stars (Bay et al.,

2004; Klanten et al., 2007; Williams and Benzie, 1998). Within regions there was also no evidence of genetic stock structure for either east or west coast sites using HVR1 as expected, given no net genetic differences on the broader spatial scale. These results confirm those derived by Johnson et al. (1993) using allozymes for *L. sebae* in Western Australian waters. Johnson et al. (1993) examined populations from 5 localities over a total distance of approximately 1400 km within Western Australia and concluded that there were no clear geographical genetic partitions and that there were extensive connections between populations separated by large distances.

4.3. Temporal scales of connectivity and spawner biomass

In the absence of genetic structure, it is difficult to identify whether there is ongoing, contemporary gene flow between reefs and/or regions or whether the reefs were relatively recently colonised from adjacent reefs or regions, because reefs on the continental shelves were re-populated only subsequent to the last glacial maximum, LGM, which was 18,000 years ago. These alternatives (ongoing gene flow within/between regions or not) have different implications for the ability of these populations to be replenished at ecological timeframes, if managed as a single population, because low contemporary levels of gene flow of a relatively young population, may be inadequate to ensure that overfished populations are replenished.

A high probability of connectivity or intermixing during the pelagic larval stages across multiple separate and distinct adult assemblages implies that the size of the total adult spawning population (i.e. the combined sum of each of the separate adult populations) could impact recruitment. Thus, fishing on any one adult population could indirectly impact fishing on any other adult population by limiting the availability of recruits resulting from a reduced total spawner biomass. This is a serious concern for shared stocks of Indo-Australian fish species. This consideration is of even greater relevance if gene flow is not as great as it appears to be at contemporary time frames, but is rather due to an accumulation of relatively low levels of gene flow over more extended, historic time frames since the LGM.

4.4. Genetic diversity considerations

Based on genetic diversity calculations, east coast *L. miniatus* and *L. sebae* from the GBR have high genetic diversity, a valuable characteristic that provides the populations with greater resilience to perturbations (Luck et al., 2003). This is consistent with findings of high genetic diversities in some other GBR fish populations studied using mtDNA HVRI (e.g. *P. leopardus*, van Herwerden et al., 2006; *N. vlamingii*, Klanten et al., 2007; *Pseudochromis fuscus*, Messmer et al., 2005; *C. sordidus*, Bay et al., 2004). In contrast, three of the four samples from Western Australia (not *L. sebae* from Montebello Island) displayed much less genetic diversity than the GBR samples. This could result from either a selective sweep such as may have been caused by varying environmental conditions across the distribution range (including past fishing pressure) or sweepstake recruitment. The selective sweep interpretation is supported by neutrality tests that rejected the assumption of selective neutrality. Regardless of the causes of the observed reductions in west Australian genetic diversity, it is noteworthy, because genetically impoverished populations are less resilient to environmental perturbations. This is of concern, especially when acknowledging future challenges to the persistence of tropical marine species due to the impacts of escalating anthropogenic and natural disturbances, such as over fishing, loss of coral reef habitats, climate change and associated acidification of the oceans.

In summary, these findings provide additional useful data for fisheries management of these species on the east and west coasts of Australia, and suggest that management strategies for *L. miniatus* should consider the east and west Australian coast populations of *L. miniatus* as distinct stocks.

A robust and precautionary approach to fisheries management for *L. miniatus* is recommended, whereby each state management agency aims to maintain an adequate total spawner biomass within each fishable population and avoid where possible localised depletion events. In Western Australia, the low level of genetic diversity indicates that these stocks of *L. miniatus* and *L. sebae* (from Browse Island) are particularly susceptible to both environmental and anthropogenic perturbations (e.g. climate change, impacts from exploitation). In addition, life history parameters determined from east coast populations of *L. miniatus* (Williams et al., 2003, 2006) are unlikely to be applicable to the west coast populations. Thus, there is a vital need to determine the life history characteristics of *L. miniatus* in the waters of Western Australia.

Acknowledgments

The authors gratefully acknowledge funding for this project from the CQU Research Development and Incentives Scheme, and SEH Faculty grant. We thank the Effects of Line Fishing Project, funded by the Cooperative Research Centre for the Sustainable Development of the Great Barrier Reef (CRC Reef), Fisheries Research and Development Corporation (FRDC) and the Great Barrier Reef Marine Park Authority for providing *L. miniatus* samples from the Great Barrier Reef. Thank you to Tom Edmistone of Yeppoon for donating fin clips of east coast *L sebae*. Logistical support was provided by the Department of Fisheries, Government of Western Australia. The authors are grateful to the commercial fishers of Western Australia for the provision of and access to specimens from a broad variety of locations.

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