Mar Biol (2011) 158:1497–1509
DOI 10.1007/s00227-011-1666-y

ORIGINAL PAPER

Negligible evidence for regional genetic population structure for two shark species *Rhizoprionodon acutus* (Rüppell, 1837) and *Sphyrna lewini* (Griffith & Smith, 1834) with contrasting biology

Jennifer R. Ovenden · Jess A. T. Morgan · Raewyn Street · Andrew Tobin · Colin Simpfendorfer · William Macbeth · David Welch

Received: 9 December 2010 / Accepted: 2 March 2011 / Published online: 2 April 2011
© Her Majesty the Queen in Rights of Australia as represented by the Government of Queensland 2011

Abstract Biodiversity of sharks in the tropical Indo-Pacific is high, but species-specific information to assist sustainable resource exploitation is scarce. The null hypothesis of population genetic homogeneity was tested for scalloped hammerhead shark (*Sphyrna lewini*, $n = 237$) and the milk shark (*Rhizoprionodon acutus*, $n = 207$) from northern and eastern Australia, using nuclear (*S. lewini*, eight microsatellite loci; *R. acutus*, six loci) and mitochondrial gene markers (873 base pairs of NADH dehydrogenase subunit 4). We were unable to reject genetic homogeneity for *S. lewini*, which was as expected based on previous studies of this species. Less expected were similar results for *R. acutus,* which is more benthic and less vagile than *S. lewini*. These features are probably driving the genetic break found between Australian and central Indonesian *R. acutus* (*F*-statistics; mtDNA, 0.751–0.903, respectively; microsatellite loci, 0.038–0.047 respectively). Our results support the spatially homogeneous monitoring and management plan for shark species in Queensland, Australia.

Communicated by S. Uthicke.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-011-1666-y) contains supplementary material, which is available to authorized users.

J. R. Ovenden (✉) · J. A. T. Morgan · R. Street
Molecular Fisheries Laboratory, Department of Employment, Economic Development and Innovation, Queensland Government, P.O. Box 6097, St. Lucia, QLD 4069, Australia
e-mail: Jennifer.Ovenden@deedi.qld.gov.au

J. A. T. Morgan
Queensland Alliance for Agriculture and Food Innovation, and Animal Research Institute, The University of Queensland, Locked Mailbag 4, Moorooka, QLD 4105, Australia

A. Tobin · C. Simpfendorfer · D. Welch
Fishing and Fisheries Research Centre, James Cook University, Townsville, QLD 4811, Australia

W. Macbeth
Cronulla Fisheries Centre, Department of Industry and Investment, New South Wales Government, P.O. Box 21, Cronulla, NSW 2230, Australia

Introduction

Australia is the custodian of a large amount of tropical marine biodiversity within the northern extent of its large exclusive economic zone. It is a significant slice of a marine biodiversity hotspot that has the richest marine fish fauna in the world (Randall 1998). Exploitation in the Australian zone is largely well managed, and many species are protected. Nations to Australia's north have large populations and small amounts of land for agriculture, which increases their reliance on marine resources for food. One group that is readily impacted by exploitation is the Elasmobranchii, but in tropical Australia, these species are abundant. For example, in Queensland on the north-eastern coastline, sharks represent about 20% (by weight) of the inshore commercial catch (Anon 2010). The shark catch largely consists of five species, but numerous other Elasmobranchii species are also caught. The government has a precautionary limit of 600 tonnes per annum for the total allowable catch of Elasmobranchii species in Queensland waters and has set additional alerts to overexploitation. These 'trigger points' are a reduction in the tonnage of landed catch by 30% over a three-year period and a significant change in the species composition of the landed catch. Preferably, management arrangements would be species-specific and based on a large body of species-specific demographic and biological

information. In the absence of this, the question remains whether exploitation limits on Elasmobranchii species are conservative enough for non-specific management to be effective.

To redress the lack of scientific information for management, this study focuses on the population structure of two commonly exploited species: *Sphyrna lewini* (scalloped hammerhead) and *Rhizoprionodon acutus* (milk shark). Population structure is relevant to setting the scale of management arrangements and once in place for monitoring the effects of exploitation on a scale that matches the amount of demographic connectedness within the range of a species. *Sphyrna lewini* is a coastal and semi-oceanic shark found worldwide in the Atlantic, Pacific and Indian Oceans (Baum et al. 2007). *Rhizoprionodon acutus* is a continental shelf species with a more restricted distribution on the western and eastern African coasts, in the Arabian Sea, the Indo-West Pacific Ocean and the northern Australian coastline (Gallo et al. 2010). There is a considerable size difference between the species: *S. lewini* is large with a maximum size of around 340 cm (TL) and *R. acutus* is much smaller (100 cm, Australia; 178 cm, Africa) (Last and Stevens 2009). Their resilience to exploitation is also different. *Sphyrna lewini* has low resilience because age at first reproduction and total lifespan is thought to be measured in tens of years (males mature at 10 years, females at 15 and some animals estimated to 30 years old), even though it has relatively high fecundity compared with other sharks (12–38 pups) (Baum et al. 2007). In contrast, *R. acutus* has smaller litters (one to eight pups), but its generation time is much shorter with rapid sexual maturity (2–3 years) and a maximum lifespan of 8 years providing resilience to exploitation (Simpfendorfer 2003). Globally, *S. lewini* is listed as endangered by the IUCN. Australian populations are thought to be well managed, but the increase in illegal, unregulated and unreported fishing in the north is of concern (Baum et al. 2007). *Rhizoprionodon acutus* is listed as Least Concern by the IUCN. The smaller body size of *R. acutus* implies lower vagility compared with *S. lewini* and combined with shorter generation times; there is the expectation of a more pronounced population subdivision in *R. acutus* compared with *S. lewini.* These biological differences between the species suggest that population structure in *R. acutus* would be different to *S. lewini*.

There has been no previous stock structure research on *R. acutus.* However, stock structure of *S. lewini* has been investigated on a worldwide and on a regional scale. Duncan et al. (2006) and Quattro et al. (2006) reported pronounced genetic differences between *S. lewini* from major ocean basins (Atlantic, Pacific and Indian Oceans) in contrast to genetic similarity between populations along continental margins. Genetic similarity among populations within the Indo-Pacific region was confirmed by Ovenden et al. (2009) between central Indonesia and northern Australia. Quattro et al. (2006) reported a possible *S. lewini* cryptic species amongst Atlantic samples. Genetic population structure on a regional scale has not been widely studied in the Carcharhinidae, with the majority of studies focusing on global phylogeography (Keeney and Heist 2006; Portnoy et al. 2010), except for studies focusing on natal philopatry (Pardini et al. 2001; Keeney et al. 2003). Genetic tools have been used to study the mating system in sharks, with some species having multiple paternity, (Portnoy et al. 2007) and genetic tools are widely used for species identification (Mendonca et al. 2009; Wong et al. 2009; Ovenden et al. 2010).

In this study, two types of genetic markers were used to test for genetic population structure in *S. lewini* and *R. acutus* on a regional scale within Australia. Sequence polymorphism in a mitochondrial gene region (ND4) plus allele frequency variation at a range of microsatellite loci was deployed on each species. The markers represent genes that are neutral with respect to selection and are biparentally (microsatellite) or strictly maternally (mtDNA) inherited. When combined, they have the potential to provide a high degree of resolution at the intra-specific level per species. To ensure that population genetic subdivision would be detected whether it were present, samples from a geographically distant population were included for each species. Samples were obtained from central Indonesia to provide this contrast within the data set. Populations of *S. lewini* and *R. acutus* were sampled from similar locations along the coastline of Queensland and New South Wales to test the expectation that the scale of genetic subdivision would be finer in the species with lower potential vagility and shorter generation times (*R. acutus*) compared with the more vagile species with longer generation time (*S. lewini*).

Methods

Collection

Rhizoprionodon acutus and *S. lewini* were sampled from regions on the eastern coast of Australia and one location (Bali) in central Indonesia (Table 1). All samples were taken from the landed catch of the shark fishing sector and were provided by commercial fishers or collected by observers on commercial boats. Sharks were sampled from artisanal markets in central Indonesia. Approximately 200 mg of muscle tissue was dissected and preserved in 1 ml of NaCl saturated solution with 20% dimethyl-sulphoxide and stored at −70°C. Total genomic DNA was extracted from ten to 25 mg of the tissue samples using a Qiagen DNeasy Blood &

Table 1 Sample size (*N*, microsatellite sample size bracketed if different), gender distribution and mean stretch total length (STL) for *Rhizoprionodon acutus* and *Sphyrna lewini* samples from Eastern Australian regions and one Indonesian region (Fig. 3)

Region	Males		Females		Unknown gender or STL	
	N	Mean STL (mm)	*N*	Mean STL (mm)	*N*	Total *N*
Rhizoprionodon acutus						
Kedonganan, Bali Indonesia	–	–	–	–	17 (15)	17 (15)
Far North	45	829	7	872	4	56
Townsville	50	757	14	634	–	64
Mackay	10	852	–	–	–	10
Fraser Burnett	24	560	27	556	9	60
Total	129		48		30 (28)	207 (205)
Sphyrna lewini						
Kedonganan, Bali Indonesia	–	–	–	–	33	33
Far North	21	1,205	12	986	14	47
Townsville	29	840	20	676	–	49
Mackay	12	680	8	588	–	20
Fraser Burnett	3	577	–	–	–	3
Brisbane	12	614, 704, 1,760[a]	13	508, 679	17	42
Northern New South Wales	23	2,200	20	1,650	–	43
Total	100		73		64	237

[a] STL for a subset of *S. lewini* samples from Brisbane is presented

Tissue Kit (Qiagen, Doncaster Victoria) into a final elution volume of 200 µl.

Microsatellite loci

Microsatellite loci developed for species from the Carcharhinidae and Sphyrnidae (Keeney and Heist 2003; Chapman et al. 2004; Ovenden et al. 2006; Portnoy et al. 2006; Nance et al. 2009) were used to genotype *R. acutus* and *S. lewini* samples (Table 2). Genetic population structure was inferred from within- and among-population variation at microsatellite loci.

Microsatellite PCR amplifications were carried out in 96-well plates using Perkin Elmer 9600 & 9700 series thermocyclers. PCR reactions (6 µl total volume) utilized Qiagen Multiplex PCR kit (Qiagen, Doncaster Victoria) and contained 3 µl of 2x Master Mix, 0.6 µl of 5x Q solution, 20 nM forward primer and 200 nM reverse primer, 200 nM FAM-labelled M13 primer and approximately 20 ng of genomic DNA template. Forward primers had an M13 extension (GAG CGG ATA ACA ATT TCA CAC AGG) at the 5′ end, enabling product amplification with the FAM-labelled M13 primer (Schuelke 2000). The DNA template and enzyme were denatured at 95°C for

Table 2 Description of the microsatellite loci screened for *Rhizoprionodon acutus* and *Sphyrna lewini*

Locus	Repeat	Reference	Species
Cli100	$(TG)_4(GT)_{10}$	Keeney and Heist (2003)	*R. acutus* and *S. lewini*
Ct06	$(CA)_{14}$	Ovenden et al. (2006)	*R. acutus* and *S. lewini*
Ct07	$(GT)_{10}(GC)(GT)(GC)(GT)_2(GC)$	Ovenden et al. (2006)	*R. acutus* and *S. lewini*
Cpl166	$(GT)_{17}$	Portnoy et al. (2006)	*R. acutus* and *S. lewini*
Cli07	$(GT)_{20}$	Keeney and Heist (2003)	*R. acutus* only
Cli107	$(GT)_{14}$	Keeney and Heist (2003)	*R. acutus* only
Cli12	$(GT)_9$	Keeney and Heist (2003)	*S. lewini* only
PGL02	Unknown	Chapman et al. (2004)	*S. lewini* only
SLE018	$(CA)_5(TA)_3(CA)_3CG(CA)_4TA(CA)_4$	Nance et al. (2009)	*S. lewini* only
SLE045	(CA) interrupted	Nance et al. (2009)	*S. lewini* only
SLE089	$(GT)_{17}(CT)_2(GT)_7$	Nance et al. (2009)	*S. lewini* only

15 min, followed by 35 cycles consisting of 94°C for 30 s, 50°C for 45 s, and 72°C for 90 s. A final extension at 72°C for 45 min was used to ensure complete addition of adenine to the PCR product, essential for consistent allele calling during genotyping. Products were separated on an ABI3130xl sequencer, and genotypes were scored and binned using GeneMapper® v4.0 software (Applied Biosystems, Foster City, CA, USA).

Mitochondrial DNA

The extent of sequence variation in the NADH dehydrogenase subunit 4 (ND4) region of mtDNA was used to examine genetic population structure. A section of the ND4 region (873 base pairs) was amplified and sequenced using primers ND4 (CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC) (Arevalo et al. 1994) and H12293-LEU (TTG CAC CAA GAG TTT TTG GTT CCT AAG ACC) (Inoue et al. 2001).

PCR amplifications were carried out in 20 µl volumes and contained 0.5 µM of each primer, combined with 10–100 ng of template DNA, 10x Taq buffer (containing 15 mM $MgCl_2$), 0.8 mM dNTP's, and 0.6 units of Taq DNA polymerase (Qiagen, Valencia, CA, USA). Thermal cycling conditions consisted of an initial denaturation (94°C for 3 min) followed by 35 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 1 min, with a final extension step of 72°C for 10 min. Cycling was performed in either a PTC200 DNA Engine (MJ Research, USA) or Perkin Elmer 9600 & 9700 series thermocyclers (Perkin Elmer Australia, Melbourne, VIC). PCR products were viewed on a 1.5% agarose TAE gel stained with GelRed (Biotium, USA). PCR products were concentrated and desalted prior to sequencing using either a DNA Clean or Concentrator-5 Kit (Zymo Research Corporation, Orange, CA, USA) or Exosap-it® (USB Corporation distributed by GE Healthcare Bio-Sciences, Rydalmere NSW, Australia). Approximately 20 ng of DNA was used in standard ABI Dye Terminator sequencing reactions using Big Dye Vers 3.1 technology (Applied Biosystems, CA, USA) and were run on an Applied Biosystems 3130xl Genetic Analyser.

Data analyses

MtDNA ND4 sequence was used to confirm the identity of all *R. acutus* and *S. lewini* samples by comparison with in-house ND4 reference sequences representing local species from the Carcharhinidae and Sphyrnidae.

Microsatellite genotypes were tested for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium using Genepop-on-the-web (Raymond and Rousset 1995; Morgan 2000). For the former, all locus *x* population combinations were tested, and for the latter, all combinations of locus pairs were assessed for each population. A Bonferroni correction for simultaneous tests was applied at an α level of 0.05 to estimate critical levels of significance.

The software Microchecker v 2.2.3 (Van Oosterhout et al. 2004) was used to explore cases of departure from Hardy–Weinberg equilibrium. When null alleles were detected, their frequency was estimated using the maximum likelihood estimator of Dempster et al. (1977) for each locus and population. Calculations were carried out in software FreeNA (Chapuis and Estoup 2007).

Population genetic structure was assessed by determining the proportion of total genetic variation that was found in pairwise comparisons between populations using an *F*-statistics approach. F_{ST} (actually theta) estimates were made following Weir and Cockerham (1984) based on allele frequency variance for microsatellites and based on Slatkin's linearized F_{ST} (Slatkin 1995) for mtDNA sequence polymorphism. We corrected for the positive bias on F_{ST} estimates caused by the presence of null alleles at microsatellite loci using the ENA (excluding null alleles) approach of Chapuis and Estoup (2007). The corrected F_{ST} estimates were made using the software FreeNA (Chapuis and Estoup 2007). After estimating frequencies per population and per locus for null and non-null alleles, the software estimated F_{ST} using non-null alleles only. Null alleles were ignored in the computation meaning that the sums of allele and genotype frequencies were less than one, which is feasible according to Chapuis and Estoup (2007) based on Weir and Cockerham (1984). Data were assumed to be missing due to technical problems rather than presence of null alleles. F_{ST} estimates were made using FreeNA with, and without the correction for null alleles, for all loci or subsets of loci (i.e. excluding those with null alleles).

Mitochondrial DNA sequence data were edited and aligned with Sequencher (v 4.8 Gene Codes Corporation, Ann Arbor, MI, USA). Haplotype parsimony networks were estimated with the assistance of TCS (Clement et al. 2000). Neighbour joining phylogenetic trees were constructed from mtDNA sequence data based on the number of polymorphic sites between haplotypes in PAUP* v 4.0b10 (Swofford 2002). Arlequin v 3.5 (Excoffier et al. 2005) was used to calculate a range of population genetic statistics relevant to sequence variation at the population level.

Results

Species identification

Genetic characterisation of shark tissue samples excluded 12 misidentified animals; five great hammerheads (*Sphyrna mokarran*), and four smooth hammerheads (*Sphyrna*

zygaena) that were confused for *S. lewini* and three *R. acutus* samples, genotyped to other genera, which were assumed to be mislabelled. Overall, there were 237 *S. lewini* and 207 *R. acutus* samples (Table 1).

Microsatellite loci

Six microsatellite loci were polymorphic for *R. acutus*. Numbers of alleles per locus varied from three to four (Ct06) to 12 to 34 alleles per population (Cpl166, Appendix 1—Electronic Supplementary Material). As expected for these dinucleotide loci, the interval between alleles was two base pairs (or multiples of two base pairs). However, some loci had alleles separated by one base pair (Cli100, Cpl166, Ct06 and Cli107), which most likely reflected the insertion or deletion of one base pair in the flanking sequence or in the repeat motif. There was no evidence of linkage disequilibrium between pairs of loci for any population of *R. acutus*.

Eight microsatellite loci were polymorphic for *S. lewini*. Three of eight microsatellite loci used to genotype *S. lewini* samples were developed for that species (SLE018, 045 and 089). Alleles separated by one base pair were detected at loci SLE018, SLE089, Cli12, Cli100, Ct07 and PGL02. The numbers of alleles per locus per population for *S. lewini* varied from two (Cpl166) to 16–24 (Cli12, Appendix 1—Electronic Supplementary Material). Out of 168 combinations of locus pairs and populations, there was evidence for linkage disequilibrium in the Townsville population for *S. lewini* at three pairs of loci (Cpl166/SLE018, Cli12/SLE045 and SLE018/SLE089). The linkage was judged to be slight because it was population specific and involved different pairs of loci; data were not excluded from analysis.

Several loci per population were out of Hardy–Weinberg equilibrium for both species. For *R. acutus*, nine of the 30 tests performed rejected the null hypothesis of Hardy–Weinberg equilibrium after Bonferroni correction. Instances of disequilibrium were spread across populations and commonly involved loci Cpl166, Cli07, Ct06 and Ct07 (Appendix 1—Electronic Supplementary Material). Analysis with Microchecker software (Van Oosterhout et al. 2004) suggested that these were not due to scoring errors (large allele dropout or stuttering), but to an excess of homozygotes at the majority of allele size classes strongly suggesting the presence of null alleles. This was confirmed by the estimated frequency of null alleles at locus x population combinations in disequilibrium. Small sample sizes affected the reliability of the test for Hardy–Weinberg equilibrium and estimation of null allele frequency. For instance, only 10 *R. acutus* were genotyped for locus Ct07 and the p-value (0.4536) suggested Hardy–Weinberg equilibrium, yet it was estimated that a null allele was present at a frequency of 0.45. For *S. lewini*, instances of Hardy–Weinberg disequilibrium occurred commonly with loci Cli12 and SLE018. Microchecker software (Van Oosterhout et al. 2004) confirmed that this was not due to scoring errors, and at these loci x population combinations, null alleles were estimated to be present (Appendix 1—Electronic Supplementary Material). In a previous study of *S. lewini*, locus SLE018 showed evidence of null alleles in samples taken from the eastern Pacific Ocean (Nance et al. 2009).

F-statistic analyses from microsatellite loci generally were unable to reject the null hypothesis of genetic homogeneity for *R. acutus* or *S. lewini* populations on the eastern Australian coastline. There was strong evidence for separate populations of *R. acutus* in eastern Australia and Bali (Indonesia), and slight evidence of separation on this scale for *S. lewini*. The global F_{ST} estimate (theta) for *R. acutus* was 0.0579 (six loci using ENA, 95% confidence interval 0.0250–0.1036), and the global F_{ST} estimate (theta) for *S. lewini* was 0.0016 (eight loci using ENA, 95% confidence interval −0.0017 to 0.0044). F_{ST} estimates (theta) for population pairwise comparisons involving *R. acutus* from central Indonesia and eastern Australia were significantly larger than zero and ranged from 0.1648 to 0.2682 (Table 3). We were unable to reject the null hypothesis of panmixia for *R. acutus* on the eastern Australian coast as F_{ST} estimates between pairs of sampling locations were generally not significantly larger than zero. The two pairwise F_{ST} estimates that were significant within the eastern coast (Table 3) most likely occurred by chance. They were low (0.0184 and 0.0797) and not consistent among types of statistical tests. The results were similar for *S. lewini*, except there was little, if any, evidence of genetically separate populations between Indonesia and the eastern coast of Australia. Pairwise F_{ST} estimates for *S. lewini* between the most spatially separated population pair (Bali, Indonesia, and northern New South Wales) were significantly larger than zero in some cases (Table 3). However, this result and the other pairwise comparison with an F_{ST} estimate larger than zero (Brisbane and northern New South Wales, Table 3) may have occurred by chance.

The presence of null alleles was predicted to inflate F_{ST} estimates by depressing intra-population genetic diversity (Chapuis and Estoup 2007; Chapuis et al. 2008). This effect occurred for comparisons between *R. acutus* from central Indonesia and eastern Australia, but it was minor (Table 3).

Mitochondrial DNA

Nucleotide polymorphism in the 873-bp fragment of the mtDNA ND4 gene was higher in *R. acutus* than in

Table 3 Pairwise F_{ST} (theta, below diagonal, with 95% confidence intervals above diagonal) using the ENA (excluding null alleles) approach of Chapuis and Estoup (2007) for (A) *Rhizoprionodon acutus* and (B). *Sphyrna lewini* from sampling locations in eastern Australia and central Indonesia

(A)

Number of loci	Region	Bali, Indonesia	Far North	Townsville	Mackay	Fraser Burnett
Six (i.e. all)						
Allele frequencies not adjusted for presence of null alleles						
	1	–	0.1068 to 0.3364	0.1002 to 0.3042	0.0520 to 0.2974	0.1025 to 0.2972
	2	**0.2220**	–	−0.0047 to 0.0253	−0.0137 to 0.0282	−0.0061 to 0.0018
	3	**0.1878**	0.0073	–	−0.0218 to 0.0025	−0.0038 to 0.0190
	4	**0.1667**	0.0024	−0.0087	–	−0.0097 to 0.0157
	5	**0.2039**	−0.0015	0.0075	0.0034	–
Allele frequencies adjusted for presence of null alleles						
	1	–	0.1069 to 0.3461	0.0733 to 0.2825	0.0412 to 0.3047	0.1021 to 0.2934
	2	**0.2227**	–	−0.0019 to 0.0547	−0.0069 to 0.2291	−0.0016 to 0.0023
	3	**0.1648**	0.0190	–	−0.0005 to 0.1855	0.0001 to 0.0423
	4	**0.1699**	0.0703	0.0562	–	−0.0017 to 0.1861
	5	**0.1982**	0.0003	**0.0184**	0.0585	–
Five (All except locus Cpl 166)						
Allele frequencies not adjusted for presence of null alleles						
	1	–	0.1598 to 0.3610	0.1210 to 0.3285	0.0740 to 0.3309	0.1680 to 0.3160
	2	**0.2682**	–	−0.0055 to 0.0293	−0.0152 to 0.0329	−0.0075 to 0.0005
	3	**0.2173**	0.0087	–	−0.0270 to 0.0043	−0.0044 to 0.0206
	4	**0.2014**	0.0059	−0.0109	–	−0.0042 to 0.0187
	5	**0.2501**	−0.0031	0.0062	0.0097	–
Allele frequencies adjusted for presence of null alleles						
	1	–	0.1517 to 0.3851	0.0883 to 0.3090	0.0521 to 0.3378	0.1697 to 0.3167
	2	**0.2671**	–	−0.0032 to 0.0630	−0.0085 to 0.2816	−0.0020 to 0.0026
	3	**0.1943**	0.0241	–	−0.0005 to 0.2146	−0.0039 to 0.0486
	4	**0.1995**	0.0924	0.0731	–	0.0039 to 0.2263
	5	**0.2425**	0.0002	0.0215	**0.0797**	–

(B)

Number of loci	Region	Bali, Indonesia	Far North	Townsville	Mackay	Brisbane	Northern NSW
Eight (i.e. all)							
Allele frequencies not adjusted for presence of null alleles							
	1	–	−0.0070 to 0.0022	−0.0010 to 0.0082	−0.0101 to 0.0082	−0.0028 to 0.0098	−0.0020 to 0.0167
	2	−0.0029	–	−0.0056 to 0.0006	−0.0085 to 0.0009	−0.0005 to 0.0154	−0.0048 to 0.0116
	3	−0.0011	−0.0027	–	−0.0125 to 0.0011	−0.0030 to 0.0032	−0.0038 to 0.0098
	4	−0.0015	−0.0044	−0.0062	–	−0.0067 to 0.0094	−0.0015 to 0.0051
	5	0.0038	0.0067	0.0004	0.0017	–	−0.0045 to 0.0127
	6	0.0069	0.0021	0.0026	0.0013	0.0031	–
Allele frequencies adjusted for presence of null alleles							
	1	–	−0.0057 to 0.00314	−0.0086 to 0.0084	−0.0093 to 0.0090	−0.0018 to 0.0099	−0.0012 to 0.0167
	2	−0.0019	–	−0.0051 to 0.0014	−0.0084 to 0.0013	−0.0009 to 0.0148	−0.0032 to 0.0113
	3	−0.0006	−0.0022	–	−0.0114 to −0.0008	−0.0025 to 0.0042	−0.0029 to 0.0114
	4	−0.0004	−0.0041	−0.0059	–	−0.0067 to 0.0096	−0.0012 to 0.0056
	5	0.0044	0.0064	0.0012	0.0017	–	−0.0037 to 0.0126
	6	0.0074	0.0026	0.0037	0.0015	0.0035	–

Table 3 continued

(B)

Number of loci	Region	Bali, Indonesia	Far North	Townsville	Mackay	Brisbane	Northern NSW
Six (excluding loci Cli12 and SLE018)							
Allele frequencies not adjusted for presence of null alleles							
	1	–	−0.0037 to 0.0046	−0.0099 to 0.0114	−0.0099 to 0.0118	−0.0033 to 0.0111	0.0004 to 0.0219
	2	−0.0003	–	−0.0043 to 0.0022	−0.0081 to 0.0034	−0.0021 to 0.0174	−0.0032 to 0.0138
	3	0.0004	−0.0013	–	−0.0140 to 0.0007	−0.0038 to 0.0037	−0.0029 to 0.0145
	4	0.0004	−0.0033	−0.0071	–	−0.0103 to 0.0113	−0.0021 to 0.0067
	5	0.0048	0.0074	0.0004	0.0001	–	−0.0018 to 0.0186
	6	**0.0109**	0.0030	0.0048	0.0015	0.0069	–
Allele frequencies adjusted for presence of null alleles							
	1	–	−0.0034 to 0.0054	−0.0038 to 0.0114	−0.0076 to 0.0127	−0.0002 to 0.0115	0.0018 to 0.0218
	2	0.0003	–	−0.0047 to 0.0028	−0.0150 to 0.0040	−0.0024 to 0.0173	−0.0022 to 0.0141
	3	0.0008	−0.0010	–	−0.0126 to 0.0003	−0.0034 to 0.0049	−0.0002 to 0.0159
	4	0.0015	−0.0029	−0.0060	–	−0.0093 to 0.0123	−0.0017 to 0.0073
	5	0.0053	0.0074	0.0012	0.0010	–	0.0020 to 0.0179
	6	**0.0109**	0.0036	0.0056	0.0021	**0.0066**	–

F-statistics are shown in bold when their 95% interval does not include zero

S. lewini. For *R. acutus*, 37 haplotypes were recorded (Appendix 2—Electronic Supplementary Material) compared with *S. lewini* samples with only 13 haplotypes (Appendix 3—Electronic Supplementary Material). A single *S. lewini* haplotype (SL01) dominated all populations, including Indonesia, with a mean abundance of 81%. The second most common *S. lewini* haplotype (SL06, 7% mean abundance) was also widely dispersed. Although often widely distributed, none of the remaining 11 *S. lewini* haplotypes were very abundant (<3%). The dominant *R. acutus* haplotype, RA01, was present in all populations along the Australian east coast, with a mean abundance of 44%. Four other common haplotypes (RA06—9.09%, RA11—7.18%, RA17—5.26% and RA18—5.26%) were also distributed across the full east coast sampling range (southern extent of distribution was Fraser Burnett). Unique Indonesian haplotypes were identified for both species; in fact, no shared haplotypes of *R. acutus* were found between eastern Australia and Indonesia (Appendix 2—Electronic Supplementary Material, Fig. 1). Greater overlap of *S. lewini* haplotypes occurred between Australia and Indonesia (Appendix 3—Electronic Supplementary Material); however, a genetically distant haplotype (SL13) was found in Indonesia. SL13 differed from SL01 at 46 nucleotide positions, but remained clustered with the *S. lewini* haplotypes in a neighbour joining tree containing sister species: *Sphyrna zygaena* and *Sphyrna mokarran* (Fig. 2). The nuclear genome of the two SL13 sharks was not divergent. They had microsatellite alleles in common with the other Indonesian and Australian samples. For example, at locus Cli12, the two SL13 samples possessed three alleles (i.e. 251/255 and 241/241) that were among the eight most common alleles out of the 40 alleles that were observed overall for that locus. For other microsatellite loci, the alleles possessed by the SL13 samples were among the most common. Genetic (mtDNA and nuclear) and morphological data are needed on a larger sample size to test whether these sharks are related to the Western Atlantic cryptic lineage reported by Quattro et al. (2006) or represent a newly discovered cryptic lineage and whether inter-lineage interbreeding and mtDNA introgression have occurred.

There was a large contrast between nucleotide diversity (the probability that two randomly chosen homologous nucleotides are different in a population) levels for the two shark species. Low nucleotide diversity (π) values for Australian east coast *S. lewini* (from 0.000372 to 0.001249) reflected the low number of polymorphic sites distinguishing the different haplotypes. In contrast, *R. acutus* populations had three- to ten times higher nucleotide diversity values (0.003445–0.003895). Haplotype diversity (the probability that two randomly chosen haplotypes are different in the sample) was greater for *R. acutus* than for *S. lewini*, as was the Watterson estimator (θ_s) suggesting a higher population mutation rate in *R. acutus* (Table 4).

The haplotype networks for *R. acutus* and *S. lewini* (Fig. 1) show characteristic differences between the species. While both species have haplotypes that were reported

in two or more Australian populations, many more rare *R. acutus* haplotypes were found. With the exception of the Indonesian population of *R. acutus*, the *R. acutus* and *S. lewini* networks show no clustering of haplotypes by sampling location. The Australian *R. acutus* haplotypes separated into three clusters with the five most common *R. acutus* haplotypes (63%) falling into two. There was no clustering pattern in the star-like *S. lewini* network; the single dominant haplotype (SL01) was centrally positioned.

As expected, Queensland populations of *R. acutus* and *S. lewini* were significantly different from the population sampled in Indonesia. Using mtDNA ND4 sequence variation, all pairwise F_{ST} estimates between Queensland *R. acutus* sampling locations and the Indonesian location were significantly different (F_{ST} estimates 0.57–0.63, Table 5). The Indonesian *S. lewini* population was genetically different from Far North, Townsville and Northern New South Wales populations, but could not be distinguished from Mackay or Brisbane populations (Table 5). Intra-specific pairwise F_{ST} comparisons from ND4 sequence data for the Australian east coast populations of the two shark species were not significant (Table 5). The distribution of dominant ND4 haplotypes for *R. acutus* and *S. lewini* highlighted the similarities among east coast populations and the contrast with the Indonesian collection location, particularly for *R. acutus* (Fig. 3).

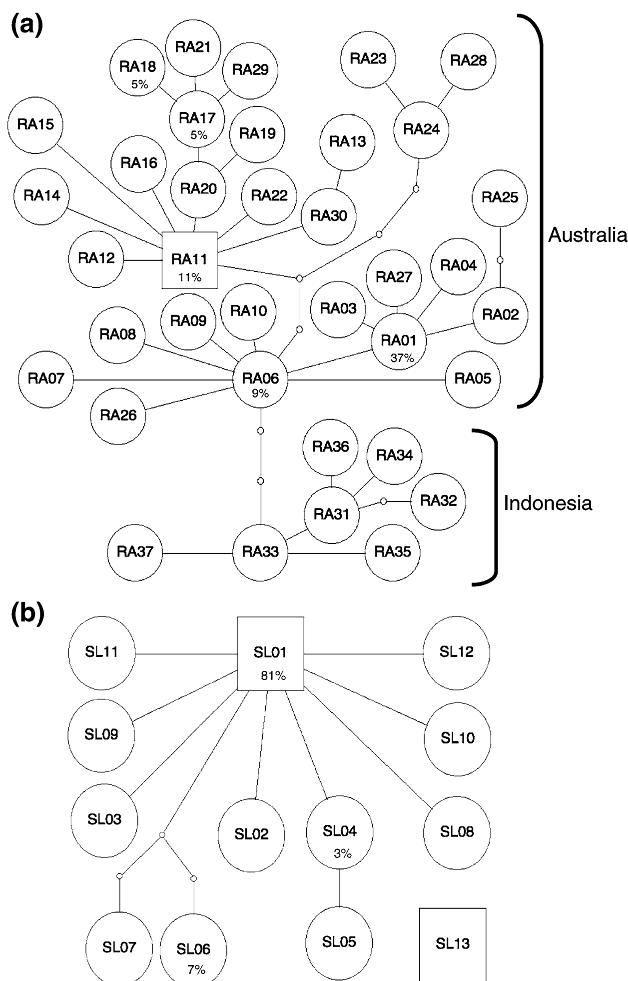

Fig. 1 Mitochondrial DNA ND4 haplotype networks for *Rhizoprionodon acutus* (**a**) and *Sphyrna lewini* (**b**) indicating the sampling location and frequency of the most common haplotypes. One *Sphyrna lewini* haplotype (ND4_SL13, Indonesia only) was too divergent (46 steps) to be included in the network. GenBank numbers for all sequences are listed in the ESM

Largely because this study relied on tissue samples from commercially caught sharks, there was little opportunity for repeated sampling at any location or species to confirm the stability of genetic population characteristics through time. Limited temporal sampling from Townsville from 2007 to 2009 was achieved for *R. acutus*. In 2007, fifteen animals were collected and nine haplotypes described. In 2008, 36 animals were collected representing 10 haplotypes of which six were new. In 2009, 13 animals were collected with nine haplotypes of which four were new. Sample sizes were insufficient to test for genetic population structure; however, haplotype RA01 dominated the catches over the 3-year time period (46, 47 and 31%, respectively), and common haplotypes RA06, RA11 and RA18 were present at all time points. Overall, 19 haplotypes were described from 64 animals collected off Townsville. The high frequency of new haplotypes across temporal samples suggested that larger sample sizes would have been needed to fully characterise *R. acutus* haplotype diversity.

Discussion

This study represents a comprehensive, regional analysis of genetic population structure in two shark species (*S. lewini* and *R. acutus*), which are an important component of the Elasmobranchii catch in northern and eastern Australian fisheries. Two types of genetic markers (mtDNA and microsatellites) were used per species, and large numbers of samples were studied from four to five regions per species on the eastern Australian coast. There was no evidence of more than one eastern Australian coast genetic stock for *S. lewini* or *R. acutus*, from samples encompassing about two thousand kilometres of coastline. Results from both types of genetic markers for both species were concordant; comparisons between pairs of eastern Australian populations showed genetic similarity based on microsatellite allele frequencies and sequence polymorphisms in the mtDNA ND4 gene region. Apparent genetic homogeneity at this large spatial scale is not matched by environmental conditions, however. The regions sampled are environmentally heterogeneous; for example, they cross gradients of sea temperatures and extent of continental shelf, and they

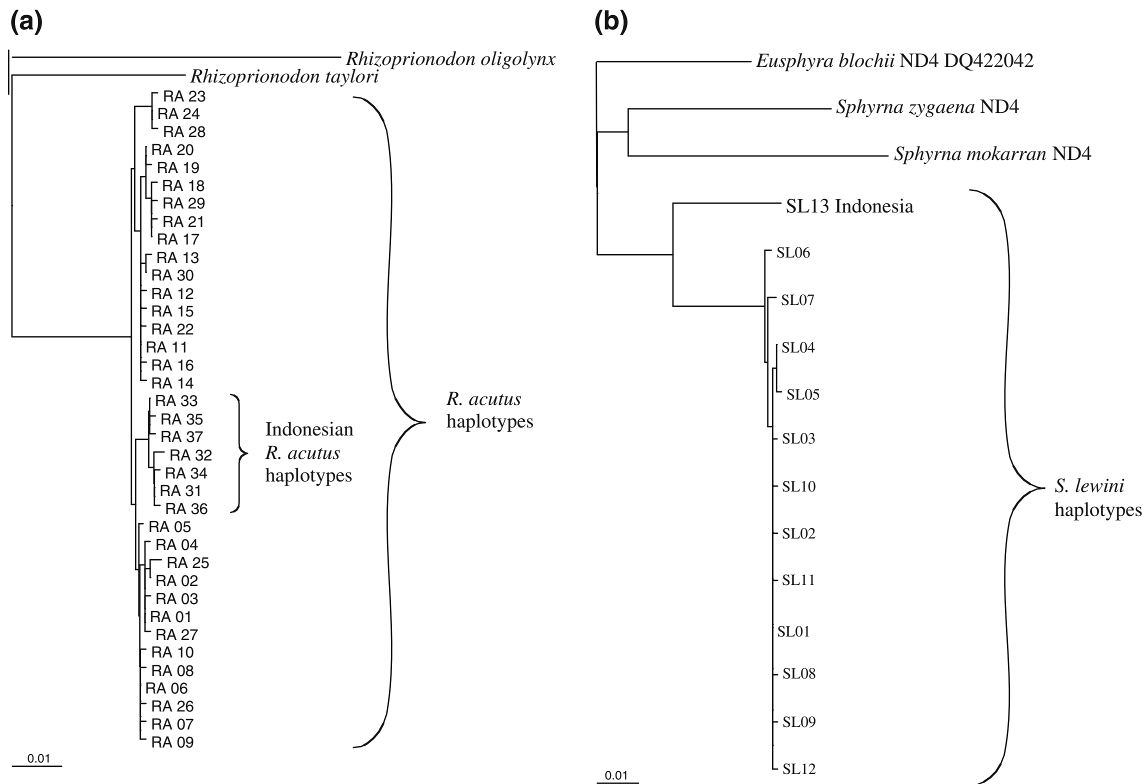

Fig. 2 Phylogenetic neighbour joining tree for *Rhizoprionodon acutus* (**a**) and *Sphyrna lewini* (**b**) mtDNA ND4 haplotypes illustrating the position of Indonesian haplotypes. *Scale* represents the proportion of polymorphic sites between haplotypes

Table 4 Mitochondrial DNA ND4 molecular diversity indices for populations of *Rhizoprionodon acutus* and *Sphyrna lewini*

Region	N	u	p	h	π	θ_S
Rhizoprionodon acutus						
Indonesia	17	7	7	0.8897 ± 0.0398	0.002089 ± 0.001414	2.070559 ± 1.029486
Far North	56	17	20	0.8331 ± 0.0418	0.003860 ± 0.002227	4.353872 ± 1.495619
Cairns	2	2	7	–	–	–
Townsville	64	19	23	0.7847 ± 0.0476	0.003536 ± 0.002065	4.864363 ± 1.601907
Mackay	10	6	9	0.7778 ± 0.1374	0.003895 ± 0.002460	3.181372 ± 1.605053
Fraser Burnett	60	15	17	0.8277 ± 0.0414	0.003445 ± 0.002022	3.645562 ± 1.285715
Total	209	37	40			
Sphyrna lewini						
Indonesia	33	4	46	0.4886 ± 0.0921	0.007120 ± 0.003859	11.33425 ± 3.742889
Far North	47	7	9	0.4098 ± 0.0885	0.001064 ± 0.000829	2.037726 ± 0.863100
Cairns	7	1	0	–	–	–
Townsville	49	6	7	0.2313 ± 0.0798	0.000372 ± 0.000424	1.569930 ± 0.715307
Mackay	20	3	4	0.1947 ± 0.1145	0.000458 ± 0.000495	1.127478 ± 0.651884
Fraser Burnett	3	1	0	–	–	–
Brisbane	42	6	9	0.4111 ± 0.0908	0.001249 ± 0.000930	2.091596 ± 0.893926
Northern New South Wales	43	7	9	0.3012 ± 0.0911	0.000581 ± 0.000558	2.080087 ± 0.887302
Total	244	13	52			

Statistics presented include the number of sharks sequenced (N), the number of unique haplotypes (u), the number of polymorphic sites (p), haplotype diversity (h, ± standard deviation), nucleotide diversity (π) and the Watterson estimator used to estimate population mutation rate using segregating sites and non-recombining DNA (θ_S)

Table 5 Mitochondrial DNA ND4 population pairwise F_{ST} estimates (below diagonal) and *p*-value (above diagonal, $\alpha = 0.05$, NS = not significant) for major sampling localities for *Rhizoprionodon acutus* and *Sphyrna lewini*

R. acutus	Indonesia $N = 17$	Far North $N = 56$	Townsville $N = 64$	Mackay $N = 10$	Fraser Burnett $N = 60$
Indonesia	–	0.00000	0.00000	0.00000	0.00000
Far North	**0.56833**	–	NS	NS	NS
Townsville	**0.57691**	0.00608	–	NS	NS
Mackay	**0.63420**	−0.03435	−0.02425	–	NS
Fraser Burnett	**0.58784**	0.00675	−0.00731	−0.02594	–

S. lewini	Indonesia $N = 33$	Far North $N = 47$	Townsville $N = 49$	Mackay $N = 20$	Brisbane $N = 42$	Northern New South Wales $N = 43$
Indonesia	–	0.02703	0.01802	NS	NS	0.00000
Far North	**0.03877**	–	NS	NS	NS	NS
Townsville	**0.07302**	0.01114	–	NS	0.03604	NS
Mackay	0.03125	0.00032	−0.00611	–	NS	NS
Brisbane	0.02876	−0.01003	**0.04730**	0.01642	–	NS
Northern New South Wales	**0.05965**	0.00007	−0.01289	−0.02348	0.02345	–

Significant F_{ST} estimates are shown in bold

cross a major transition zone between subtropical rocky coast and the tropical Great Barrier Reef.

Genetic population subdivision has been reported along this coastline for marine species. For example, Sumpton et al. (2008) reported a weak genetic disjunction on the north coast of New South Wales between populations of pink snapper (*Pagrus auratus*) and general isolation by distance on the eastern Australian coast. For species studied within the Great Barrier Reef, genetic homogeneity has been the rule rather than the exception. For example, genetic homogeneity was reported for two popular recreational species, coral trout (*Plectropomus maculatus*) and stripey snapper (*Lutjanus carponotatus*) (Evans et al. 2010), and only limited genetic subdivision was found in goby species (*Eviota queenslandica* and *E. albolineata*) that were specifically selected as a candidates for population genetic structure because of their cross-shelf distribution and short lifespan (Farnsworth et al. 2010). The most likely explanation for the lack of population genetic structure at large spatial scales for fish species is semi-passive dispersal of early life history stages in long-shore and oceanic currents as well as possible active dispersal of mature life forms. This is counter-intuitive to the process of allopatric speciation, driven by vicariant events, that is thought to have shaped the evolutionary history of the majority of marine organisms. In reality, it is the imprint of marine vicariant events, such as the collision of the African and Asian continents in the Miocene (23 to five million years ago) that shaped the evolution of species within the genus *Rhizoprionodon* (Gallo et al. 2010), as well as marine dispersal processes that drive marine species diversity (Barber and Bellwood 2005; Bourjea et al. 2007; Schluessel et al. 2010).

Sharks have a considerable potential for active dispersal, most likely as mature adults, although juveniles of some species (e.g. *Carcharodon carcharias*, white shark) also have the capacity to move long distances (Bruce 2008). Long-shore dispersal and subsequent panmixia along the continuous eastern Australian coast are the most likely explanation of implied genetic homogeneity observed in this study for eastern Australian *S. lewini* and *R. acutus*. Other techniques for studying dispersal (e.g. tag-release-recapture) or biological similarities at a population level (e.g. otolith microchemistry, parasite abundance) provide information about movement during the lifespan of individuals and provide an interesting contrast to genetic analyses. For example, more population subdivision was suggested by parasite (Charters et al. 2010) and otolith (Newman et al. 2010) analyses than by population genetic studies (Broderick et al. 2011) for grey mackerel (*Scomberomorus semifasciatus*) on the Australian northern coastline. For *S. lewini* and *R. acutus*, variation in the microchemical composition of vertebrae appeared to suggest limited lifespan movement along the eastern Australian coastline (Schroeder et al. 2011), implying that sporadic or low levels of dispersal may be maintaining genetic homogeneity.

From genetic data alone, however, there are obvious limits to dispersal at least for *R. acutus*. While *S. lewini*

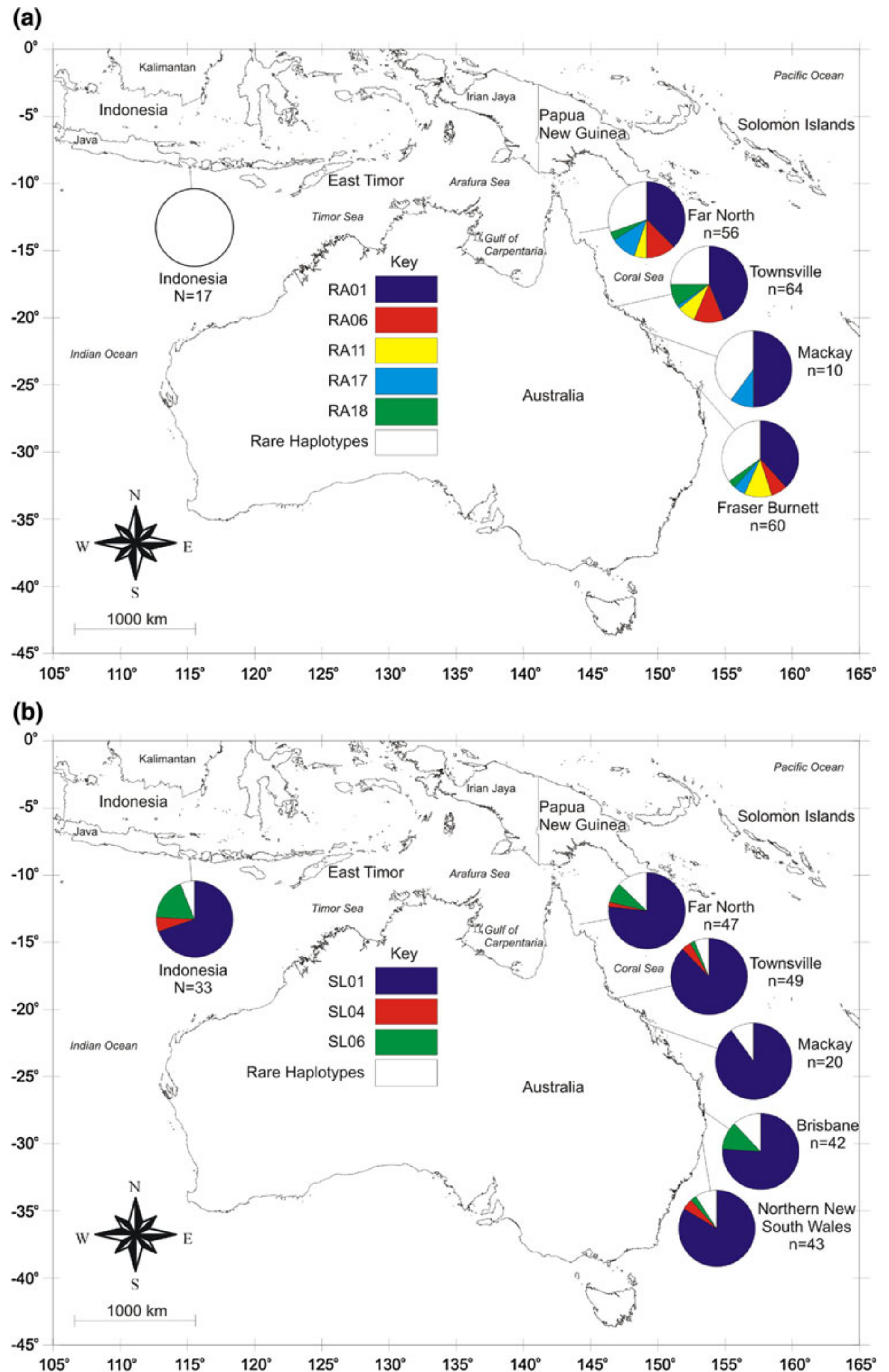

Fig. 3 Relative distribution of the most common *Rhizopriondon acutus* (**a**) and *Sphyrna lewini* (**b**) mtDNA ND4 haplotypes from the major Australian east coast sampling sites and Indonesia

may disperse widely between Australia and Indonesia, as shown here and by Ovenden et al. (2009), the population of *R. acutus* in central Indonesia was significantly different to the eastern Australian population of *R. acutus.* Ovenden et al. (2009) sampled the spot-tail shark (*Carcharhinus sorrah*) from several locations in northern Australia and from two locations in central Indonesia. Using a combination of microsatellites and mtDNA sequence (control region), *F*-statistics for *C. sorrah* ranged from 0.751 to 0.903 (mtDNA, control region) to 0.038 to 0.047 (microsatellite

loci). *F*-statistics between Indonesian and Australian *R. acutus* were similar to that of *C. sorrah* (0.58–0.63; mtDNA, ND4 region), and no mtDNA haplotypes were shared between locations. *F*-statistics for *R. acutus* were more pronounced for microsatellite loci (0.16–0.27). This strongly indicates that dispersal does not occur between Indonesia and Australia for *R. acutus.* Future studies should test for finer scale genetic breaks by sampling *R. acutus* from the Gulf of Carpentaria, northern and western Australia and other Indonesian locations. Compared with *S. lewini*, *R. acutus* showed higher levels of haplotype diversity (0.78–0.89 compared with 0.20–0.49), and it had a more extensively branched haplotype network. In the network, Indonesian *R. acutus* haplotypes formed a well-resolved clade and there was evidence of two to three further clades among the Australian samples whose members were spatially overlapping. The *R. acutus* haplotype network did not show the characteristic 'star-burst' pattern often linked with rapidly expanding populations (Posada and Crandall 2001).

For two of the most commonly landed Elasmobranchii species in Queensland, *S. lewini* and *R. acutus*, this study has not been able to refute widespread genetic homogeneity. Against expectations, the results are the same for both species from northern New South Wales (*S. lewini*) or southern Queensland (*R. acutus*) to far north Queensland. The smaller body size, and hence presumed lower vagility of *R. acutus*, has not been responsible for genetic subdivisions within its range in Queensland. Despite passing through a heterogeneous environment encompassing temperate, subtropical and tropical ecosystems, this genetic study has found no obvious barriers to gene flow in either species within their range in Queensland. The distinction between the two species becomes apparent, however, when comparisons were made between the population on the eastern Australian coast and central Indonesia. As predicted, the smaller species (*R. acutus*) appears to be genetically subdivided, whereas the more oceanic larger species (*S. lewini*) is not affected. Our results show that the spatial extent of the current generic Queensland State government shark fishery management and monitoring plan is appropriate for two of many shark species under commercial exploitation. We urge fisheries authorities to support further stock structure and biological research on the remainder of the largely unstudied Elasmobranchii fauna in tropical and subtropical Australia and to continually refine management strategies to ensure that this unique part of Indo-Pacific biodiversity has a sustainable future in Australian waters.

Acknowledgments We are indebted to the many fishers and scientists who collected tissue samples for this project, including those associated with the Marine and Tropical Science Research Facility project 4.8.4 (GBRWHA Inshore fisheries), James Cook University and the fisheries observer programmes in Queensland and New South Wales. The Australian Fisheries Research and Development Corporation supported this study (project 2007/035).

References

Anon (2010) Annual status report 2009. East coast inshore fin fish fishery. Department of Employment, Economic Development and Innovation, Brisbane

Arevalo E, Davis S, Sites JJ (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst Biol 43:387–418

Barber PH, Bellwood DR (2005) Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (Halichoeres: Labridae) in the Indo-Pacific and new world tropics. Mol Phylogenet Evol 35:235–253

Baum J, Clarke S, Domingo A, Ducrocq M, Lamónaca AF, Gaibor N, Graham R, Jorgensen S, Kotas JE, Medina E, Martinez-Ortiz J, Monzini Taccone di Sitizano J, Morales MR, Navarro SS, Pérez JC, Ruiz C, Smith W, Valenti SV, Vooren CM (2007) *Sphyrna lewini*. IUCN red list of threatened species. http://www.iucnredlist.org. Accessed 11th Oct 2010

Bourjea J, Lapegue S, Gagnevin L, Broderick D, Mortimer JA, Ciccione S, Roos D, Taquet C, Grizel H (2007) Phylogeography of the green turtle, Chelonia mydas, in the Southwest Indian Ocean. Mol Ecol 16:175–186

Broderick D, Ovenden JR, Buckworth RC, Newman SJ, Lester RJG, Welch DJ (2011) Genetic population structure of grey mackerel (*Scomberomorus semifasciatus* Macleay, 1883) in northern Australia. J Fish Biol (Submitted)

Bruce BD (2008) The biology and ecology of the white shark *Carcharodon carcharias*. In: Cambi MD, Pikitch EK, Babcock EA (eds) Sharks of the open ocean. Blackwell, Oxford, pp 69–81

Chapman DD, Prodohl PA, Gelsleichter J, Manire CA, Shivji MS (2004) Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. Mol Ecol 13:1965–1974

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Chapuis M-P, Lecoq M, Michalakis Y, Loiseau A, Sword GA, Piry S, Estoup A (2008) Do outbreaks affect genetic population structure? A worldwide survey in *Locusta migratoria*, a pest plagued by microsatellite null alleles. Mol Ecol 17:3640–3653

Charters RA, Lester RJG, Buckworth RC, Newman SJ, Ovenden JR, Broderick D, Kravchuk O, Ballagh A, Welch DJ (2010) The stock structure of grey mackerel *Scomberomorus semifasciatus* in Australia as inferred from its parasite fauna. Fish Res 101:94–99

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the EM algorithm. J R Stat Soc Ser B-Methodol 39:1–38

Duncan KM, Martin AP, Bowen BW, De Couet HG (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). Mol Ecol 15:2239–2251

Evans RD, van Herwerden L, Russ GR, Frisch AJ (2010) Strong genetic but not spatial subdivision of two reef fish species targeted by fishers on the Great Barrier Reef. Fish Res 102:16–25

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Farnsworth CA, Bellwood DR, van Herwerden L (2010) Genetic structure across the GBR: evidence from short-lived gobies. Mar Biol 157:945–953

Gallo V, Cavalcanti MJ, da Silva RFL, da Silva HMA, Pagnoncelli D (2010) Panbiogeographical analysis of the shark genus *Rhizoprionodon* (Chondrichthyes, Carcharhiniformes, Carcharhinidae). J Fish Biol 76:1696–1713

Inoue JG, Miya M, Tsukamoto K, Nishida M (2001) A mitogenomic perspective on the basal teleostean phylogeny: resolving higher-level relationships with longer DNA sequences. Mol Phylogenet Evol 20:275–285

Keeney DB, Heist EJ (2003) Characterization of microsatellite loci isolated from the blacktip shark and their utility in requiem and hammerhead sharks. Mol Ecol Notes 3:501–504

Keeney DB, Heist EJ (2006) Worldwide phylogeography of the blacktip shark (*Carcharhinus limbatus*) inferred from mitochondrial DNA reveals isolation of western Atlantic populations coupled with recent Pacific dispersal. Mol Ecol 15:3669–3679

Keeney DB, Heupel M, Hueter RE, Heist EJ (2003) Genetic heterogeneity among blacktip shark, *Carcharhinus limbatus*, continental nurseries along the US Atlantic and Gulf of Mexico. Mar Biol 143:1039–1046

Last PR, Stevens JD (2009) Sharks and rays of Australia. CSIRO Australia, Collingwood

Mendonca FF, Hashimoto DT, Porto-Foresti F, Oliveira C, Gadig OBF, Foresti F (2009) Identification of the shark species *Rhizoprionodon lalandii* and *R. porosus* (Elasmobranchii, Carcharhinidae) by multiplex PCR and PCR-RFLP techniques. Mol Ecol Resour 9:771–773

Morgan E (2000) Genepop on the web. http://wbiomed.curtin.edu.au/genepop. Accessed 6th May 2009

Nance HA, Daly-Engel TS, Marko PB (2009) New microsatellite loci for the endangered scalloped hammerhead shark, *Sphyrna lewini*. Mol Ecol Resour 9:955–957

Newman SJ, Wright IW, Rome BM, Mackie MC, Lewis PD, Buckworth RC, Ballagh AC, Garrett RN, Stapley J, Broderick D, Ovenden JR, Welch DJ (2010) Stock structure of Grey Mackerel, *Scomberomorus semifasciatus* (Pisces: Scombridae) across northern Australia, based on otolith stable isotope chemistry. Environ Biol Fish 89:357–367

Ovenden JR, Broderick D, Street R (2006) Microsatellite primers for two carcharinid sharks (*Carcharinus tilstoni* and *C. sorrah*) and their usefulness across a wide range of shark species. Mol Ecol Notes 6:415–418

Ovenden JR, Kashiwagi T, Broderick D, Giles J, Salini JP (2009) The extent of population genetic subdivision differs among four co-distributed shark species in the Indo-Australian archipelago. BMC Evol Biol 9:40

Ovenden JR, Morgan J, Kashiwagi T, Broderick D, Salini J (2010) Towards better management of Australia's shark fishery: genetic analyses reveal unexpected ratios of cryptic blacktip species *Carcharhinus tilstoni* and *C. limbatus*. Mar Freshw Res 61:253–262

Pardini AT, Jones CS, Noble LR, Kreiser B, Malcolm H, Bruce BD, Stevens JD, Cliff G, Scholl MC, Francis M, Duffy CAJ, Martin AP (2001) Sex-biased dispersal of great white sharks—in some respects, these sharks behave more like whales and dolphins than other fish. Nature 412:139–140

Portnoy DS, McDowall JR, Thompson K, Musick JA, Graves JE (2006) Isolation and characterization of five dinucleotide microsatellite loci in the sandbar shark, *Carcharhinus plumbeus*. Mol Ecol Notes 6:431–433

Portnoy DS, Piercy AN, Musick JA, Burgess GH, Graves JE (2007) Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic and Gulf of Mexico. Mol Ecol 16:187–197

Portnoy DS, McDowell JR, Heist EJ, Musick JA, Graves JE (2010) World phylogeography and male-mediated gene flow in the sandbar shark, *Carcharhinus plumbeus*. Mol Ecol 19:1994–2010

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. Trends Ecol Evol 16:37–45

Quattro JM, Stoner DS, Driggers WB, Anderson CA, Priede KA, Hoppmann EC, Campbell NH, Duncan KM, Grady JM (2006) Genetic evidence of cryptic speciation within hammerhead sharks (Genus *Sphyrna*). Mar Biol 148:1143–1155

Randall JE (1998) Zoogeography of shore fishes of the Indo-Pacific region. Zool Stud 37:227–268

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Schluessel V, Broderick D, Collin SP, Ovenden JR (2010) Evidence of extensive population structure in the white spotted eagle ray *Aetobatus narinari* within the Indo-Pacific inferred from mitochondrial gene sequences. J Zool 281:46–55 (London)

Schroeder R, Simpfendorfer CA, Welch D (2011) Population structure of two inshore shark species (*Sphyrna lewini* and *Rhizoprionodon acutus*) using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) along the east coast of Queensland, Australia. In: Welch D, Ovenden JR, Simpfendorfer CA, Tobin A, Morgan J, Street R, White J, Harry A, Schroeder R, Macbeth GM (eds) Stock structure of exploited shark species in north-eastern Australia. Final Report to the Fisheries Research & Development Corporation, Project 2007/035. Fishing & Fisheries Research Centre Technical Report No. 12, Townsville, Australia, pp 39–48

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234

Simpfendorfer CA (2003) *Rhizoprionodon acutus*. IUCN red list of threatened species. http://www.iucnredlist.org. Accessed 11th Oct 2010

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462

Sumpton WD, Ovenden JR, Keenan CP, Street R (2008) Evidence for a stock discontinuity of snapper (*Pagrus auratus*) on the East coast of Australia. Fish Res 94:92–98

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Wong EHK, Shivji MS, Hanner RH (2009) Identifying sharks with DNA barcodes: assessing the utility of a nucleotide diagnostic approach. Mol Ecol Resour 9:243–256