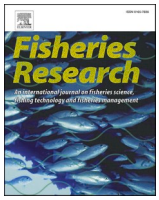
Fisheries Research 251 (2022) 106305

Contents lists available at ScienceDirect

Fisheries Research

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



Updated connectivity assessment for the scalloped hammerhead (*Sphyrna lewini*) in Pacific and Indian Oceans using a multi-marker genetic approach

M.E. Green [a,b,c,*], S.A. Appleyard [a,d], W.T. White [a,d], S.R. Tracey [b,c], M.R. Heupel [e], J. R. Ovenden [f]

[a] CSIRO Oceans & Atmosphere, Castray Esplanade, Battery Point, Hobart, TAS 7001, Australia
[b] Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia
[c] Centre for Marine Socioecology, University of Tasmania, Private Bag 49, Hobart TAS 7001, Australia
[d] CSIRO Australian National Fish Collection, National Research Collections Australia, Castray Esplanade, Hobart, TAS 7001, Australia
[e] Integrated Marine Observing System (IMOS), University of Tasmania, Private Bag 110, Hobart, TAS 7000
[f] Molecular Fisheries Laboratory, School of Biomedical Sciences, University of Queensland, Chancellors Pl, St. Lucia, Brisbane, OLD 4072, Australia

ARTICLE INFO

Handled by Dr. J Viñas

Keywords:
Elasmobranch
SNP
Microsatellite
Mitochondrial DNA
Conservation

ABSTRACT

Patterns of genetic connectivity can be used to define the geographic boundaries of fishes and underpin management decisions. This study used a genetic multi-marker approach to investigate the population structure of scalloped hammerheads (*Sphyrna lewini*) in the Indo–Pacific. Samples from 541 *S. lewini* were collected from 12 locations across the Indo–Pacific. Samples were analysed using two regions of the mitochondrial genome, nine microsatellite loci and two sets of Single Nucleotide Polymorphisms (SNP). Our study has four key findings; (1) genetic structure of *S. lewini* across the Indo–Pacific is affected by oceanic basins and can be separated into four distinct regions. (2) Within the central Indo–Pacific, connectivity is facilitated along continental shelves and strong signals of Isolation-By-Distance (IBD) were observed. (3) Mitochondrial haplotypes previously thought only to exist in the Atlantic Ocean are observed in Indo–Pacific populations, suggesting the haplotype should be reconsidered as more widespread than initially thought. (4) Results from microsatellites and SNPs largely agree, however a few differences are apparent with SNPs identifying more discrete population subdivision. Our findings suggest management at the spatial scales and boundaries identified in this study will necessitate international and national cooperation to conserve *S. lewini* populations.

1. Introduction

Knowledge of the biological stock structure of highly mobile marine species provides a basis for informed management for fisheries or conservation commitments. The identification of biological stock structure is challenging for many broad ranging species, given a lack of obvious barriers to dispersal (Cowen et al., 2006). Until formally tested, it is assumed a species belongs to a single panmictic stock, which may result in complex and challenging international management requirements (Chin et al., 2017; Reiss et al., 2009; Ward, 2000). Despite a lack of physical barriers preventing shark dispersal, we often see patterns of stock structure driven by subtle environmental barriers relating to an individual's requirements of habitat, food and reproduction (Pember et al., 2020). For large-bodied sharks that are subject to intense harvest pressure, biological stocks can be found to occur across Exclusive Economic Zones (EEZs) of a number of countries, including international waters requiring cross-jurisdictional consultation and management (Chin et al., 2017; Ovenden et al., 2015; Vaudo et al., 2017).

The scalloped hammerhead (*Sphyrna lewini*) is one of ten recognised hammerhead shark species. It is a large-bodied shark with a circumglobal distribution in tropical and warm-temperate waters (Last and Stevens, 2009). Adults are often found occupying oceanic seamounts and continental shelves to depths of more than 275 m, with reports of aggregation and long distance dispersive behaviours (Bessudo et al., 2011; Compagno et al., 2005; Hearn et al., 2010; Klimley and Nelson, 1981; López et al., 2022). There are a number of studies describing the seasonal migrations of adult females into sheltered coastal waters to give birth (Bessudo et al., 2011; Clarke, 1971; Yates et al., 2015) and it has been suggested that the species demonstrates female-mediated philopatry (Daly-Engel et al., 2012). Multiple mating events in a single

* Corresponding author at: CSIRO Oceans & Atmosphere, Castray Esplanade, Battery Point, Hobart, TAS 7001, Australia.
E-mail address: madeline.green@utas.edu.au (M.E. Green).

https://doi.org/10.1016/j.fishres.2022.106305
Received 1 November 2021; Received in revised form 10 March 2022; Accepted 17 March 2022
Available online 29 March 2022

season can occur for *S. lewini*, leading to litters containing pups sired by multiple fathers (Green et al., 2017). Young-of-the-year (YOY), neonate and juvenile *S. lewini* remain in shallow coastal areas in depths of less than 100 m, likely providing protection from large predators (Heupel et al., 2018, 2007). A number of pupping grounds have been identified for *S. lewini* including in the Gulf of California (Baum et al., 2007), inshore regions of the east Australian coast (Simpfendorfer and Milward, 1993; Yates et al., 2015), Fiji (Brown et al., 2016; Marie et al., 2017), the Galapagos Islands (Hearn et al., 2010) and an inshore region in Florida, USA (Wargat, 2021). Ontogenetic and sex-biased shifts in habitat use have been described for *S. lewini* within the central Indo–Pacific; whereby the majority of northern Australian waters contain male neonates and juveniles, while a high proportion of adult females are found in Indonesia and Papua New Guinea (Chin et al., 2017; Green et al., 2017). Recent diet analyses also described ontogenetic changes in diet and habitat for *S. lewini* in the tropical East Pacific Ocean, highlighting the complex patterns of behaviour and habitat use for the species (Estupiñán-Montaño et al., 2021).

Listed as Critically Endangered under the International Union for the Conservation of Nature's (IUCN) *Red List of Threatened Species*, all life stages of *S. lewini* are vulnerable to capture with a variety of fishing methods near and offshore (Rigby et al., 2019). As such, *S. lewini* is listed as a conservation concern with many international and national bans and restrictions established including: Appendix II listing within the Conservation on International Trade in Endangered Species (CITES, 2014), Appendix II for the Conservation of Migratory Species (CMS, 2015), listing in the U.S. Endangered Species Act (79 FR 38213) (NOAA, 2014) and listed as Endangered under the New South Wales (Australia) Fisheries Management Act (1994). Fishing is considered the major threat for *S. lewini* with gears such as trawling, purse-seining, gillnetting, longlining (bottom and pelagic) and inshore artisanal fishing capturing *S. lewini* (Baum et al., 2007; Roberson et al., n.d.). Slow life history characteristics (low growth rate, late maturation, low natural mortality) in combination with a preference to aggregate during mating and inshore parturition mean *S. lewini* are highly susceptible to fishing pressure and are less likely to replenish local population losses at a sufficient rate (Barker and Schluessel, 2005). Additionally, the fins of *S. lewini* are highly valuable due to their high fin-ray count leading to the species being one of the most commonly traded in the Hong Kong fin market (Cardeñosa et al., 2020; Clarke et al., 2006).

In the Western Indian Ocean, population size estimates based on fisheries independent surveys (shark control nets) found a trend of population decline across a 25 year period (1978–2003) (Dudley and Simpfendorfer, 2006). Catch data from South Africa, northwest and western central Atlantic and Brazil has also reported significant population declines between 50% and 90% over 32 years (Baum et al., 2007). Total catches throughout central Indo–Pacific locations; Indonesia, Papua New Guinea (PNG) and Australia are unknown, however given the high number of elasmobranch catches reported for Indonesia the species is estimated to be at risk of being overfished in at least Indonesia waters (White et al., 2008). Recent regional IUCN assessments have calculated positive population change in some locations (North Atlantic[1], South Pacific[2]) indicating recovery may be possible for the species (Rigby et al., 2019).

Genetic methods have delivered important assessments of the biological stock structure for circumglobal *S. lewini* populations (Daly-Engel et al., 2012; Duncan and Holland, 2006; Ovenden et al., 2009). In *S. lewini*, maternally inherited mitochondrial DNA (mtDNA; considered a single marker) and bi-parentally inherited nuclear DNA (i.e. multi-allelic microsatellites) have shown female mediated gene flow is thought to be restricted to oceanic basins and along continental shelves (Duncan et al., 2006b; Ovenden et al., 2009; Daly-Engel et al., 2012; Hadi et al., 2020). Thus, female residency or female philopatry is likely for the species. Conversely, studies primarily using microsatellite loci have described genetic homogeneity for *S. lewini* across a broad area encompassing the Indo–Pacific region, proposed to be driven by male biased dispersal (Daly-Engel et al., 2012). As an emerging nuclear genetic marker, Single Nucleotide Polymorphisms (SNPs) have recently been applied in *S. lewini* studies of juvenile hammerhead sharks to examine kinship within Fijian populations (Marie et al., 2019). SNPs are single base-pair mutations which occur across an individual's genome. Cost effective and accessible in the thousands, SNPs are becoming a common tool for population genetic assessments of elasmobranchs (Devloo-Delva et al., 2019; Díaz-Jaimes et al., 2021; Green et al., 2019; Momigliano et al., 2017; Pazmiño et al., 2018).

Using a species whose global genetic stock structure is well documented, this study compared and contrasted genetic connectivity for *S. lewini* using a multi-marker approach (mitochondrial DNA, microsatellites and SNPs). Given the global Critically Endangered status of *S. lewini* and its Data Deficient listing within the Oceania region, this novel research in which we test new regional samples alongside samples from previously published studies of Duncan et al. (2006b), Daly-Engel et al. (2012) and Ovenden et al. (2009) is the most comprehensive population genetic study for *S. lewini* to date. Our aim was to provide updated genetic knowledge and inputs for *S. lewini* conservation and management, primarily in the Australasian region.

2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 541 *S. lewini* DNA samples were obtained from 12 locations across the Indo–Pacific (Fig. 1 and.). To compare our multi-marker study with that of previous *S. lewini* genetic assessments, we accessed samples used in Daly-Engel et al. (2012) and Ovenden et al. (2009) with permission from lead authors of these respective studies. Therefore, across all locations, collection occurred between 1999 and 2016. Collection protocols for samples from the Seychelles (SEY), Philippines (PH), Taiwan (TW), Hawaii (HAW) and Gulf of California (GOC) are described in Daly-Engel et al. (2012), while sampling of individuals from Indonesia (IN), Western Australia (WA) and Princess Charlotte Bay, Australia (PCB) are outlined in Ovenden et al. (2009). Samples from Papua New Guinea (PNG) were collected on-board fishing vessels by fisheries observers and from coastal fisheries during dedicated surveys as part of an Australian Centre for International Agricultural Research (ACIAR) project (project number FIS/2012/102). For sharks landed by commercial and coastal fishers a piece of vertebrae chord or muscle was collected. DNA was extracted from all samples using the Wizard© SV Genomic DNA Purification system (Promega, Australia) using SV minicolumns. Total genomic DNA (gDNA) was eluted in DNAse free water and quantified (ng/ul) on a Nanodrop 8000 (Thermo Fisher Scientific, Australia) with A260:A280 ratios reflecting DNA quality. Working stocks of DNA were maintained at 4 °C while archival DNA stocks (in water) and samples (in 95% ethanol) are stored at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) marine laboratories at − 80 °C.

2.2. Mitochondrial DNA

To measure the matrilineal genetic similarity between samples from various locations we amplified two portions of mitochondrial DNA. Two markers; the Control Region (CR) and NADH dehydrogenase subunit 4 (ND4), were used to capture sufficient diversity within the mitochondrial DNA to distinguish between putative genetically similar and divergent populations. For the CR portion forward and reverse primers PRoL2 and PheCacaH2 were used (Pardini et al., 2001), while the forward and reverse primers; ND4 and Leu-Scyliorhinus were used for ND4 amplification (Naylor et al., 2005). For both mitochondrial DNA regions Polymerase Chain Reactions (PCR) were conducted in 25 μL reactions with 15–25 ng of gDNA, GoTaq® Green Master Mix (Promega, USA), 1 μL Bovine Serum Albumin (Promega) and 10 μM primers. PCR used the following thermocycler parameters (for both CR and ND4); initial hold at 94 °C/ 5 min, 35 cycles of 94 °C/ 30 s, 52 °C/ 30 s, 72 °C/ 1 min,

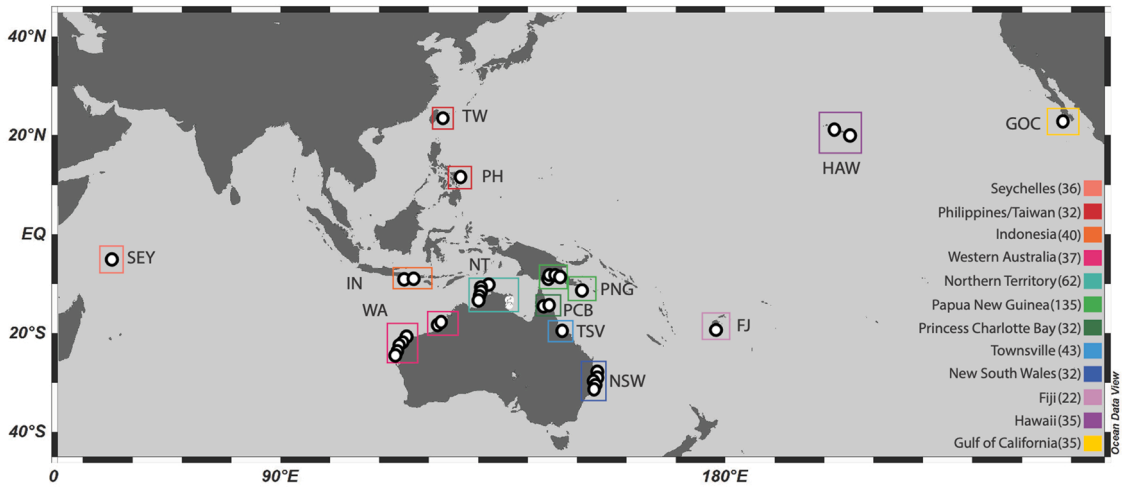


Fig. 1. Sample collections for *S. lewini* within the Indian and Pacific Oceans. Coloured squares represent locations of sample collection, white dots represent sample collection sites, numbers in brackets indicate total sample size (for sample size per marker type see Table 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

followed by final extension of 72 °C/ 10 min. After PCR products were cleaned with Agencourt AMPure magnetic beads (Beckman Coulter, Australia), successfully amplified PCR products were Sanger sequenced bi-directionally for CR and unidirectional (forward) for ND4 using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Invitrogen Life Technologies, USA) and an annealing stage of 52 °C/5 s for 25 cycles. Cycled sequence products were cleaned using CleanSEQ magnetic beads (Beckman Coulter, Australia) and run on an ABI 3130XL AutoDNA sequencer (Applied Biosystems, USA) at the CSIRO marine laboratories, Hobart, Australia. Sequences were screened and aligned using Geneious v10.2.3 (Biomatters Ltd, New Zealand).

In order to find the best-fit substitutional model for mitochondrial DNA sequences MEGA v5.2 was used (Tamura et al., 2011). We calculated molecular diversity indices such as haplotype and nucleotide diversities using Arlequin v3.5 (Excoffier and Lischer, 2010). To visualize haplotype networks, median-Joining network analysis was constructed using POPart v1.7 (http://popart.otago.ac.nz) (Bandelt et al., 1999). Estimates of genetic differentiation between locations was calculated using pairwise Φ_{ST} for mitochondrial DNA in Arelquin v3.5 (Excoffier and Lischer, 2010). Each analysis consisted of 10,000 bootstraps generating confidence intervals and *p* values for each pairwise comparison. Significance of pairwise tests for mitochondrial DNA and nuclear markers was considered when $p = < 0.01$ and $p = < 0.001$ respectively, to be comparable with Daly-Engel et al. (2012) and Ovenden et al. (2009).

2.3. Microsatellites

Microsatellite loci were one of two types of nuclear genome markers used to test for population distinctiveness among individuals across sample locations. Samples were genotyped using nine polymorphic microsatellite loci initially described in Nance et al. (2009) for *S. lewini*. PCR amplifications were performed in three multiplex reactions and forward primers were labelled with a single proprietary fluorophore dye; 6-FAM, VIC, NED, PET (Applied Biosystems, USA). PCR conditions consisted of 1X GoTaq© Colourless Master Mix (Promega), 1 μL Bovine Serum Albumin (Promega,), 0.2 μM of each individual F and R primer, and 0.8 ng/μl DNA in a 25 μL reaction volume. Thermal cycling (in an Eppendorf Mastercycler®, Eppendorf, Germany) consisted of initial denaturation at 94 °C/3 min, 35 cycles of 94 °C/1 min, 58 °C/30 s, 72 °C/1 min and a final extension of 72 °C/10 min. Amplification success was visualised on agarose gels containing SYBR Safe DNA gel stain (ThermoFisher Scientific, USA). Following PCR amplifications in each of the *S. lewini* individuals, GeneScan™ 500 LIZ ™ size standard (Thermofisher Scientific) and formamide were added to 3 μL of each PCR reaction and 20 μL sample volumes were run on an ABI 3130XL AutoDNA sequencer (Thermofisher).

Genotypes were scored using the Microsatellite plug-in program in Geneious R10.2.3 (Biomatters Ltd). To check for potential scoring errors (due to stuttering and large allele drop out) and the presence of null alleles, we used MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004). At each locus and each location we calculated the number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosities, allelic richness (A_R), fixation indices (F_{IS}) and deviations from Hardy-Weinberg Equilibrium (HWE_p) using R-Package '*diveRsity*' (Keenan et al., 2013). Allele frequencies are available in Supplementary material (Table S2). To detect non-random associations of genotypes among pairs of loci, exact tests for linkage disequilibrium were undertaken using GENEPOP on the web v4.2 (Raymond and Rousset, 1995).

Genetic differentiation between locations was calculated with pairwise F_{ST} for microsatellites using the R-package '*diveRsity*' (Keenan et al., 2013). Each analysis consisted of 100,000 bootstraps generating 95% confidence intervals for each pairwise comparison. The number of genetic groups was estimated using Bayesian clustering algorithms implemented in STRUCTURE v2.3.4 (Pritchard et al., 2000). STRUCTURE analysis was initially run using an admixture models with correlated allele frequencies, a burn-in length of 50,000, followed by 1000, 000 MCMC with K (number of clusters) set between 1 and 15 and 8 iterations per value. Optimum K was determined by LnP and ΔK outputs using Structure Harvester v.0.6.94 (Earl and VanHoldt, 2012; Evanno et al., 2005). We also conducted an alternative assessment of genetic clusters for microsatellites using a Discriminant Analysis of Principle Components (DAPC*)* in R-package '*adegenet*' (Jombart et al., 2010). DAPC identifies clusters by sequential clustering and model selection, the multivariate analyses do not require populations to be in HWE or linkage equilibrium (Jombart, 2008; Jombart et al., 2010) As per instructions from Jombart et al. (2010), the number of Principle Components (PC) retained was selected by dividing the number of individuals by three (PC = 115) and 10 eigenvalues were used.

2.4. Single nucleotide polymorphisms (SNPs)

We used a reduced-representation next generation sequencing (NGS) approach to obtain SNPs from across the genome. This enabled us to target a subset of orthologous regions across the genome for the selected samples and to capture and genotype SNP loci from those regions. We sent genomic

DNA to the Diversities Arrays Technology Pty. Ltd (Canberra, Australia) for library preparation and sequencing using the standard DArTSeq Protocol. DArTSeq is a genotype-by-sequencing approach that uses Diversity Arrays (DArT) restriction enzymes (Jaccoud, 2001) and next-generation sequencing on an Illumina platform (Sansaloni et al., 2011). All resulting SNPs were filtered using R-Package '*dartR*' (Gruber et al., 2018) according to criteria described in the Supplementary materials (Methods S.1, Table S2). Broad and fine scale population structure was examined using two different suites of SNPs filtered separately. Broad scale gene flow was assessed among all locations using a final set of 5689 SNPs, while fine scale gene flow was assessed among central Indo–Pacific locations only (PNG, PHTW, IN, WA, NT, PCB, TSV and NSW) using a set of 5969 SNPs. The central Indo–Pacific dataset was generated to sweep for regionally important SNPs that may be lost in the dataset containing distant (and likely) genetically distinct populations. The outcome of each filtering step for each set of SNPs is described in Supplementary materials (Table S2, Fig. S1).

Summary statistics including, H_E and H_O, F_{IS} and A_R were calculated using R-package '*diveRsity*' (Keenan et al., 2013). To describe genetic differentiation between locations we calculated pairwise F_{ST} using the R-package '*StAMPP*'(Excoffier and Heckel, 2006; Pembleton et al., 2013). Each analysis consisted of > 10,000 bootstraps generating confidence intervals and *p* values for each pairwise comparison. To investigate the possible ancestry of each individual and number of genetic groups in our SNP datasets we used the unsupervised maximum likelihood algorithm implemented in ADMIXTURE with K varying from one to 14 and 10,000 bootstraps (Alexander and Lange, 2011). A 100-fold cross-validation (CV) was set to determine the optimal number of clusters for successfully reassigning individuals to their original group (i.e. lowest CV error). Alternative clustering assessment for SNPs was undertaken using DAPC in the R-package '*adegenet*' (Jombart et al., 2010). One hundred and three PCs (a third of the number of individuals in the dataset) were retained and 10 discriminant eigenvalues were used.

2.5. Isolation-by-distance

The relationship between genetic and geographic distance was explored using Mantel tests, where the null hypothesis is that genetic difference is not correlated with geographical distance (Mantel, 1967). Analyses were undertaken following Slatkin (1995) in Arlequin v3.5 (Excoffier and Heckel, 2006) whereby genetic distance was calculated as $F_{ST}/(1\text{-}F_{ST})$ (or $\Phi_{ST}/(1\text{-}\Phi_{ST})$ for mitochondrial DNA) and geographic distance was measured using Google Earth (2019). Using microsatellites and SNPs two spatial scenarios were examined; firstly, a broad scale dataset (consisting of all locations), secondly a finer scale dataset (central Indo–Pacific locations only). Central Indo–Pacific locations included PNG, PHTW, IN, WA, NT, PCB, TSV and NSW. The latter dataset was generated to identify regionally important SNPs that may otherwise be lost in the global dataset. The central Indo-Pacific dataset is more suitable for measuring genetic patterns in the unique biogeographical region which is under strong harvest pressure and where little is known about local populations of *S. lewini*. Genetic distance in relation to geographic distance was measured for SNPs using the *gl.ibd* function in R-package '*dartR*' (Gruber et al., 2018). Similar to Arlequin, '*dartR*' estimates isolation by distance (IBD) based on mantel tests where genetic distance is calculated as $F_{ST}/(1\text{-}F_{ST})$. Geographic distance is represented as the log of distance in metres. Two differently filtered datasets consisting of various combinations of locations were used to understand the effect of large and fine scale structure on IBD analysis (See Supplementary materials for description of SNP sets).

3. Results

The number of samples successfully analysed for each marker type is described in Table 1. Differences in sample size per location and per marker are likely due to a number of factors affecting sequencing and genotype success (i.e. poor quality gDNA). Due to reduced sample sizes for the Philippines (PH) and Taiwan (TW), individuals from these sampling locations were grouped together to allow the representation of the north Indo–Pacific region in the analyses (hereafter abbreviated to PHTW). Separate pairwise F_{ST} analysis was conducted to ensure no difference between locations occurred; since no significance was identified, samples were combined.

3.1. Mitochondrial DNA

To describe the relationship among mitochondrial genomes of individual *S. lewini* we sequenced a 964 bp of the control region CR and 853 bp of ND4 and subsequently concatenated for analysis (total concatenated sequence of 1817 bp in length). Maximum likelihood fits of nucleotide substitutions models found the Tamura model to have the lowest Bayesian Information Criterion (BIC) values for both CR and ND4 portions and was therefore used in further testing of the concatenated sequences. A total of 359 individuals from 12 populations were successfully amplified at both mitochondrial DNA regions resulting in 43 haplotypes. A large break of 19 mutations between haplotypes separated all individuals from SEY and some individuals from IN, PHTW and PNG (Fig. 2). Upon further investigation these haplotypes were identified as very similar to those of the previously described CR 'Atlantic Ocean' haplotype of *S. lewini* (Quattro et al., 2013, 2006) (). An additional branch, divergent by seven mutations, included a mixture of central Indo–Pacific locations and almost all the WA samples. Overall, the majority of other haplotypes were found to be shared in individuals from different locations. The number of haplotypes varied greatly from $H = 33$ in PNG to $H = 3$ in Hawaii and the GOC. Nucleotide diversity greatly varied from $\pi = 0.014$ in PHTW to $\pi = 0.003$ in GOC (Table 1).

An assessment of the fixation indices for spatial population structure across the 12 locations, found a moderate and significant global Φ_{ST} of 0.622 ($p < 0.010$). Pairwise Φ_{ST} estimates for the westernmost location (SEY) and the easternmost location (GOC) were very high and significantly different across all locations ($\Phi_{ST} = 0.265$–0.988, $p < 0.010$) (Table 2). HAW and WA were also found to be genetically dissimilar from east Australia and Fiji locations (PCB, TSV, NSW & FJ) ($\Phi_{ST} = 0.193$–0.379, $p < 0.010$).

3.2. Nuclear markers – microsatellites & SNPs

To determine the extent to which genetic markers from the nuclear genome distinguished biological stocks, two types of markers were compared: microsatellite and SNP loci. A total of 354 individuals from 12 locations successfully genotyped at nine polymorphic microsatellite loci (as in Daly-Engel et al., 2012, with exception of SLE053 and SLE081 which were poorly resolved in our samples and not in HWE). Each locus was highly variable as indicated with A_R ranging from 6.99 to 9.57 and H_O 0.612–0.787 (Table 1). All loci were checked for departures from HWE, after Bonferroni correction of *p* values ($BFp = 0.05/108$), 12 tests were considered out of HWE within some populations (). No single locus was out of HWE at each of the 12 locations, however, WA did have the highest number of departures for a single location (3 loci with $HWE_p < 0.002$), which were rechecked to ensure no heterozygous alleles were missed during genotyping. Per loci (across all locations), no significant departures from HWE were observed, therefore all loci were retained in analyses.

The global F_{ST} estimated across 12 locations using microsatellites was quite small ($F_{ST} = 0.010$). Pairwise F_{ST} comparisons showed the westernmost location SEY and easternmost locations HAW and GOC to have the highest pairwise F_{ST} values compared to the remaining locations ($F_{ST} = 0.010 - 0.064$), with many of the pairwise comparisons found to be highly significant ($p = < 0.001$) (Fig. 3, Table S4). Evidence of high gene flow as reflected in low and non-significant F_{ST} values was apparent between closely located regions within the central Indo–Pacific (PHTW, IN, WA, NT, PNG, PCB, TSV, NSW) ($F_{ST} = 0.000$–0.006). DAPC analysis identified some overlap between all locations, however GOC,

Table 1
Summary of various measures of genetic diversity (averages given) for mitochondrial DNA, microsatellites and SNP datasets in *S. lewini* across the twelve sampling locations.

			mitochondrial DNA (CR+ND4, 1817 bp)					Microsatellites (9 loci)					SNPs (5689 loci)				
Ocean	Site	Abbr.	*n*	S	H	*h*	π x 10^2	*n*	A_R	H_O	H_E	F_{IS}	HWE_p	*n*	S	A_R	H_O	H_E	F_{IS}
Indian	Seychelles	SEY	22	4	7	0.653	0.055	26	9.28	0.768	0.801	0.045	0.516	14	3753	1.91	0.128	0.167	0.177
	Indonesia	IN	35	75	18	0.908	0.675	23	8.65	0.614	0.715	0.165	0.248	23	4507	1.91	0.121	0.161	0.214
	West Australia	WA	10	17	6	0.780	0.478	27	9.13	0.612	0.761	0.220	0.173	21	4491	1.92	0.130	0.166	0.178
Pacific	Philippines/Taiwan	PHTW	19	75	10	0.776	1.419	29	8.86	0.697	0.784	0.125	0.237	21	4421	1.92	0.130	0.165	0.177
	North Australia	NT	54	27	20	0.882	0.233	33	9.13	0.743	0.783	0.048	0.204	28	4812	1.92	0.126	0.165	0.206
	Papua New Guinea	PNG	77	82	33	0.912	0.267	37	9.57	0.746	0.800	0.073	0.416	67	5315	1.93	0.126	0.166	0.228
	Princess Charlotte Bay	PCB	25	24	12	0.838	0.167	29	9.15	0.717	0.783	0.093	0.406	17	4223	1.91	0.123	0.163	0.189
	Townsville	TSV	39	29	25	0.934	0.165	43	8.61	0.686	0.785	0.134	0.241	33	4916	1.92	0.129	0.167	0.202
	New South Wales	NSW	25	23	13	0.774	0.135	30	9.09	0.738	0.788	0.076	0.311	26	4686	1.92	0.126	0.165	0.196
	Fiji	FJ	21	5	7	0.712	0.064	22	9.43	0.770	0.792	0.027	0.383	19	4263	1.92	0.126	0.165	0.192
	Hawaii	HAW	14	5	3	0.439	0.055	28	8.05	0.787	0.758	0.040	0.502	25	4351	1.93	0.127	0.169	0.215
	Gulf of California	GoC	18	2	3	0.537	0.033	27	6.99	0.673	0.709	0.048	0.370	16	3236	1.91	0.116	0.162	0.227
	Total *n*		359					354						310					

The number of individuals successfully amplified per marker and total (n), the observed (HO) and expected heterozygosity (HE), the number of polymorphic sites (S); for SNPs one site equals one locus, number of Haplotypes (H), haplotype diversity (h), nucleotide diversity (π), allelic richness (AR), inbreeding coefficient (FIS) and Hardy-Weinberg significance value (HWEp).

WA, SEY and IN were more separated in cluster space than all other locations (Fig. 4). Based on the STRUCTURE analyses, K = 3 was the most likely number of clusters detected based on the Evanno method (Supplementary Fig. S2). In all plotted scenarios (Fig. 6, K = 2–6) GOC was separated into its own distinct cluster, while almost all the other locations were similarly assigned to other clusters. Genetic and geographic distances were found to correlate when all populations were included in the analysis (Fig. 5). The all location dataset based on microsatellites was found to be significantly correlated with r values of 0.74 ($p = < 0.001$). No significant relationship between genetic and geographic distance could be identified between central Indo–Pacific locations (Fig. 5).

DArTSeq processing returned a total of 53,729 SNP loci for 352 individuals from 12 populations. After additional stringent quality filtering (Supplementary Methods S.1) we identified two datasets containing 5689 and 5969 SNPs (Supplementary Fig. S1, Table. S2). Summary statistics are reported for the total SNP dataset (5689) in Table 1. The number of polymorphic loci per population was similar for most locations, however PNG and GOC had the highest (5315) and lowest (3236) respectively (Table 1). For bi-allelic SNP markers, A_R and H_O did not vary greatly between locations ($A_R = 1.91–1.93$, $H_O = 0.116–0.130$), further heterozygosity (H_O) values were consistently low suggesting a deficit across populations.

Pairwise location comparisons based on SNPs were similar to those observed with microsatellites (global F_{ST} = 0.010 and F_{ST} = 0.011 respectively). Again, the easternmost location, GOC was the most genetically differentiated from all locations and F_{ST} values based on SNPs were comparable (albeit slightly higher) than with microsatellites ($F_{ST} = 0.044–0.072$) (Fig. 3,). Comparisons between *S. lewini* from SEY and HAW to all other locations revealed a lack of gene flow with moderate and significant F_{ST} values (F_{ST} = 0.009–0.023, $p = < 0.001$). Despite somewhat similar trends in F_{ST} values, SNPs and microsatellites differed greatly in the statistical significance of comparisons. The majority of pairwise tests for SNPs were found to be significant ($p = < 0.001$), except for closely located regions in Indonesia, Australia and PNG – (IN, NT, PCB, NSW, TSV and PNG). The central Indo–Pacific SNP set estimated similar pairwise F_{ST}'s as the all location SNP set with the largest difference between F_{ST} values being + /- 0.001 ().

The unsupervised clustering algorithm run using ADMIXTURE software yielded more visibly structured clusters based on geographic location than STRUCTURE plots for the microsatellites (using all location SNP set). CV error value were lowest for clusters K = 1–5 suggesting these clusters has the best predictive accuracy (Supplementary material S.3) (Alexander et al., 2020). Like pairwise F_{ST} tests indicating genetic distinctiveness, SEY, GOC and HAW all belong to clearly defined clusters from K = 3 onwards. This is dissimilar from Microsatellite STRUCTURE output where HAW and SEY did not form distinct clusters. ADMIXTURE plots also show a high level of homogeneity among many of the central Indo–Pacific locations (PHTW, IN, WA, NT, PNG, PCB, TSV, NSW and FJ) sharing assigned clusters. DAPC plots also support the findings of the pairwise F_{ST}'s and ADMIXTURE analyses for SNPs. Clustering is consistent with geographic locations showing SEY, GOC and HAW most clearly separated from a central Indo–Pacific cluster (Fig. 7). Testing exclusively central Indo–Pacific locations (5969 SNPs) we found PHTW, WA and FJ cluster slightly away from other overlapping locations. Contrary to the microsatellite results, both SNP datasets found significant correlations between genetic and geographic distance. Correlation value (*r*) varied between groupings with all locations and central Indo–Pacific locations showing significant r values of 0.67 and 0.73, respectively (Fig. 5).

4. Discussion

Population subdivision identified in our study using three genetic marker types has updated our understanding of *S. lewini* connectivity across the Indo–Pacific. The results presented here clearly show little

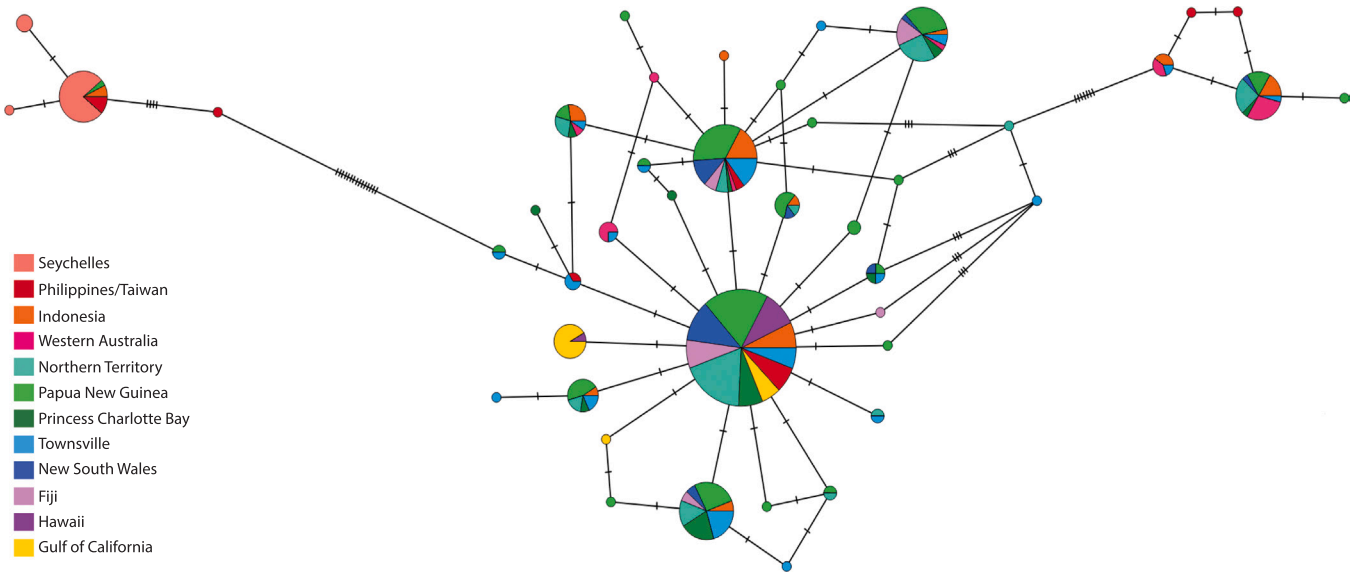


Fig. 2. Mitochondrial DNA (CR and ND4) Median-Joining network analysis from POPart v1.7. *S. lewini* haplotype frequencies are relative to the size of the circles, colours represent sampling locations. Number of strokes joining nodes represents number of mutations between two haplotypes (across the concatenated 1817 bp fragment). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Pairwise genetic differences (Φ_{ST}) calculated from concatenated 1817 bp mitochondrial DNA CR and ND4 sequences for *S. lewini*.

	SEY	PHTW	IN	WA	NT	PNG	PCB	TSV	NSW	FJ	HAW	GOC
SEY	*	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
PHTW	0.764	*	0.140	0.118	**0.002**	**0.001**	**0.009**	**0.001**	**0.008**	**0.002**	0.027	**0.000**
IN	0.877	0.048	*	0.181	0.086	0.039	0.042	0.011	0.041	0.032	0.020	**0.000**
WA	0.951	0.099	0.018	*	0.014	0.003	**0.001**	**0.001**	**0.001**	**0.001**	**0.000**	**0.000**
NT	0.951	0.207	0.025	0.177	*	0.377	0.164	0.169	0.249	0.186	**0.008**	**0.000**
PNG	0.941	0.215	0.037	0.220	0.000	*	0.406	0.777	0.564	0.644	**0.002**	**0.000**
PCB	0.969	0.157	0.045	0.291	0.018	0.002	*	0.740	0.677	0.251	**0.000**	**0.000**
TSV	0.966	0.203	0.054	0.309	0.013	0.000	0.000	*	0.748	0.577	**0.000**	**0.000**
NSW	0.974	0.166	0.045	0.307	0.007	0.000	0.000	0.000	*	0.659	**0.000**	**0.000**
FJ	0.984	0.171	0.057	0.379	0.020	0.000	0.009	0.000	0.000	*	**0.000**	**0.000**
HAW	0.985	0.158	0.111	0.418	0.171	0.133	0.193	0.199	0.209	0.362	*	**0.000**
GOC	0.988	0.265	0.290	0.598	0.498	0.442	0.582	0.593	0.639	0.788	0.805	*

Above diagonal; p values, below diagonal; pairwise ΦST values, significant values ($p = < 0.010$) are in bold.

gene flow is occurring between the westernmost (Seychelles), easternmost (Hawaii and Gulf of California) and central Indo–Pacific locations (Australia, Indonesia, Philippines, Taiwan, Papua New Guinea and Fiji). The implication is that neither sex migrates effectively across the Indian Ocean as mitochondrial haplotypes and biparental genotypes from the Seychelles were distinct from the remainder. The lack of gene flow is most likely driven by the large distances involved, inferring that *S. lewini* do not cross ocean basins. In contrast, the closely located continental shelves of Australia, Papua New Guinea and Indonesia provide well connected habitat enabling dispersive behaviours between the Pacific and eastern Indian Oceans explaining the gene flow patterns across marker types identified in the central Indo–Pacific region.

This study used a number of samples and similar genetic markers from previous work including Duncan et al. (2006a), Daly-Engel et al. (2012) and Ovenden et al. (2009) as well as newly collected samples from Seychelles, Papua New Guinea and Townsville (Australia) and newly developed genomic markers. Overall, our results are broadly equivalent with previous studies, finding structure between ocean basins and connectivity along continental shelves using mitochondrial DNA. Additionally, our findings of ocean basin and Indo–Pacific connectivity identified using microsatellites are similar to descriptions in Daly-Engel et al. (2012) and Ovenden et al. (2009) respectively. The new information in this study, based on the addition of over 5600 SNP markers enabled direct comparisons to previous interpretations of *S. lewini* connectivity across all 12 locations. In contrast with microsatellite results, SNP markers revealed no genetic connectivity across the broader Indian and Pacific ocean basins suggesting initial conclusions of male biased dispersal across Indian and Pacific oceans (Daly-Engel et al., 2012) will need to be updated.

Of great interest is the mitochondrial DNA haplotypes thought only to exist in the Atlantic Ocean, shown here to be apparently 'leaking' into the Indian Ocean. Previously, the divergent haplotype described for individuals from North and South Carolina, Florida, Louisiana and the Ivory Coast led authors to suggest Atlantic *S. lewini* are a distinct divergent lineage (Quattro et al., 2006). However, Duncan et al. (2006b) and Daly-Engel et al. (2012) noted a western Indian Ocean haplotype clustering within the Atlantic lineage. In our current study, when assessing mitochondrial DNA CR haplotypes from individuals sampled in Seychelles, mitochondrial DNA data demonstrated all individuals from the Seychelles were represented by the same 'Atlantic' haplotype over two separate time periods of sampling (2010 and 2017). Additionally, one individual from Indonesia and PNG and three individuals from the Philippines all had similar haplotypes to the 'Atlantic' lineage. Given the evidence suggesting *S. lewini* centre of origin was likely from the Indo–Pacific (Duncan et al., 2006), it is possible the Atlantic haplotype could have originated in the Indo–Pacific and through migration westward past South Africa moved into the Atlantic Ocean. Alternatively, ancestors of the 'Atlantic' lineage migrated westward

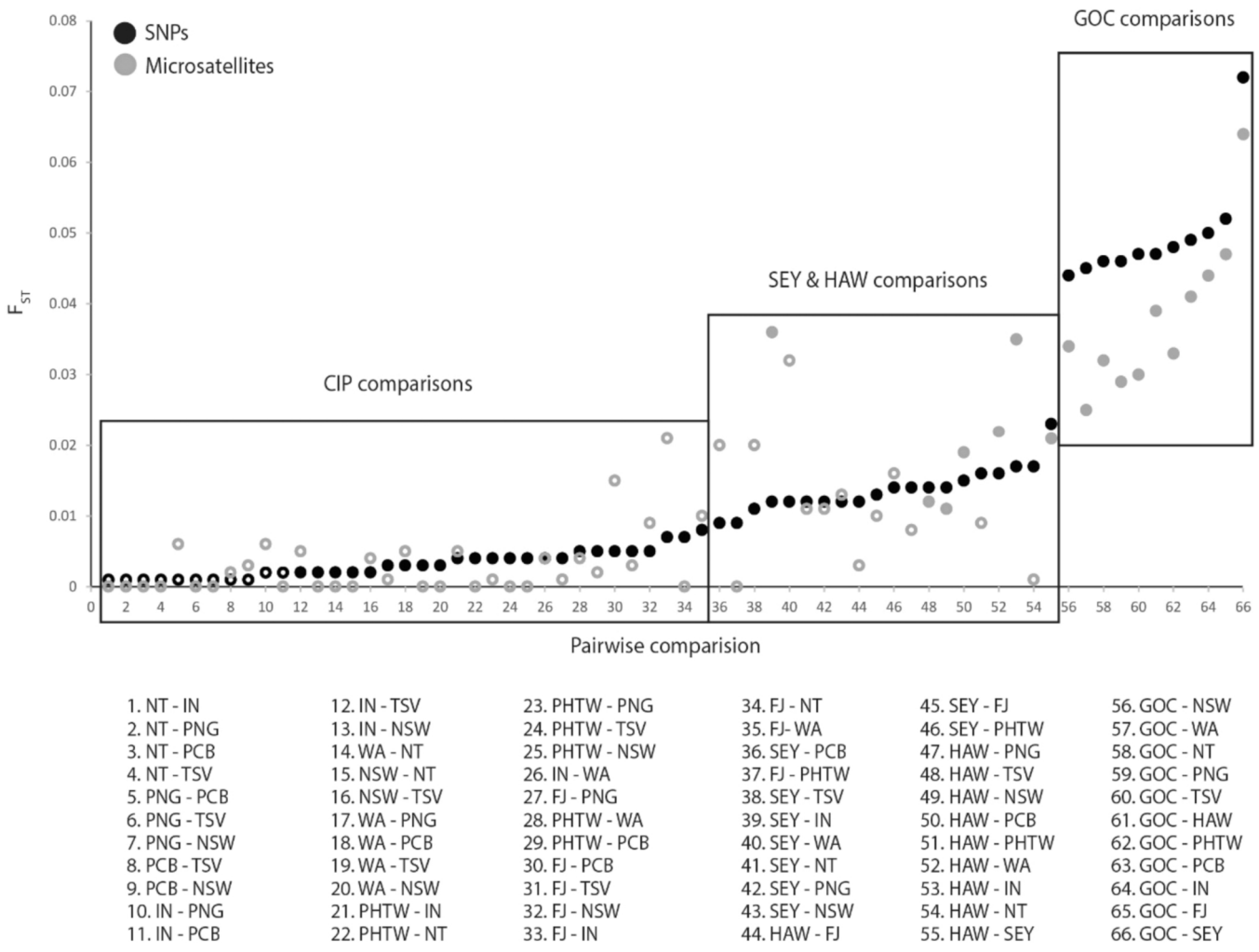


Fig. 3. Estimates of pairwise genetic differentiation (F_{ST}) between all sampled locations for *S. lewini* using SNP (black) and microsatellite (grey) loci. Where CIP = central Indo-Pacific, SEY = Seychelles, HAW = Hawaii and GOC = Gulf of California. Comparisons are arranged in ascending order of SNP F_{ST} values (x-axis). Filled circles indicate significant *p*-values where $p = < 0.001$ and boxes represent pairwise comparisons between grouped locations (note 37 is the only CIP comparison within the SEY & HAW section).

where evolution in isolation occurred followed by possible eastward leakage. It is unknown which direction the 'Atlantic' haplotype came from, however, results presented here suggest the haplotype should be reconsidered as more widespread than initially thought.

4.1. Indo–Pacific population structure

The findings of significant population structure based on SNPs, between the Seychelles and Hawaii with the central Indo–Pacific clearly defined using SNPs adds new knowledge to our understanding of *S. lewini* dispersive behaviour. Until now, microsatellites have only detected population structure between the region of the Gulf of California and other locations used in this study. Previously, Seychelles and Hawaii were found to be connected with central Indo–Pacific locations, suggesting some level of male-mediated connectivity occurs across ocean basins (Daly-Engel et al., 2012). Using SNP markers, our results identified individuals from the Seychelles and Hawaii belonging to distinct clusters corroborated with large and significant pairwise F_{ST}'s (analogous with mitochondrial DNA results presented here). Similarly, SNP DAPC and ADMIXTURE plots clearly define Seychelles and Hawaii as separate and distinct populations from the central Indo–Pacific. Therefore, connectivity of *S. lewini* across the Indo–Pacific may be more restricted than initially proposed.

The number of populations estimated using ADMIXTURE and STRUCTURE for SNPs and microsatellites respectively were not concordant. Differences in the number of loci and therefore informativeness of each marker likely attributed to varied clusters/populations identified (Morin et al., 2004). Bayesian clustering algorithms are reported to be inaccurate in the presence of low levels of genetic differentiation (Latch et al., 2006), therefore many scenarios of K need to be modelled to assess for biological importance. Scenarios of K = 4–6 populations using SNPs appear reasonable, with the Seychelles, Hawaii and Gulf of California all distinct populations and the central Indo–Pacific (9 locations) making up a homogeneously mixed population. Philippines, Taiwan, Western Australia and Fiji begin to differ at K = 6, however this differentiation is only slight. DAPC and pairwise F_{ST}'s support K scenarios identifying Seychelles, Hawaii, Gulf of California, Fiji and some comparisons between Philippines, Taiwan and Western Australia to be genetically distinct. We therefore propose four major genetic stocks across the Pacific and Indian Oceans; 1. West Indian (Seychelles), 2. Central Indo–Pacific (Papua New Guinea, Philippines, Taiwan, Australia, Fiji), 3. Central Pacific (Hawaii), and 4. East Pacific (Gulf of California). We also note structure occurring within the central Indo–Pacific (i.e. Western Australia and Fiji), however this is less clear (discussed below). The genetic stock groupings suggested here are largely ocean basin focused. A number of other shark species have been found to have limited gene flow across ocean basins (using microsatellites), including many of the Carcharhinidae family (Pember et al.,

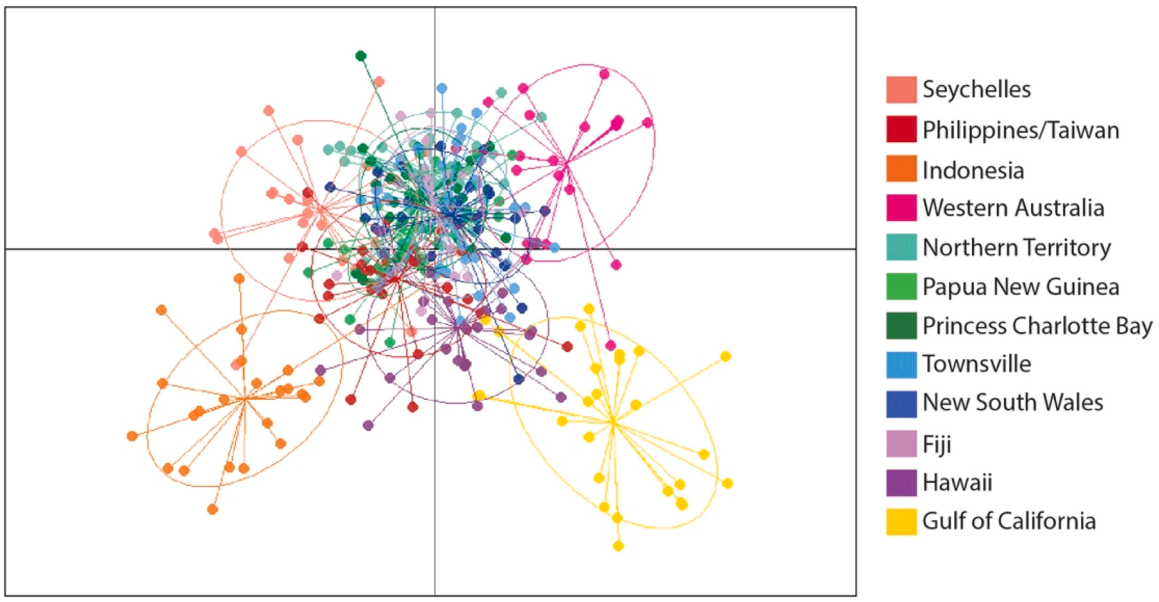


Fig. 4. Scatterplot created using DAPC showing variation between *S. lewini* individuals (dots) and populations (colours) for 9 microsatellite loci. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

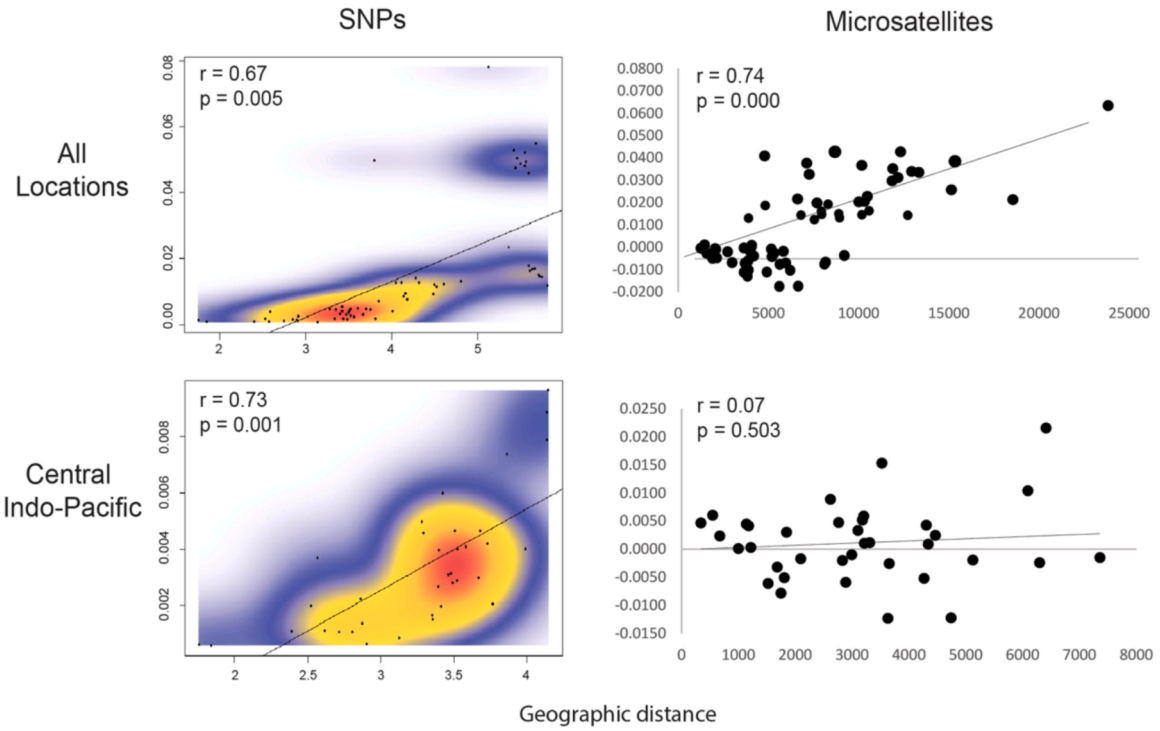


Fig. 5. Isolation by Distance (IBD) plots showing the relationship between genetic distance (y-axis) and geographic distance (x-axis) for SNPs (left) and microsatellites (right) across two population scenarios; all locations (top) and central Indo-Pacific locations (bottom). SNP IBD plots were generated using dartR package (Gruber et al., 2018) where geographic distance is represented as the log of distance in metres.

2020), lemon shark *Negaprion brevirostris* (Schultz et al., 2008), common blacktip shark *C. limbatus* (Keeney and Heist, 2006) and tiger shark *Galeocerdo cuvier* (Bernard et al., 2016). Similar to *S. lewini*, these species rely on coastal and/or reef habitat for food, protection and reproduction. It is possible that the strong reliance on these habitats for critical physiological and ecological functions explains why large scale oceanic movements are rare (Ketchum et al., 2014).

It would be beneficial to link genetic and genomic findings with movement data, similar to that of Corrigan et al. (2018) who combined genetic and telemetry methods to describe connectivity of shortfin mako sharks *Isurus oxyrinchus* across the Indo–Pacific. Much of the available telemetry and mark-recapture data for *S. lewini* have focused on young-of-the-year and juveniles from Hawaii (Clarke, 1971; Duncan and Holland, 2006; Holland et al., 1993; Klimley and Nelson, 1981; Kohler and Turner, 2001; Lowe, 2002) and the South African coast (Diemer et al., 2011). However, recent tagging undertaken in Australia deployed eight tags on adult *S. lewini* (7 males, 1 female) and found tracked individuals did not move greater than 200 km suggesting relatively high

Fig. 6. Average population for *S. lewini* clustering based on ADMIXTURE (SNPs) and STRUCTURE (Microsatellite) outputs for 5689 SNPs (left) and 9 microsatellites (right) respectively. Colours represent different clusters as defined by K values. Each column represents a different location. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

residency (Heupel et al., 2020). Adult movements tracked within the Galapagos Islands found the majority of individuals remained resident at their capture reef (Ketchum et al., 2014). A few large females moved significant distances (700–1200 km) and returned to resident reefs. Parasite analyses for *S. lewini* in Australia, Indonesia and Papua New Guinea also support a high degree of residency similar to that of tagging studies (Heupel et al., 2020). The largest difference in parasite assemblages were found along the east Australian coast and driven by

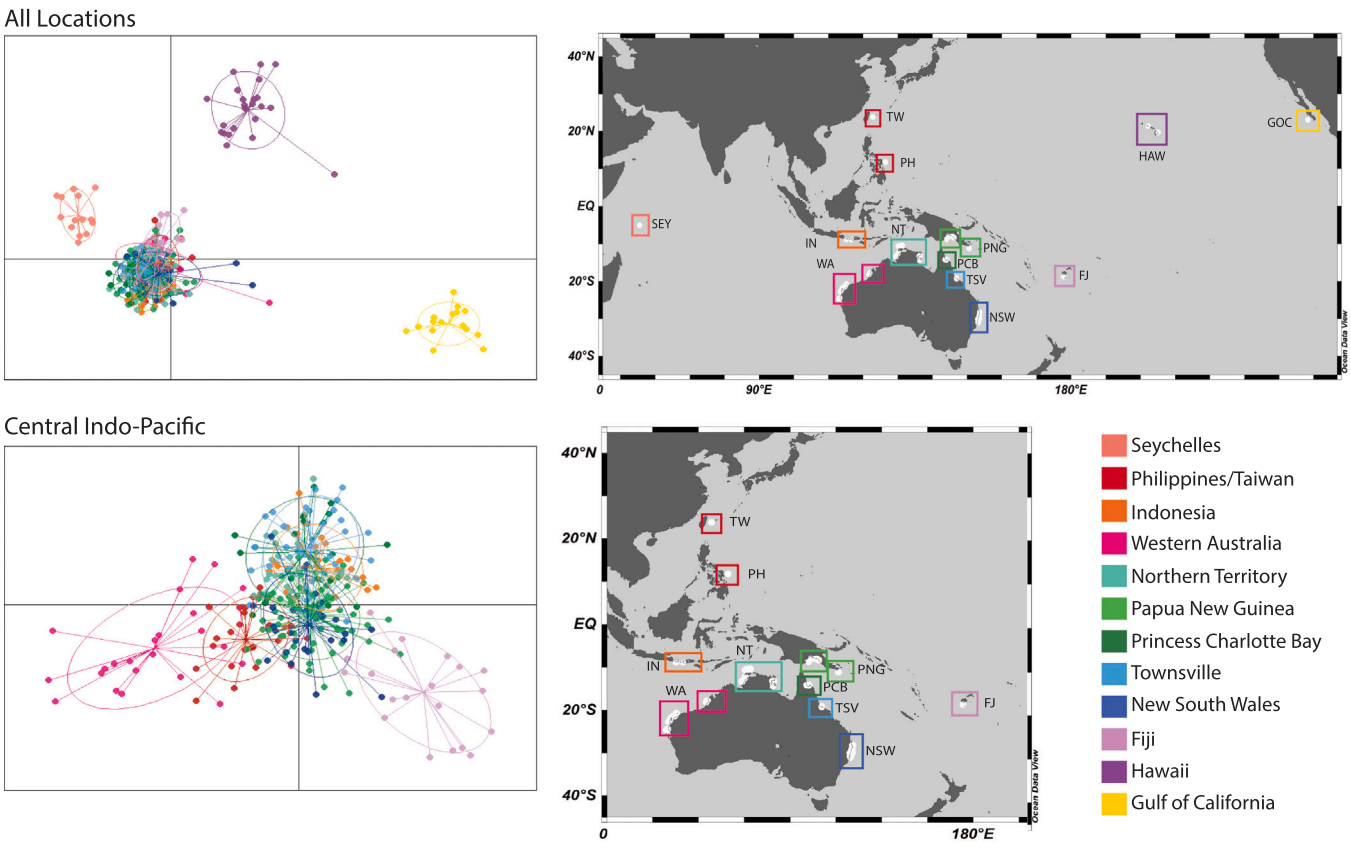


Fig. 7. Scatterplot created using DAPC showing variation between *S. lewini* individuals (dots) and populations (colours) for two SNP datasets with accompanying map of locations. Top- All locations (5689 SNPs), bottom- central Indo-Pacific locations (5969 SNPs). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ontogenetic shifts from juveniles to adults (Heupel et al., 2020). Although these studies have limited sample sizes, the available demographic evidence (i.e. telemetry, parasites) suggests *S. lewini* do not traverse large distances and instead show site attachment and residency. The vast genetic connectivity identified in this study, and other population genetic analyses of *S. lewini,* is therefore likely to represent a small number of individuals migrating and successfully breeding over long periods of time.

4.2. Central Indo–Pacific population structure

The removal of distant locations (Seychelles, Hawaii and Gulf of California) and re-filtering of SNPs enabled our study to generate a central Indo–Pacific dataset. By assessing genetic information of individuals closely located, our study identified 931 informative SNPs exclusive to the central Indo-Pacific region. Findings from the central Indo-Pacific SNP set in combination with microsatellites and mitochondrial DNA have described the genetic connectivity present in a critical region for *S. lewini*. Regional assessment of *S. lewini* in the central Indo–Pacific is important given the data deficient status in Oceania and closely located EEZ's of Indonesia, Papua New Guinea and Australia.

SNPs, microsatellites and mitochondrial DNA identified patchy gene flow across the central Indo–Pacific countries of Australia, Papua New Guinea, Indonesia, Philippines, Taiwan and Fiji. Connectivity across the Indo–Pacific has been noted for *S. lewini* previously (Daly-Engel et al., 2012; Ovenden et al., 2009) as well as a number of other shark species including *G. cuvier* (Holmes et al., 2017), short fin mako *Isurus oxyrinchus* (Corrigan et al., 2018), *C. amblyrhynchos* (Momigliano et al., 2017), *C. albimarginatus* (Green et al., 2019) and *P. glauca* (Ovenden et al., 2009). Within the central Indo–Pacific region, *S. lewini* are continuously distributed (Last and Stevens, 2009) with no perceived contemporary barriers hindering dispersal along continental shelves. Interestingly, SNP markers revealed a subtle level of population structure within the central Indo–Pacific. The majority of SNP pairwise comparisons between central Indo–Pacific locations were significantly different, meaning gene flow between these locations may be limited. Only some of the geographically closest locations including the Northern Territory, east Australian coast, Indonesia and PNG comparisons are not significantly partitioned, likely driving the observed pattern of Isolation by Distance (IBD). Supported by F_{ST}, DAPC and ADMIXTURE plots, our results show Fiji, Western Australia and Philippines/Taiwan to have restricted gene flow with other central Indo–Pacific locations. If we accept that IBD is driving genetic diversity patterns among the central Indo–Pacific, it is likely gene flow across the region is facilitated by stepping-stone migrations as purported for *C. amblyrhynchos* (Momigliano et al., 2017). Reef coverage is continuous between eastern/northern Australia, PNG and Indonesia, however, coral reef habitat in Western Australia is sparser, separated by hundreds of kilometres of unsuitable habitat (Momigliano et al., 2017). The distance and lack of suitable reef structure between Western Australia, Fiji and Philippines/Taiwan and other central Indo–Pacific locations may reduce dispersal ability and therefore gene flow between these locations. In contrast, high gene flow identified between east/north Australia, PNG and Indonesia suggests movement of *S. lewini* between these locations is likely.

4.3. Microsatellite vs. SNP markers for population assignment

Results from nuclear microsatellites and SNPs largely agree, however a few differences were identified. First, the clusters inferred by SNP DAPC are much more distinct than those observed with microsatellites. Second, while global F_{ST} values were similar and pairwise F_{ST}'s followed

similar trends, the pairwise significances were highly varied. Third, the IBD pattern detected among populations in the central Indo–Pacific was much stronger in the SNP data. Forth, assignment of clusters using Bayesian programs ADMIXTURE and STRUCTURE identified different K values and population assignment between K = 1–6 varied, with SNPs more accurately assigning individuals to their geographic locations. A number of studies comparing SNPs and microsatellites have found similar patterns with SNPs more clearly defining DAPC and PCA clusters (Benestan et al., 2015; Jeffries et al., 2016; Malenfant et al., 2015), IBD correlations (Coates et al., 2009; Jeffries et al., 2016) and larger, significant F_{ST} values (Malenfant et al., 2015; Vendrami et al., 2017). These differences can be attributed to SNPs more densely sampling the genome, SNP genotyping identifying a large set of loci, highly differentiated and providing fine-scale resolution and estimations of population structure (Hohenlohe et al., 2018; Xing et al., 2005). The significance of SNP F_{ST} values is likely due to the sheer number of SNP loci lowering residuals and creating more confidence in calculated observations. Similar to many previous studies, here SNPs provided fine-scale discrimination of population structure for a widely distributed marine species where weakly differentiated microsatellites did not.

4.4. Management implications and conclusion

Determining genetic connectivity provides important information for fisheries management. Population structure can be used to define the geographic boundary of a biological stock and underpin management decisions (Ovenden et al., 2009). Here we have defined the biological stock structure of *S. lewini* into four regions: the Western Indian (Seychelles), the central Indo–Pacific (Papua New Guinea, Indonesia, Australia, Philippines, Taiwan and Fiji), Central Pacific (Hawaii) and the tropical East Pacific (Gulf of California). As was done for the central Indo–Pacific, it would be of great interest to estimate intra-regional connectivity of individuals from geographically close locations in the Western Indian (around the Seychelles) and Eastern Pacific (around Gulf of California). Future studies should incorporate samples near regions tested within our study to determine the extent of connectivity within ocean basins.

Within the central Indo–Pacific, only subtle population structure was identified; connectivity appears to be present between east and north Australia, PNG and Indonesia, however, small breaks in gene flow were observed between the west coast of Australia and Fiji. Conceptual models developed to explain patterns of *S. lewini* distribution described four possible models of movement (see Chin et al., 2017). Based on the findings of our current study, we assume Model 1 and Model 2, suggesting panmixia and limited movement respectively are unlikely. Instead, our results support Model 3; continental shelf movement enabling connectivity between Australia, PNG and eastern Indonesia, but not with the Pacific Islands (i.e. Fiji). In addition to Model 3, the results presented here suggest connectivity to Western Australia appears to be limited. Ultimately, adopting management at the spatial scales and boundaries identified in this study for the central Indo–Pacific region will necessitate international and national cooperation.

A key objective of this study was to assess whether differences in genetic patterns could be observed using a multi-marker approach. The work presented here indicates differences between microsatellites and SNPs do occur, with SNPs identifying more discrete population subdivision than microsatellites. The ability of genomic techniques to capture a large subset of highly differentiated markers provides a robust approach to identify population structure (Hohenlohe et al., 2018). Our results suggest increased sampling regimes and an increased number of microsatellite loci are required if choosing to undertake population structure analyses exclusively with genetic markers. Therefore, undertaking a genomic approach using SNPs may be more suited for shark and ray population structure studies given the challenges faced (expense and accessibility) when obtaining adequate sample sizes. Future studies assessing population connectivity using alternative demographic methods (i.e. telemetry, parasites) should be undertaken alongside genomic approaches to estimate the level of demographic connectivity between regions with varying levels of fishing pressure and capacity for management.

Ethics statement

Samples collected from the following locations were taken from sharks harvested as part of commercial fishing activity and therefore do not require permits or ethics approval; Seychelles, Philippines, Taiwan, Indonesia, Australia, Papua New Guinea, Fiji and Gulf of California. Samples collected in Hawaii were collected under Permit #2008–99 issued by the State of Hawaii's Division of Aquatic Resources to the Hawaii Institute of Marine Biology.

Declaration of Funding

This project was funded by the Australian Centre for International Agricultural Research (ACIAR; Project FIS/2012/102), National Fisheries Authority Papua New Guinea (NFA), the Australian National Environmental Science Program (NESP), the University of Tasmania (UTAS) and the Holsworth Wildlife Research Endowment (Grant number HWRE2016R2050) . M. E. Green was supported by the CSIRO–University of Tasmania Ph.D. Program in Quantitative Marine Science Elite Scholarship.

Lay summary

Samples were collected from twelve locations across the Indo-Pacific to provide an updated assessment of the genetic connectivity for the critically endangered *S. lewini*. Three different genetic methods were used to better understand how to integrate new and old genetic technologies for population assignment. Results suggest large oceanic barriers separate populations, while continuous continental shelves facilitate connectivity.

CRediT authorship contribution statement

M.E. Green: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **S.A. Appleyard:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **W. White:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **S. Tracey:** Funding acquisition, Resources, Supervision, Writing – review & editing. **M.R. Heupel:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **J.R. Ovenden:** Conceptualization, Data curation, Methodology, Resources, Supervision, Writing – review & editing.

Data Availability

All raw genetic data has been submitted to the CSIRO Data Access Portal and can be accessed here https://data.csiro.au/collections/collection/CIcsiro:52099v1.

Acknowledgements

Special thanks to Chris Barlow and Jes Sammut for their support of this project. The authors thank Brian Kumasi, Luanah Yaman, Leban Gisawa and Ludwig Kumoru from the National Fishing Authority for their continued support and collaboration on this project. The authors also thank the fishers of the longline vessels, with a special thanks to the NFA on-board fisheries observers who collected the data and tissues samples: Jackson Mara-vee, Noah Lurang Jr, Daniel Sau, Murphy John,

Paliau Parkop, Towai Peli and Udill Jotham. A special thanks to Toby Daly-Engel and Brian Bowen who provided samples from various other locations. The authors also thank Jon Neville at Environment Seychelles for providing samples from the Seychelles. The authors thank Mario Espinoza for Australian samples that were collected during a project funded by the Australian Government's National Environmental Research Program (Tropical Ecosystems Hub Project 6.1). Lastly, we thank the anonymous reviewers who took time out of their schedules to provide helpful and constructive feedback.

Conflict of Interest

There are no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2022.106305.

References

Alexander, D.H., Lange, K., 2011. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinforma. 12. https://doi.org/10.1186/1471-2105-12-246.

Alexander, D.H., Shringarpure, S.S., Novembre, D.J., Lange, K., 2020. ADMIXTURE 1.3 Software Manual.

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Barker, M.J., Schluessel, V., 2005. Managing global shark fisheries: suggestions for prioritizing management strategies. Aquat. Conserv. Mar. Freshw. Ecosyst. 15, 325–347. https://doi.org/10.1002/aqc.660.

Baum, J.K., Clarke, S., Domingo, A., Ducrocq, M., Lamónaca, A.F., Gaibor, N., Graham, R., Jorgensen, S., Kotas, J., Medina, E., Martinez-Ortiz, J., Monzini, D., Accone, J., Morales, M.R., Navarro, S.S., Pérez-Jiménez, J.C., Ruiz, C., Smith, W., Valenti, S.V., Vooren, C.M., 2007. Sphyrna lewini [WWW Document]. IUCN Red List Threat. Species.

Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., Bernatchez, L., 2015. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (Homarus americanus). Mol. Ecol. 24, 3299–3315. https://doi.org/10.1111/mec.13245.

Bernard, A.M., Feldheim, K.A., Heithaus, M.R., Wintner, S.P., Wetherbee, B.M., Shivji, M. S., 2016. Global population genetic dynamics of a highly migratory, apex predator shark. Mol. Ecol. 25, 5312–5329. https://doi.org/10.1111/mec.13845.

Bessudo, S., Soler, G.A., Klimley, A.P., Ketchum, J.T., Hearn, A., Arauz, R., 2011. Residency of the scalloped hammerhead shark (Sphyrna lewini) at Malpelo Island and evidence of migration to other islands in the Eastern Tropical Pacific. Environ. Biol. Fish. 91, 165–176. https://doi.org/10.1007/s10641-011-9769-3.

Brown, K.T., Seeto, J., Lal, M.M., Miller, C.E., 2016. Discovery of an important aggregation area for endangered scalloped hammerhead sharks, Sphyrna lewini, in the Rewa River estuary, Fiji Islands. Pac. Conserv. Biol. 22, 242–248. https://doi.org/10.1071/PC14930.

Cardeñosa, D., Fields, A.T., Babcock, E.A., Shea, S.K.H., Feldheim, K.A., Chapman, D.D., 2020. Species composition of the largest shark fin retail-market in mainland China. Sci. Rep. 10, 1–10. https://doi.org/10.1038/s41598-020-69555-1.

Chin, A., Simpfendorfer, C.A., White, W.T., Johnson, G.J., McAuley, R.B., Heupel, M.R., 2017. Crossing lines: a multidisciplinary framework for assessing connectivity of hammerhead sharks across jurisdictional boundaries. Sci. Rep. 7, 1–14. https://doi.org/10.1038/srep46061.

Clarke, S.C., McAllister, M.K., Milner-Gulland, E.J., Kirkwood, G.P., Michielsens, C.G.J., Agnew, D.J., Pikitch, E.K., Nakano, H., Shivji, M.S., 2006. Global estimates of shark catches using trade records from commercial markets. Ecol. Lett. 9, 1115–1126. https://doi.org/10.1111/j.1461-0248.2006.00968.x.

Clarke, T.A., 1971. The ecology of the Scalloped Hammerhead Shark. Pac. Sci. 25, 133–144.

Coates, B.S., Sumerford, D.V., Miller, N.J., Kim, K.S., Sappington, T.W., Siegfried, B.D., Lewis, L.C., 2009. Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis. J. Hered. 100, 556–564. https://doi.org/10.1093/jhered/esp028.

Compagno, L.J.V., Dando, M., Fowler, S.L., 2005. Sharks of the World. Princeton University Press.

Corrigan, S., Lowther, A.D., Beheregaray, L.B., Bruce, B.D., Cliff, G., Duffy, C.A., Foulis, A., Francis, M.P., Goldsworthy, S.D., Hyde, J.R., Jabado, R.W., Kacev, D., Marshall, L., Mucientes, G.R., Naylor, G.J.P., Pepperell, J.G., Queiroz, N., White, W. T., Wintner, S.P., Rogers, P.J., 2018. Population connectivity of the highly Migratory Shortfin Mako (Isurus oxyrinchus Rafinesque 1810) and implications for management in the Southern Hemisphere. Front. Ecol. Evol. 6, 1–15. https://doi.org/10.3389/fevo.2018.00187.

Cowen, R.K., Paris, C.B., Srinivasan, A., 2006. Scaling of connectivity in marine populations. Science 311, 522–527.

Daly-Engel, T.S., Seraphin, K.D., Holland, K.N., Coffey, J.P., Nance, H.A., Toonen, R.J., Bowen, B.W., 2012. Global phylogeography with mixed-marker analysis reveals male-mediated dispersal in the endangered scalloped hammerhead shark (Sphyrna lewini). PLoS One 7, 1–11. https://doi.org/10.1371/journal.pone.0029986.

Devloo-Delva, F., Maes S., H.I., G.E., McAllister, J.D., Gunasekera, R.M., Grewe, P.M., Thomson, R.B., Feutry, P., 2019. Accounting for kin sampling reveals genetic connectivity in Tasmanian and New Zealand school sharks, Galeorhinus galeus. Ecol. Evol. 1–9. https://doi.org/10.1002/ece3.5012.

Díaz-Jaimes, P., Bayona-Vásquez, N.J., Escatel-Luna, E., Uribe-Alcocer, M., Pecoraro, C., Adams, D.H., Frazier, B.S., Glenn, T.C., Babbucci, M., 2021. Population genetic divergence of bonnethead sharks Sphyrna tiburo in the western North Atlantic: implications for conservation. Aquat. Conserv. Mar. Freshw. Ecosyst. 31, 83–98. https://doi.org/10.1002/aqc.3434.

Diemer, K., Mann, B., Hussey, N., 2011. Distribution and movement of scalloped hammerhead Sphryna lewini and smooth hammerhead Sphyrna zygaena sharks along the east coast of southern Africa. Afr. J. Mar. Sci. 33, 229–238. https://doi.org/10.2989/1814232X.2011.600291.

Dudley, S.F.J., Simpfendorfer, C.A., 2006. Population status of 14 shark species caught in the protective gillnets off KwaZulu–Natal beaches, South Africa, 1978–2003. Mar. Freshw. Res. 57, 225. https://doi.org/10.1071/MF05156.

Duncan, K., Holland, K., 2006. Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks Sphyrna lewini in a nursery habitat. Mar. Ecol. Prog. Ser. 312, 211–221. https://doi.org/10.3354/meps312211.

Duncan, K.M., Martin, A.P., Bowen, B.W., De Couet, H.G., 2006. Global phylogeography of the scalloped hammerhead shark (Sphyrna lewini). Mol. Ecol. 15, 2239–2251. https://doi.org/10.1111/j.1365-294X.2006.02933.x.

Earl, D.A., VanHoldt, B.M., 2012. Structure harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361. https://doi.org/10.1007/s12686-011-9548-7.

Estupiñán-Montaño, C., Galván-Magaña, F., Elorriaga-Verplancken, F., Zetina-Rejón, M., Sánchez-González, A., Polo-Silva, C., Villalobos-Ramírez, D., Rojas-Cundumí, J., Delgado-Huertas, A., 2021. Ontogenetic feeding ecology of the scalloped hammerhead shark Sphyrna lewini in the Colombian Eastern Tropical Pacific. Mar. Ecol. Prog. Ser. 663, 127–143. https://doi.org/10.3354/meps13639.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Excoffier, L., Heckel, G., 2006. Computer programs for population genetics data analysis: a survival guide. Nat. Rev. Genet. 7, 745–758. https://doi.org/10.1038/nrg1904.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Green, M.E., Appleyard, S.A., White, W., Tracey, S., Ovenden, J., 2017. Variability in multiple paternity rates for grey reef sharks (Carcharhinus amblyrhynchos) and scalloped hammerheads (Sphyrna lewini). Sci. Rep. 7, 1–8. https://doi.org/10.1038/s41598-017-01416-w.

Green, M.E., Appleyard, S.A.A., White, W.T., Tracey, S., Devloo-Delva, F., Ovenden, J.R., 2019. Novel multimarker comparisons address the genetic population structure of silvertip sharks (Carcharhinus albimarginatus). Mar. Freshw. Res. 70, 1007–1019. https://doi.org/10.1071/MF18296.

Gruber, B., Unmack, P.J., Berry, O.F., Georges, A., 2018. dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Mol. Ecol. Resour. 18, 691–699. https://doi.org/10.1111/1755-0998.12745.

Hadi, S., Andayani, N., Muttaqin, E., Simeon, B.M., Ichsan, M., Subhan, B., Madduppa, H., 2020. Genetic connectivity of the scalloped hammerhead shark Sphyrna lewini across Indonesia and the Western Indian Ocean. PLoS One 15. https://doi.org/10.1371/journal.pone.0230763.

Hearn, A., Ketchum, J., Klimley, A.P., Espinoza, E., Peñaherrera, C., 2010. Hotspots within hotspots? Hammerhead shark movements around Wolf Island, Galapagos Marine Reserve. Mar. Biol. 157, 1899–1915. https://doi.org/10.1007/s00227-010-1460-2.

Heupel, M., Simpfendorfer, C., Chin, A., Appleyard, S., Barton, D., Green, M., Johnson, G., McAuley, R., White, W., 2020. Examination of connectivity of hammerhead sharks in northern Australia, National Environmental Science Programme.

Heupel, M.R., Carlson, J.K., Simpfendorfer, C.A., 2007. Shark nursery areas: concepts, definition, characterization and assumptions. Mar. Ecol. Prog. Ser. 337, 287–297.

Heupel, M.R., Kanno, B., C., S., Martins, B., A.P.B., Simpfendorfer, C.A., 2018. Advances in understanding the roles and benefits of nursery areas for elasmobranch populations. Mar. Freshw. Res. https://doi.org/10.1071/MF18081.

Hohenlohe, P.A., Hand, B.K., Andrews, K.R., Luikart, G., 2018. Population genomics provides key insights in ecology and evolution. Popul. Genom. Concepts Approaches Appl. 1–28. https://doi.org/10.1007/13836_2018_20.

Holland, K.N., Wetherbee, B.M., Peterson, J.D., Lowe, C.G., 1993. Movements and distribution of Hammerhead Shark Pups on their natal grounds. Copeia 1993, 495–502. https://doi.org/10.2307/1447150.

Holmes, B.J., Williams, S.M., Otway, N.M., Nielsen, E.E., Maher, S.L., Bennett, M.B., Ovenden, J.R., 2017. Population structure and connectivity of tiger sharks (Galeocerdo cuvier) across the Indo-Pacific Ocean basin. R. Soc. Open Sci. 4, 1–9. https://doi.org/10.1098/rsos.170309.

Jaccoud, D., 2001. Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res. 29. https://doi.org/10.1093/nar/29.4.e25.

Jeffries, D.L., Copp, G.H., Lawson Handley, L., Olsén, K.H., Sayer, C.D., Hänfling, B., 2016. Comparing RADseq and microsatellites to infer complex phylogeographic patterns, an empirical perspective in the Crucian carp, Carassius carassius. L. Mol. Ecol. 25, 2997–3018. https://doi.org/10.1111/mec.13613.

Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24 (11), 1403–1405.
Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11. https://doi.org/10.1186/1471-2156-11-94.
Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., Prodöhl, P.A., 2013. diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol. Evol. 4, 782–788. https://doi.org/10.1111/2041-210X.12067.
Keeney, D.B., Heist, E.J., 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. https://doi.org/10.1111/j.1365-294X.2006.03036.x.
Ketchum, J.T., Hearn, A., Klimley, A.P., Peñaherrera, C., Espinoza, E., Bessudo, S., Soler, G., Arauz, R., 2014. Inter-island movements of scalloped hammerhead sharks (Sphyrna lewini) and seasonal connectivity in a marine protected area of the eastern tropical Pacific. Mar. Biol. 161, 939–951. https://doi.org/10.1007/s00227-014-2393-y.
Klimley, P., Nelson, D., 1981. Schooling of the scalloped hammerhead, Sphyrna lewini, in the Gulf of California. Fish. Bull. 79, 356–360.
Kohler, N.E., Turner, P.A., 2001. Shark tagging: A review of conventional methods and studies. Environ. Biol. Fishes 60, 191–223. https://doi.org/10.1023/A:1007679303082.
Last, P.R., Stevens, J.D., 2009. Sharks and Rays of Australia, Second ed.,. CSIRO Publishing.
Latch, E.K., Dharmarajan, G., Glaubitz, J.C., Rhodes, O.E., 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conserv. Genet. 7, 295–302. https://doi.org/10.1007/s10592-005-9098-1.
López, N.A., McAuley, R.B., Meeuwig, J.J., 2022. Identification of the southernmost aggregation of scalloped hammerhead sharks (Sphyrna lewini) in Australia. Austral Ecol. 1–6. https://doi.org/10.1111/aec.13149.
Lowe, C.G., 2002. Bioenergetics of free-ranging juvenile scalloped hammerhead sharks (Sphyrna lewini) in Kāne'ohe Bay, Ō'ahu, HI. J. Exp. Mar. Biol. Ecol. 278, 141–156. https://doi.org/10.1016/S0022-0981(02)00331-3.
Malenfant, R.M., Coltman, D.W., Davis, C.S., 2015. Design of a 9K illumina BeadChip for polar bears (Ursus maritimus) from RAD and transcriptome sequencing. Mol. Ecol. Resour. 15, 587–600. https://doi.org/10.1111/1755-0998.12327.
Mantel, N., 1967. The detection of disease clustering and generalized regression approach. Cancer Res. 27, 209–220. https://doi.org/10.1136/bmj.2.5524.1260-d.
Marie, A.D., Miller, C., Cawich, C., Piovano, S., Rico, C., 2017. Fisheries-independent surveys identify critical habitats for young scalloped hammerhead sharks (Sphyrna lewini) in the Rewa Delta, Fiji. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-17152-0.
Marie, A.D., Stockwell, B.L., Rico, C., 2019. DNA analysis of Juvenile Scalloped Hammerhead Sharks Sphyrna lewini (Griffith, 1834) reveals multiple breeding populations and signs of adaptive divergence in the South Pacific. Front. Mar. Sci. 6. https://doi.org/10.3389/fmars.2019.00718.
Momigliano, P., Harcourt, R., Robbins, W.D., Jaiteh, V., Mahardika, G.N., Sembiring, A., Stow, A., 2017. Genetic structure and signatures of selection in grey reef sharks (Carcharhinus amblyrhynchos). Heredity 1–12. https://doi.org/10.1038/hdy.2017.21.
Morin, P.A., Luikart, G., Wayne, R.K., 2004. SNPs in ecology, evolution and conservation. Trends Ecol. Evol. 19, 208–216. https://doi.org/10.1016/j.tree.2004.01.009.
Nance, H.A., Daly-Engel, T.S., Marko, P.B., 2009. New microsatellite loci for the endangered scalloped hammerhead shark, Sphyrna lewini. Mol. Ecol. Resour. 9, 955–957. https://doi.org/10.1111/j.1755-0998.2008.02510.x.
Naylor, G.J.P., Ryburn, J.A., Fedrigo, O., Lopez, A., 2005. Phylogenetic relationships among the major lineages of modern elasmobranchs. In: Reproductive Biology and Phylogeny of Chondrichthyes. Department of Ecology, Evolution and Organismal Biology. Iowa State University, Ames Iowa, p. 25.
Ovenden, J.R., Berry, O., Welch, D.J., Buckworth, R.C., Dichmont, C.M., 2015. Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. Fish Fish. 16, 125–159. https://doi.org/10.1111/faf.12052.
Ovenden, J.R., Kashiwagi, T., Broderick, D., Giles, J., Salini, J., 2009. The extent of population genetic subdivision differs among four co-distributed shark species in the Indo-Australian archipelago. BMC Evol. Biol. 9, 40. https://doi.org/10.1186/1471-2148-9-40.
Pardini, A.T., Jones, C.S., Noble, L.R., Kreiser, B., Malcolm, H., Bruce, B.D., Stevens, J.D., Cliff, G., Scholl, M.C., Francis, M., Duffy, C.A., Martin, A.P., 2001. Sex-biased dispersal of great white sharks. Nature 412, 139–140. https://doi.org/10.1038/35084125.
Pazmiño, D.A., Maes, G.E., Green, M.E., Simpfendorfer, C.A., Hoyos-Padilla, E.M., Duffy, C.J.A., Meyer, C.G., Kerwath, S.E., Salinas-De-León, P., Van Herwerden, L., 2018. Strong trans-Pacific break and local conservation units in the Galapagos shark (Carcharhinus galapagensis) revealed by genome-wide cytonuclear markers. Heredity 120, 407–421. https://doi.org/10.1038/s41437-017-0025-2.
Pember, B.M., Chaplin, J.A., Loneragan, N.R., Braccini, M., 2020. Population genetic structure of Indo-West Pacific carcharhinid sharks: What do we know and where to from here? Pac. Conserv. Biol. 26, 319–337. https://doi.org/10.1071/PC19046.
Pembleton, L.W., Cogan, N.O.I., Forster, J.W., 2013. StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Mol. Ecol. Resour. 13, 946–952. https://doi.org/10.1111/1755-0998.12129.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Quattro, J.M., Driggers, W.B.I., Grady, J.M., Ulrich, G.F., Roberts, M.A., 2013. Sphyrna gilberti sp. nov., a new hammerhead shark (Carcharhiniformes, Sphyrnidae) from the western Atlantic Ocean. Zootaxa 3702, 159–178.
Quattro, J.M., Stoner, D.S., Driggers, W.B., Anderson, C.A., Priede, K.A., Hoppmann, E. C., Campbell, N.H., Duncan, K.M., Grady, J.M., 2006. Genetic evidence of cryptic speciation within hammerhead sharks (Genus Sphyrna). Mar. Biol. 148, 1143–1155. https://doi.org/10.1007/s00227-005-0151-x.
Raymond, M., Rousset, F., 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.
Reiss, H., Hoarau, G., Dickey-Collas, M., Wolff, W.J., 2009. Genetic population structure of marine fish: Mismatch between biological and fisheries management units. Fish Fish. 10, 361–395. https://doi.org/10.1111/j.1467-2979.2008.00324.x.
Rigby, C.L., Dulvy, N.K., Barreto, R., Carlson, J., Fernando, D., Fordham, S., Francis, M. P., Herman, K., Jabado, R.W., Liu, K.M., Marshall, A., Pacoureau, N., Romanov, E., Sherley, R.B., Winker, H., 2019. Sphyrna lewini. https://doi.org/e.T39385A2918526.
Roberson, L., Boussarie, G., Wilcox, C., Kark, S., Kaschner, K., Garilao, C., Rousseau, Y., Dugan, E., Gonzalez, K., Vallentyne, D., Green, M.E., Watson, J.E.M., Klein, C.J., Kiszka, J.J., n.d. Spatially-explicit risk assessment of interactions between marine megafauna and tuna fisheries in the Indian Ocean. Press.
Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D., Kilian, A., 2011. Diversity arrays technology (DArT) and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus. BMC Proc. 5, 1–2. https://doi.org/10.1186/1753-6561-5-S7-P54.
Schultz, J.K., Feldheim, K. a, Gruber, S.H., Ashley, M.V., McGovern, T.M., Bowen, B.W., 2008. Global phylogeography and seascape genetics of the lemon sharks (genus Negaprion). Mol. Ecol. 17, 5336–5348. https://doi.org/10.1111/j.1365-294X.2008.04000.x.
Simpfendorfer, C.A., Milward, N.E., 1993. Utilisation of a tropical bay as a nursery area by sharks of the families Carcharhinidae and Sphyrnidae. Environ. Biol. Fish. 37, 337–345. https://doi.org/10.1007/BF00005200.
Tamura, K., Peterson, D., Perterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 10, 2731–2739. https://doi.org/10.1093/molbev/msr121.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.
Vaudo, J.J., Byrne, M.E., Wetherbee, B.M., Harvey, G.M., Shivji, M.S., 2017. Long-term satellite tracking reveals region-specific movements of a large pelagic predator, the shortfin mako shark, in the western North Atlantic Ocean. J. Appl. Ecol. 54, 1765–1775. https://doi.org/10.1111/1365-2664.12852.
Vendrami, D.L.J., Telesca, L., Weigand, H., Weiss, M., Fawcett, K., Lehman, K., Clark, M. S., Leese, F., McMinn, C., Moore, H., Hoffman, J.I., 2017. RAD sequencing resolves fine-scale population structure in a benthic invertebrate: implications for understanding phenotypic plasticity. R. Soc. Open Sci. 4, 1–16. https://doi.org/10.1098/rsos.160548.
Ward, R.D., 2000. Genetics in fisheries management. Hydrobiologia 420, 191–201. https://doi.org/10.1023/A:1003928327503.
Wargat, B., 2021. Characterization of a scalloped hammerhead (Sphyrna lewini) nursery habitat in portions of the Atlantic Intracoastal Waterway. University of North Florida.
White, W.T., Bartron, C., Potter, I.C., 2008. Catch composition and reproductive biology of Sphyrna lewini (Griffith & Smith) (Carcharhiniformes, Sphyrnidae) in Indonesian waters. J. Fish. Biol. 72, 1675–1689. https://doi.org/10.1111/j.1095-8649.2008.01843.x.
Xing, C., Schumacher, F.R., Xing, G., Lu, Q., Wang, T., Elston, R.C., 2005. Comparison of microsatellites, single-nucleotide polymorphisms (SNPs) and composite markers derived from SNPs in linkage analysis. BMC Genet. 6, 1–5. https://doi.org/10.1186/1471-2156-6-S1-S29.
Yates, P.M., Heupel, M.R., Tobin, A.J., Moore, S.K., Simpfendorfer, C.A., 2015. Diversity in immature-shark communities along a tropical coastline. Mar. Freshw. Res. 66, 399–410. https://doi.org/10.1071/MF14033.