Identifying population connectivity of shark bycatch species in Northern Territory waters

Sam Banks[1], Amy Kirke[1,3], Fernanda Alves[1,4], Grant Johnson[2,5] and David Crook[1,6]

[1] Research Institute for the Environment and Livelihoods, Faculty of Science and Technology, Charles Darwin University, Northern Territory, Australia

[2] Department of Industry, Tourism and Trade, Fisheries Division, Northern Territory, Australia

[3] Current address: Department of Industry, Tourism and Trade, Fisheries Division, Northern Territory, Australia

[4] Current address: Australian National University, Fenner School of Environment and Society, Canberra, Australian Capital Territory, Australia

[5] Current address: Australian Institute of Marine Science, Arafura Timor Research Facility, Northern Territory, Australia

[6] Current address: Department of Primary Industries and Regional Development, Narrandera Fisheries Centre, Narrandera, NSW, Australia,

October 2024

FRDC Project No **2020-036**

ISBN **978-1-922684-78-3 (Print), 978-1-922684-79-0 (Web)**

Identifying population connectivity of shark bycatch species in Northern Territory waters

2020-036

2024

Researcher contact details

Sam Banks

Research Institute for the Environment and Livelihoods, College of Engineering, IT and the Environment, Charles Darwin University, NT, 0909

08 8946 7166

sam.banks@cdu.edu.au

FRDC contact details

25 Geils Court, Deakin ACT 2600

02 6122 2100

frdc@frdc.com.au

frdc.com.au

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

Tables

Figures

Acknowledgments

Dr Rick Buckworth, Dr Thor Saunders and Dr Vinay Udyawer contributed to the conceptualisation and development of this research project. Dr Will White and Dr Alistair Harry contributed samples of both species from outside the Northern Territory. Australia Bay Seafoods kindly provided samples for this study for the Northern Territory. Charles Darwin University and the Northern Territory Department of Industry, Tourism and Trade provided in-kind support in terms of research staff time and research facilities.

Abbreviations and acronyms

BIC	Bayesian Information Criterion
DAPC	Discriminant analysis of principal components (an analysis of genetic information to detect the presence of discrete populations)
DITT	Northern Territory Department of Industry, Tourism and Trade
EEZ	Exclusive Economic Zone
F_{ST}	A measure of genetic differentiation among locations typically used to identify the presence of discrete 'structuring' of population units
H_O, H_E, H_S, H_T	Categories of heterozygosity, which is a measure of genetic variation at a point ('locus') in the genome. Heterozygosity relates to whether an individual has a different allele at its maternal and paternal copies of a locus. H_O is the observed heterozygosity and H_E is the expected level of heterozygosity given allele frequencies in a population. H_S refers to expected heterozygosity within local sub-populations and H_T refers to expected heterozygosity calculated across all samples in the study.
HWE	Hardy–Weinberg equilibrium
LD	Linkage disequilibrium
NT	Northern Territory
PCoA	Principal Coordinate Analysis
PNG	Papua New Guinea
SNP	Single-nucleotide polymorphism
WA	Western Australia

Executive summary

Charles Darwin University and the Northern Territory (NT) Department of Industry, Tourism and Trade (DITT) Fisheries Division used genetic data to investigate the population structure of two small tropical shark species (Milk Shark [*Rhizoprionodon acutus*] and Australian Blackspot Shark [*Carcharhinus coatesi*]), which are caught as bycatch from commercial fisheries in the NT.

The aim of this study was to gain information on the genetic stock structure to inform the future management of these two species in the NT. This project was conducted in parallel with a PhD project investigating the biology and ecology of both species for applications to fisheries management. There is motivation by the NT Government to develop these two shark species into a commercial product. This project used genetic analysis to understand the patterns of connectivity of populations of these two shark species in NT waters and adjacent regions, including northern Western Australia and Papua New Guinea.

Background

These two shark species that are captured as bycatch in the NT Demersal Fishery have the potential to be developed into a byproduct to add value to that fishery. A sustainable commercial harvest of these two species could greatly reduce the waste from fisheries, where they are currently abundant and caught in relatively large numbers. We address current knowledge gaps in biological information about populations of *R. acutus* and *C. coatesi* to inform the potential development of a byproduct fishery for these two species in the NT.

Aims

Our research aimed to:

- identify the genetic population structure for *R. acutus* and *C. coatesi* in NT waters
- develop capacity for genetic research and monitoring of shark species in the NT
- provide baseline information on genetic structure to inform potential genetic monitoring of these species, including initial estimates of effective population size.

Methods

We used single-nucleotide polymorphism genetic analyses to measure genetic structure among *R. acutus* and *C. coatesi* samples obtained from commercial trawl fishing in NT waters between May 2018 and November 2019. Our aim was to determine whether the two species each occur as a single population in NT waters or as a set of discrete populations that may warrant separate monitoring and management. We also analysed samples of these species from Western Australia and Papua New Guinea to provide broader context for the degree of genetic differentiation among the samples from different regions in the NT.

Our secondary aim was to provide a baseline for deciding whether genetic estimates of effective population size could be used to monitor trends in abundance of these species, and whether samples

from across the NT could be combined for the genetic estimation of effective population size for this purpose.

Results

Genetic data from *R. acutus* and *C. coatesi* strongly suggest that each species exists as a single, highly connected population in the NT. Genetic differentiation among the sampling locations for each species was low, and genetic clustering analyses provided strong support for a single population of each species in the region. Sharks of both species captured within a single location (within 50 km of one another) were more genetically related than those further apart; however, this does not constitute evidence for multiple, spatially discrete populations of either species in NT waters. Preliminary applications of effective population size estimators were used, but further work is needed to determine if these can be used to indicate trends in abundance.

Implications for relevant stakeholders

The immediate implications of our research are for fisheries scientists and managers. Our results indicate that these two shark species can be monitored and managed in the NT under the assumption that each species occurs as a single population in this region. Parasite and vertebral chemistry data collected as part of a PhD project conducted in parallel with this project suggest that, for *C. coatesi*, individuals may be resident within certain regions (eastern versus western NT waters) but the genetic data collected here suggest that, on a generational timescale, both species occur as highly-connected populations across in the NT region.

Our research has potential implications for commercial fishers, particularly from the NT Demersal Fishery. The information from our research will flow through to the industry by contributing to the information required to develop a byproduct fishery for the two species, by utilising bycatch and increasing economic return.

Recommendations

Future research could develop genetic methods, such as effective population size or close-kin mark-recapture, for population monitoring. We recommend assessing the need for further sampling for such analyses to cover geographic gaps in sample coverage for these analyses. Comparing the genetic data against other data that indicate individual movement patterns on shorter timescales would help develop a holistic understanding of shark movement and population connectivity to inform sustainable harvest strategies.

Keywords

Milk Shark, Australian Blackspot Shark, genetics, fisheries management, bycatch, stock assessment, population structure.

1 Introduction

Our project aimed to provide information on the genetic structure of populations of two small tropical shark species (Milk Shark [*Rhizoprionodon acutus*] and Australian Blackspot Shark [*Carcharhinus coatesi*]) in northern Australia. This could inform the assessment of a potential byproduct fishery of these two species, which are commonly captured as trawl bycatch. Trawl fisheries are often criticised for their indiscriminate nature, catching a broad range of target and bycatch species. Increasing community scrutiny has made it imperative for many trawl fisheries to address bycatch issues to maintain a social licence to operate. Reducing bycatch using mitigation measures, such as square mesh windows and bycatch reduction grids, is well documented. A complementary approach is to retain and utilise species that were previously discarded, increasing the efficiency of use of species captured. Benefits of this include providing a supply of local seafood to the community and generating an economic return for fishers by using previously discarded species. Before this development of the fishery can occur, however, managers and stakeholders need reassurance that the harvest of these small shark species is sustainable.

There are several regulatory management barriers that can limit utilisation of bycatch. Foremost among these relates to the availability of reliable, scientific information regarding the sustainability of commercial exploitation. This information is sparse for *R. acutus* and *C. coatesi*, which are caught in the trawl component of the Northern Territory (NT) Demersal Fishery. This fishery primarily targets Saddletail Snapper and Crimson Snapper, but it also interacts with over 300 bycatch species. *R. acutus* and *C. coatesi* are two of the most significant bycatch species (Zhou & Griffiths, 2008), comprising 10% of the total bycatch. Access rights to the NT's shark resource have been allocated to the Offshore Net and Line Fishery. Consequently, retention of sharks by other fisheries, including the Demersal Fishery, is either limited or banned. The Offshore Net and Line Fishery has recently transitioned to an individual transferable-quota management system, and there is potential for quota trading between fisheries in the future. This may provide the opportunity for the Demersal Fishery to develop markets for sharks that are currently discarded.

A current impediment to the development of commercial fisheries for *R. acutus* and *C. coatesi* is the increasing body of evidence suggesting that shark species are susceptible to overfishing. A critical component of the development of sustainable shark fisheries is a sound knowledge of the species' biology. Despite being common bycatch, relatively little is known about the biology of *R. acutus* and *C. coatesi*, particularly in a regional context. This void of knowledge has made it difficult for the NT Department of Industry, Tourism and Trade (DITT) Fisheries Division to develop management strategies that could underpin sustainable harvest of these species.

Recognising the potential for *R. acutus* and *C. coatesi* to become retained species, DITT and Charles Darwin University co-invested in a PhD project in 2018 to investigate the population structure and biology of these two species. A large number of sharks were collected for the project by trawl vessels operating in the Demersal Fishery. Samples taken from these sharks were used to investigate age and growth, reproductive biology, and population connectivity though the use of biological tags (parasite assemblages) and vertebrae microchemistry. In conjunction with that PhD research, our project was

developed to apply population genetics analyses to collected tissue samples to examine population connectivity, which provides important information for understanding stock structure.

Information about population connectivity for sharks in NT waters is lacking for many species, including *R. acutus* and *C. coatesi*. Such information provides necessary context for fisheries management by identifying whether a species occurs as a single mixed population or a set of isolated or semi-isolated populations (Barton et al., 2018). This lack of information is particularly relevant for bycatch shark species, which are managed in the NT as complexes of multiple species instead of as individual species (Northern Territory Government, 2018). Without a basic understanding of population connectivity, biologically improper management can result in over-harvest of particular populations or parts of populations (Laikre et al., 2005).

Genetic information is increasingly used to determine biological stocks of fish, as it provides information on population connectivity over diverse timescales, depending on the sampling scheme, genetic data and analyses used (Barton et al., 2018; Cowen & Sponaugle, 2009; Laikre et al., 2005). Genetic data can provide information about individual dispersal patterns and long-term inter-generational migration rates, and they can be used to estimate demographic parameters such as abundance (Bravington et al., 2016) and effective population size, a genetic concept relating to the size of an idealised population that would yield the observed patterns of genetic diversity (Wang et al., 2016). These data can also inform the conservation of genetic diversity, which is critical for adaptive capacity in the face of environmental change (Domingues et al., 2018; Laikre et al., 2005).

2 Objectives

Our objectives were to:

- identify genetic population structure for *R. acutus* and *C. coatesi* in NT waters
- develop capacity for genetic research and monitoring of shark species in the NT
- provide baseline information on genetic structure to inform potential genetic monitoring of these species, including initial estimates of effective population size.

3 Methods

3.1 Sample collection

We took tissue samples from 196 individual *R. acutus* (Figure 1) and 634 individual *C. coatesi* (Figure 2) that were collected in NT waters between May 2018 and November 2019. These sharks were caught as bycatch from the commercial trawl fisheries from Australia's Exclusive Economic Zone (EEZ) and retained for our research use. Australia's EEZ around the NT encompasses the Timor Sea in the north-west, the Arafura Sea in the north and the Gulf of Carpentaria in the east (Figure 1, Figure 2). For initial analyses of patterns of population genetics, we grouped the NT samples into four regions. We included three additional regions (Pilbara, Kimberley and Papua New Guinea [PNG]) for

R. acutus (Figure 1) and two additional regions (Kimberley and PNG) for *C. coatesi* (Figure 2). We included these additional samples from Western Australia (WA) and PNG to provide broader context to help understand the degree of genetic differentiation and connectivity among populations in NT waters relative to a broader sample across the regional distribution of these species. The Western Australian samples were provided by Alister Harry (WA Department of Primary Industries and Regional Development), and the PNG samples were provided by Will White (CSIRO).

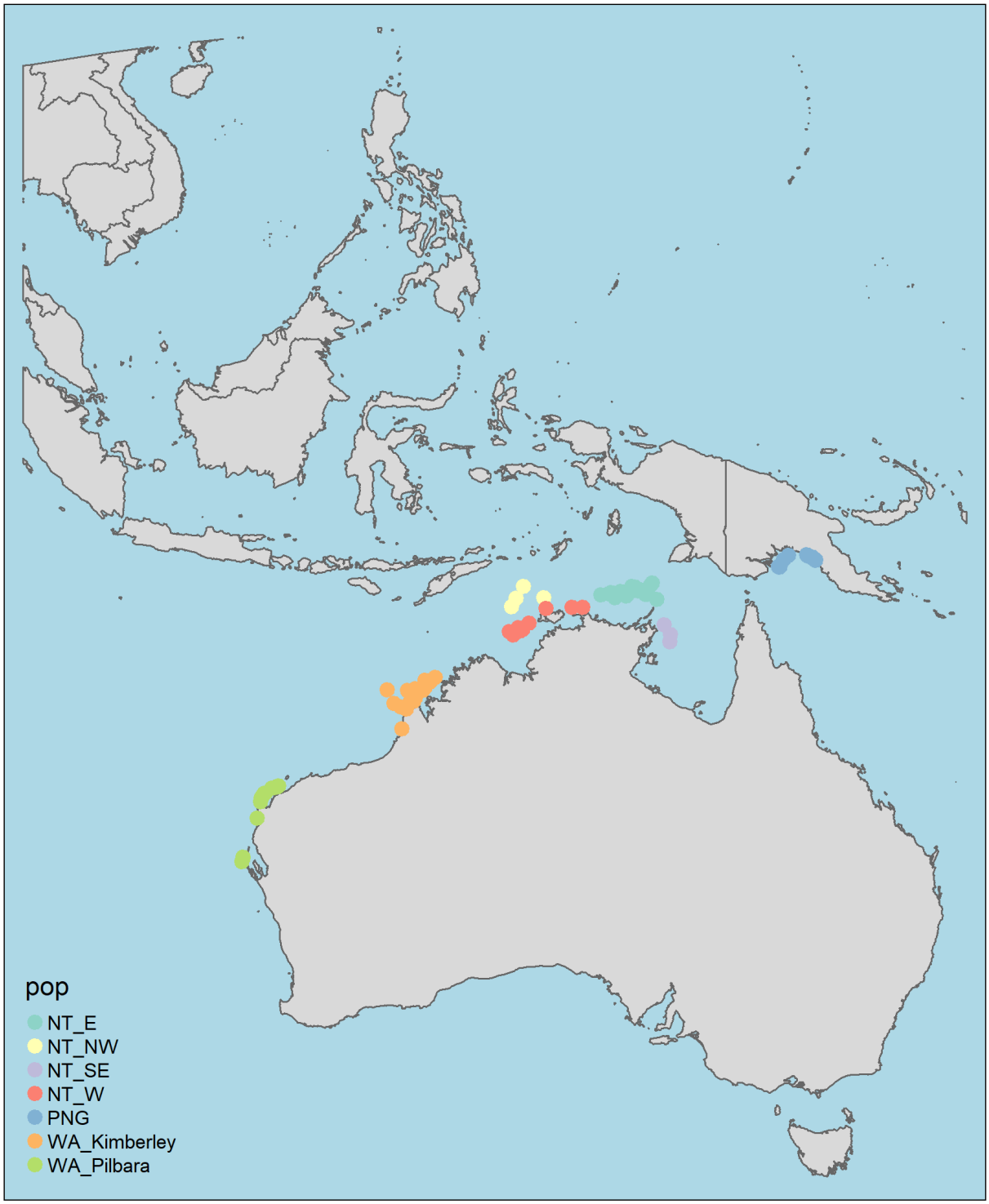

Figure 1. Map of the sampling region for *Rhizoprionodon acutus* across northern Australia and Papua New Guinea, with each sample area shown in a different colour.

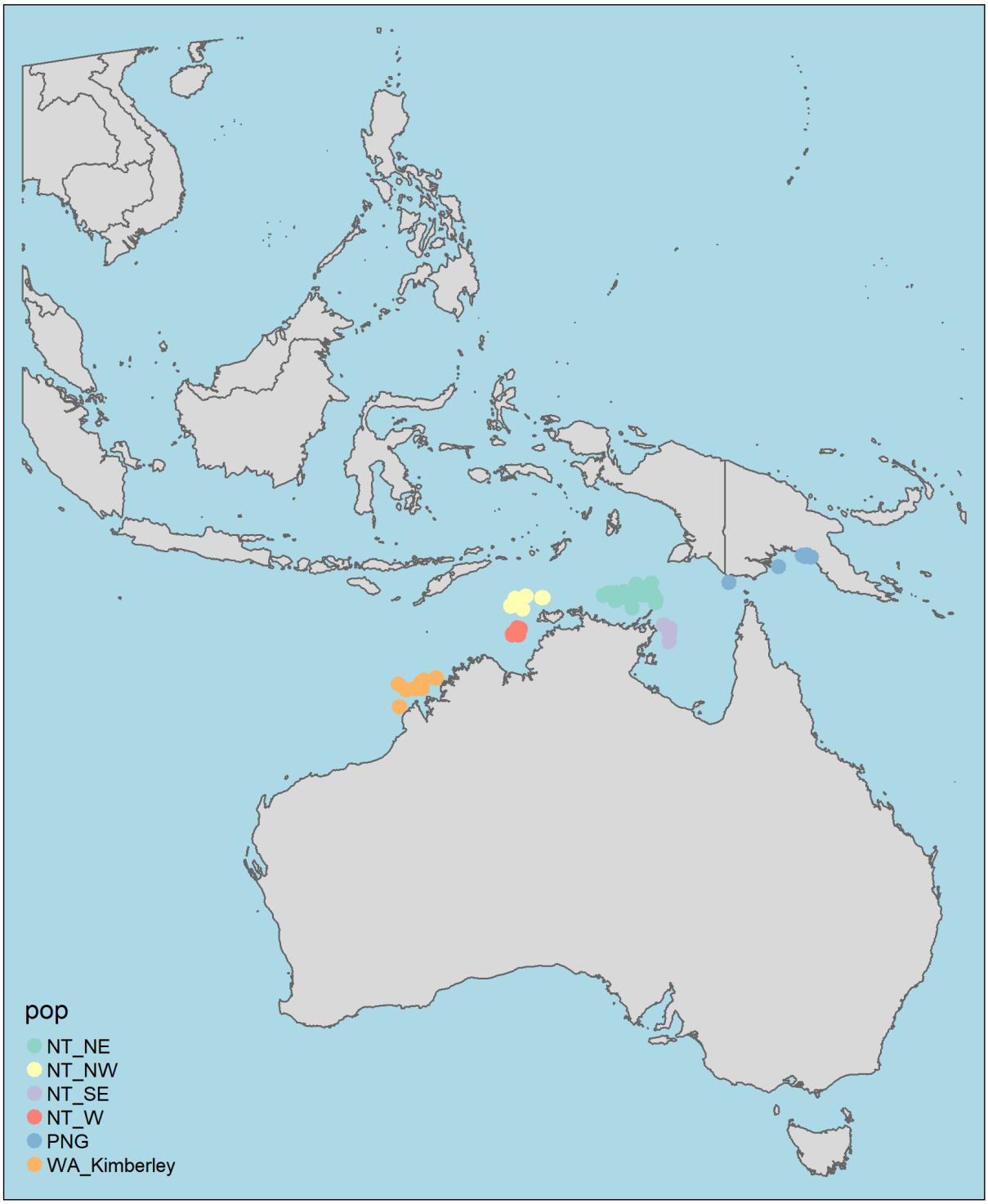

Figure 2. Map of the sampling region for *Carcharhinus coatesi* across northern Australia and Papua New Guinea, with each sample area shown in a different colour.

Sharks were kept frozen on board the trawlers until we collected them from the wharf in Darwin. We transported them to DITT's Fisheries Division laboratories and stored them at -20 °C until processing. For processing, sharks were defrosted, and the location of catch, date of catch, total length in millimetres, fork length in millimetres and weight in grams were recorded for each individual. We determined the sex and maturity stage of each shark by assessing the gonads and classifying them

according to the criteria of Walker (2005). A ~5-mg portion of muscle tissue was collected from each shark for DNA extraction.

3.2 Extracting DNA from shark tissue samples and building the genetic database

We extracted DNA for single-nucleotide polymorphism (SNP) genotyping using the DArTSeq protocol through Diversity Arrays P/L (Kilian et al., 2012). We sent tissue samples in 100% ethanol to Diversity Arrays for DNA extraction and genomic library preparation for sequencing. DArTSeq involves an initial step of 'genome reduction' to subset a small fraction of the genome of each individual for high-throughput sequencing, followed by bioinformatics analysis to identify DNA sequences containing single nucleotide positions that vary among individuals within and among populations. The DArTSeq protocols for *Carcharhinus* and *Rhizoprionodon* are available for future projects via Diversity Arrays.

The initial output of the DArTSeq protocol from Diversity Arrays typically features between 20,000 and 100,000 SNP genetic markers per individual from across the genome of each species. Before any analyses are conducted on the data to estimate patterns of connectivity among populations, there is an initial step of filtering the SNPs to remove those that do not meet quality thresholds for data analysis. Using custom R scripts and the *dartR* package in the R statistical software environment (Gruber et al., 2018; R Core Team, 2024), we filtered SNPs by repeatability (95% repeatability based on consistency among 30% of replicated samples), removing non-variable loci within the sample set, minimising missing data (per-locus call rate > 0.90), dropping secondary SNPs in same DNA sequence fragment (retaining the SNP with highest polymorphic information content), and dropping individuals according to the amount of missing data (call rate > 0.90). We also filtered by minimal total sequencing depth across alleles (threshold = 10) and by allele depth ratio (ratio of the mean allele sequencing depth, given the allele has been observed; threshold = 2), which is an indicator of bias in detection of one allele over another at a SNP locus. For all analyses other than the effective population size estimation, we filtered SNPs on linkage disequilibrium (LD) using the *gl.filter.ld* function in *dartR*, dropping the SNP with lowest polymorphic information content in each SNP pair exceeding the LD R-squared threshold of 0.2. For the LD calculations, we considered SNPs with minor allele frequency greater than 0.05 and treated all SNPs as being on the same chromosome and all samples as belonging to the same population. We did not filter SNPs on Hardy–Weinberg equilibrium (HWE) or F_{ST} (a metric of genetic differentiation among populations) outlier criteria, because such approaches are known to cause bias in the detection of population structure in reduced-representation SNP data (Pearman et al., 2022). Essentially, such approaches risk generating false signals of genetic structure by retaining SNPs conforming to the population stratification scheme used for HWE calculations. We did not consider our sampling locations to be an appropriate proxy for population structure for this purpose. Using the filtered dataset, we calculated overall genetic diversity statistics, including observed and expected heterozygosity (H_O and H_E) overall and within each population, as well as the inbreeding coefficient (F_{IS}) and the fixation index (F_{ST}).

3.3 Genetic analyses to measure population structure and infer connectivity

We used a series of analyses to quantify patterns of genetic differentiation ('genetic structure') among the individuals and regions sampled for our study. Fundamentally, these analyses measure

the patterns of genetic similarity or differentiation among individuals across the study area, and these patterns reflect the underlying processes of individual movement and connectivity.

First, we used genetic spatial autocorrelation analysis, which uses a matrix of geographic distances and genetic distances among individuals to test whether individuals sampled geographically close to one another are genetically more similar to those further apart (Smouse & Peakall, 1999). This is a powerful approach to measuring the strength and spatial scale of clustering of genetically similar individuals, which can reflect underlying dispersal patterns. We classified the pairwise comparisons among individuals into intervals from 0–50 km up to 3,500 km apart, and used 1,000 random permutations of the data to test whether observed spatial autocorrelation patterns differed significantly from a random pattern. We conducted this analysis using the software GenAlEx v6.5 (Peakall & Smouse, 2012).

We then conducted a principal coordinate analysis (PCoA) to visualise patterns of genetic similarity among individuals and regions using the R packages *dartR* and *adegenet* (Gruber et al., 2018; Jombart & Ahmed, 2011). The region-level PCoA analyses were conducted using pairwise F_{ST} values among populations. We then used discriminant analysis of principal components (DAPC) in the R package *adegenet* to identify the presence of discrete genetic groupings (clusters) of individuals that would reflect discrete populations. We evaluated support for scenarios of different numbers of discrete genetic clusters from 1 to 8 using the Bayesian Information Criterion (BIC).

As an alternative approach to evaluating the presence of population structure, we conducted an analysis using the BIC method in the STRUCTURE program (Pritchard et al., 2000), implemented via the R package *strataG* (Archer et al., 2017). The objective of this analysis is similar to the DAPC method, but the underlying model focusses on resolving groups that maximise conformation to theoretical population genetics expectations (HWE and LD). Using the *structureRun* function, we ran a model assuming uncorrelated allele frequency, which may have higher power to detect low levels of structure (Pritchard et al., 2000). We used a burn-in period of 20,000 and 80,000 Markov Chain-Monte Carlo iterations and used the admixture model. To account for the uneven number of samples from the different collection sites, we followed Wang (2017) and set the parameter alpha to 0.5 (1/'expected number of pops'; 2 expected populations in our case for both species) and used the alternative ancestry prior (uniprioralpha = 0). We ran analyses for K = 1–5 for *R. acutus* and K = 1–6 for *C. coatesi* (given the greater geographic range used for sampling), each replicated five times, and used diagnostic plots of number of groups (K) and first-order and second-order changes in likelihood (LnP(K)) as described in (Evanno et al., 2005) to choose the most likely number of clusters. We then used the function CLUMPP in strataG (Archer et al., 2017) to average the results and minimise variance across iterations (Jakobsson & Rosenberg, 2007).

3.4 Effective population size (N_e)

We estimated effective population size using the single-sample bias-corrected LD method (Waples, 2006; Waples & Do, 2010), implemented in the software N_eESTIMATOR v2.1 (Do et al., 2014). We conducted analyses under the assumption of random mating and screened out rare alleles (which can create upward bias in LD estimates) with minor allele frequency cutoffs of two critical values (P_{crit} = 0.02 and 0.05; Do et al., 2014).

4 Results

4.1 Genetic connectivity of *Rhizoprionodon acutus*

4.1.1 Data filtering and exploratory analysis

DNA sequencing and SNP-calling using the DArTSeq process for *R. acutus* produced 84,932 SNP loci, of which 9,915 SNPs were retained after filtering (seven individual samples were dropped during filtering and were not used for analysis). Summary statistics before and after filtering are presented in Table 1. Overall observed heterozygosity (H_O) and mean expected heterozygosity (H_E) across the entire sample set were estimated at 0.16 and 0.17, respectively. H_O values were similar across populations (Table 2). Mean F_{ST} was less than 0.01 (pairwise values are presented in Table 3), and F_{IS} (population-level inbreeding coefficient) was 0.06.

The pairwise F_{ST} values among sampling regions indicate incredibly low genetic differentiation within the study region for each species. Analyses described in the following sections explore these patterns in more detail, but the low F_{ST} patterns between sampling regions are indicative of very high connectivity and lack of population genetic structure.

Table 1. Mean and standard deviation of genetic diversity metrics for *Rhizoprionodon acutus* before and after filtering. Expected heterozygosity values are presented as means within sub-populations, defined by sampling location (H_S) and overall means (H_T).

	No. loci	**Mean H_S**	**Mean H_O**	**Mean H_T**	**Mean F_{IS}**	**Mean F_{ST}**	**Mean call rate**	**Mean depth**	**Mean allele ratio**	**Mean repeat-ability**
Before filter	84,932	0.09 ± 0.13	0.06 ± 0.11	0.10 ± 0.13	0.42 ± 0.43	0.03 ± 0.28	0.87 ± 0.17	13.9 ± 6.6	1.68 ± 0.74	0.98 ± 0.02
After filter	9,915	0.16 ± 0.17	0.15 ± 0.17	0.16 ± 0.17	0.05 ± 0.2	0.01 ± 0.28	0.98 ± 0.01	16.6 ± 5.33	1.47 ± 0.34	0.98 ± 0.01

Table 2. Estimated observed heterozygosity (H_O), expected heterozygosity within sub-populations (H_S) and inbreeding coefficient (F_{IS}) for *Rhizoprionodon acutus* for each geographic area sampled over a total of 9,915 variable single-nucleotide polymorphisms across sites.

Location	Sample size	Number of polymorphic loci	H_O	H_S	F_{IS}
NT_E	67	6,713	0.16	0.17	0.08
NT_NW	20	8,169	0.16	0.16	0.06
NT_SE	21	8,316	0.17	0.16	0.03
NT_W	28	7,876	0.17	0.17	0.03
PNG	19	8,310	0.15	0.16	0.10
WA_Kimberley	19	8,069	0.14	0.16	0.11
WA_Pilbara	15	8,364	0.16	0.17	0.07

Table 3. Pairwise estimates of genetic differentiation (F_{ST}) between sampled locations.

Location	NT_SE	NT_NW	NT_E	NT_W	WA_Kimberley	WA_Pilbara
NT_SE						
NT_NW	0.0031					
NT_E	0.0018	0.0021				
NT_W	0.0017	0.0012	0.0010			
WA_Kimberley	0.0036	0.0029	0.0021	0.0026		
WA_Pilbara	0.0044	0.0029	0.0020	0.0029	0.0015	
PNG	0.0023	0.0020	0.0010	0.0014	0.0020	0.0010

4.1.2 Population genetic structure

The spatial autocorrelation analysis identified significant but very weak spatial autocorrelation among *R. acutus* samples within 50 km of one another but no spatial patterns in genetic similarity beyond this scale (Figure 3). This indicates a low level of clustering of genetically related individuals on a very local scale.

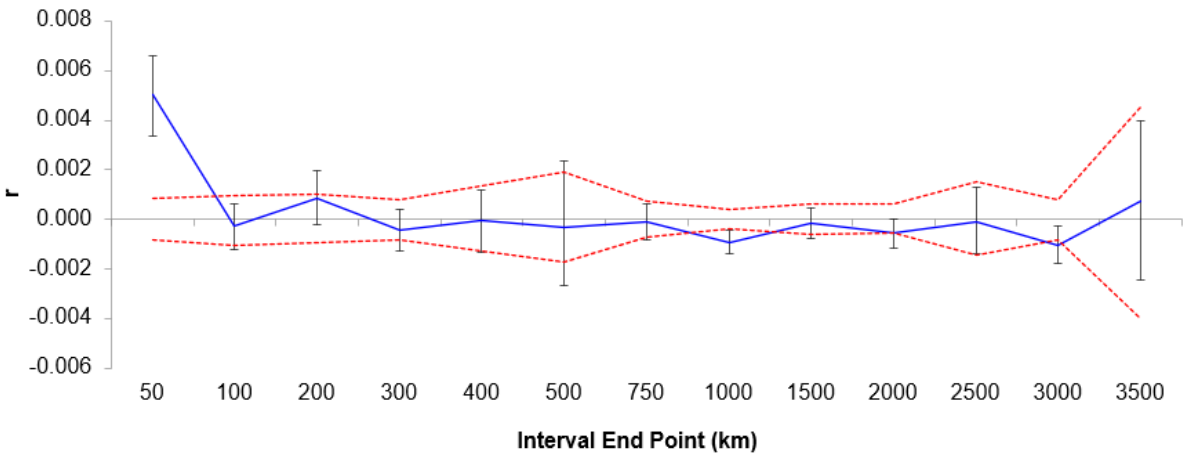

Figure 3. Genetic spatial autocorrelation analysis for *Rhizoprionodon acutus*, showing significant positive spatial genetic structure among individuals sampled within 50 km of each other.

The PCoA on the individual *R. acutus* data (Figure 4) revealed no major genetic clustering among samples corresponding to geographic origin. The first two principal coordinates explained less than 2% of the variation among samples, which is very low. This is in agreement with an analysis of genetic differentiation among the major sampling regions, which identified a F_{ST} value (a common estimate of genetic differentiation) of 0.001 (Table 3). This value is scaled from 0 to 1, and an estimate of 0.001 indicates almost no genetic population structure. However, the PCoA conducted on genetic distances among populations revealed greater similarity among regional groups of samples from the NT and PNG relative to samples from WA.

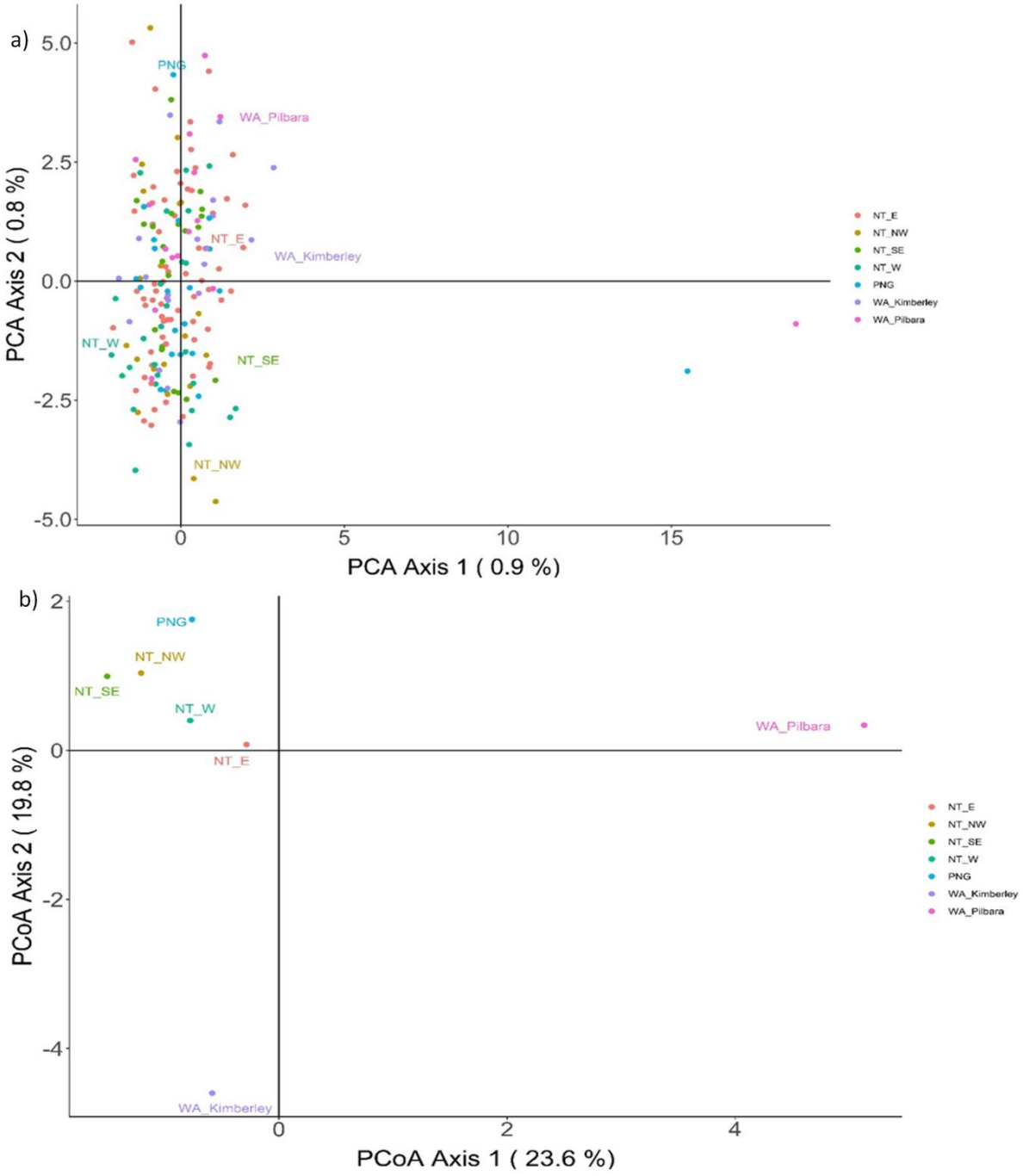

Figure 4. Principal coordinate analysis (PCoA) for *Rhizoprionodon acutus*, showing individual PCoA (top panel) and regional-level PCoA (bottom panel).

The discriminant analysis of principal components for *R. acutus* identified no discrete clusters of individuals, with a model featuring a single group of individuals receiving the lowest (best) BIC value (Table 4).

Table 4. Bayesian Information Criterion (BIC) scores for discriminant analysis of principal components models for *Rhizoprionodon acutus*, showing greatest support (lowest BIC) for a scenario of a single genetic group.

K	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
R. acutus	3899.9	3904.2	3909.1	3914	3919.1	3924.1	3929.2	3934.3

Results from STRUCTURE revealed K = 2 (two groups) as the most likely number of genetic clusters (Figure 5). However, the ancestry membership plot shows that all samples were nearly 100% assigned to one of those two groups (Figure 6). Simulation studies have shown that the ΔK method of Evanno et al. (2005) is unable to resolve the correct number of populations when migration rate is higher than 0.005, and therefore cannot evaluate a scenario of one single genetic group (Cullingham et al., 2020). Thus, we interpret these results with caution, given the lack of evidence for population structure in the PCoA and values of F_{ST}. We suggest that these results essentially indicate no genetic structure within the sampled *R. acutus*, and that individuals of this species within the region belong to a single genetic group.

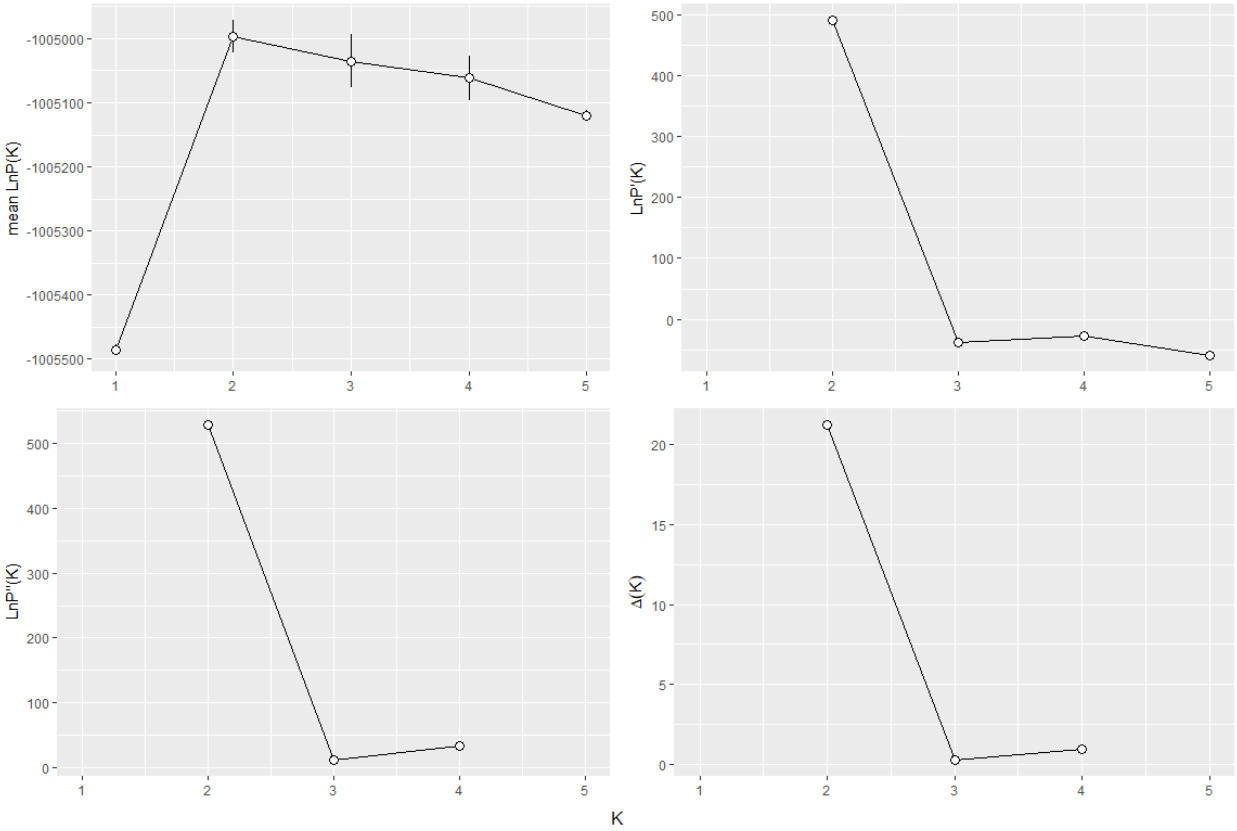

Figure 5. Diagnostic plots showing first-order and second-order changes in the likelihood for K (number of groups) for STRUCTURE analysis following Evanno et al. (2005).

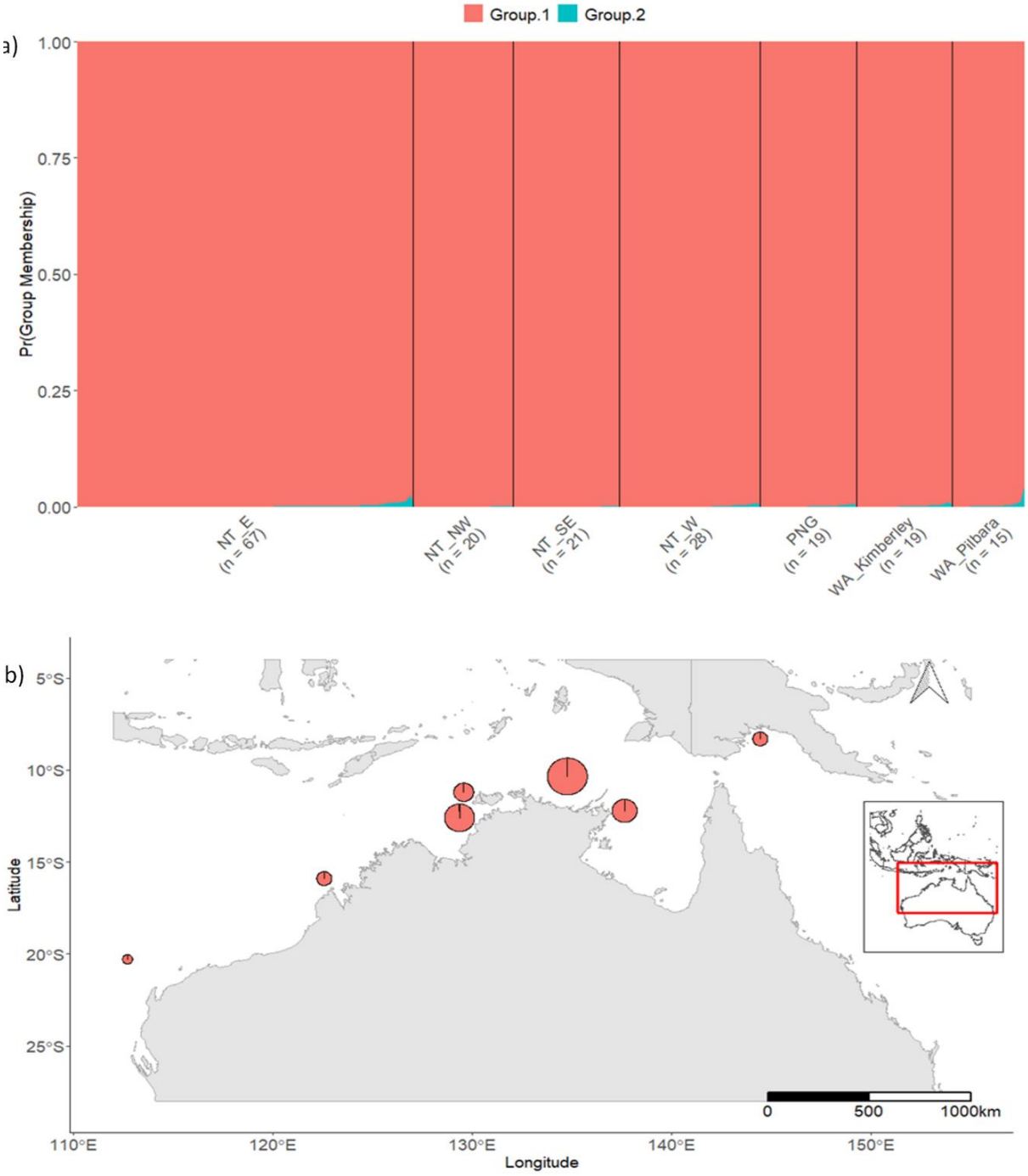

Figure 6. Results of STRUCTURE for *Rhizoprionodon acutus*, showing distribution of the probability of group membership for individuals in each population (a). Numbers in brackets represent sample sizes. Each pie chart (b) represent a sampled location, showing no evidence of multiple genetic groups in this species over the sampled region.

4.1.3 Effective population size (N_e)

We grouped all individuals into one population to estimate effective population size, given that the results across the different population structure analyses did not show evidence of population structure. For *R. acutus*, we estimated an N_e of 2,848 (95% confidence interval 2,468–2,952) for P_{crit} = 0.05 and 2,872 (2,287–2,617) for P_{crit} = 0.02, where the P_{crit} values are minor allele frequency thresholds below which SNP loci were excluded.

4.2 Genetic connectivity of *Carcharhinus coatesi*

4.2.1 Data filtering and exploratory analysis

From an initial 66,493 SNP loci for *C. coatesi*, 10,238 SNPs were retained after filtering (33 individual samples were dropped during filtering). Summary statistics before and after filtering are presented in Table 5. Overall observed heterozygosity (H_O) and mean expected heterozygosity (H_S) were estimated at 0.19. H_O values were similar across populations (Table 6). Mean F_{ST} was 0.001 (pairwise values are presented in Table 7) and F_{IS} (population-level inbreeding coefficient) was 0.02.

Table 5. Mean and standard deviation of genetic diversity metrics for *Carcharhinus coatesi* before and after filtering. Expected heterozygosity values are presented as means within sub-populations, defined by sampling location (H_S) and overall means (H_T).

	No. loci	**Mean H_S**	**Mean H_O**	**Mean H_T**	**Mean F_{IS}**	**Mean F_{ST}**	**Mean call rate**	**Mean depth**	**Mean allele ratio**	**Mean repeat-ability**
Before filter	66,493	0.11 ± 0.14	0.08 ± 0.13	0.10 ± 0.14	0.33 ± 0.40	-1.25 ± 2.63	0.91 ± 0.16	15.1 ± 7.3	1.75 ± 0.69	0.98 ± 0.02
After filter	10,238	0.18 ± 0.17	0.18 ± 0.18	0.19 ± 0.18	0.04 ± 0.17	-0.3 ± 1.76	0.98 ± 0.02	17.8 ± 6.54	1.45 ± 0.43	0.98 ± 0.01

Table 6. Estimated observed heterozygosity (H_O), expected heterozygosity within sub-populations (H_S) and inbreeding coefficient (F_{IS}) for *Carcharhinus coatesi* for each geographic area sampled over a total of 10,238 variable single-nucleotide polymorphisms across sites.

Location	**Sample size**	**Number of polymorphic loci**	**H_O**	**H_S**	**F_{IS}**
NT_NE	331	4,682	0.20	0.19	0.04
NT_NW	53	7,583	0.20	0.19	0.00
NT_SE	117	6,834	0.20	0.19	0.03
NT_W	100	6,948	0.19	0.19	0.04
PNG	19	8,234	0.17	0.18	0.09
WA_Kimberley	11	8,490	0.18	0.19	0.07

Table 7. Pairwise estimates of genetic differentiation (F_{ST}) between sampled locations.

	PNG	NT_NE	NT_SE	NT_W	NT_NW
PNG					
NT_NE	0.0035				
NT_SE	0.0036	0.0005			
NT_W	0.0040	0.0004	0.0007		
NT_NW	0.0045	0.0008	0.0011	0.0011	
WA_Kimberley	0.0037	0.0033	0.0038	0.0036	0.0047

4.2.2 Population genetic structure

The spatial autocorrelation analysis identified significant but very weak spatial autocorrelation among *C. coatesi* samples within 50 km of one another but no spatial patterns in genetic similarity beyond this scale (Figure 7**Error! Reference source not found.**). This pattern was weaker than that observed in *R. acutus* and indicates a low level of clustering of genetically related individuals on a very local scale.

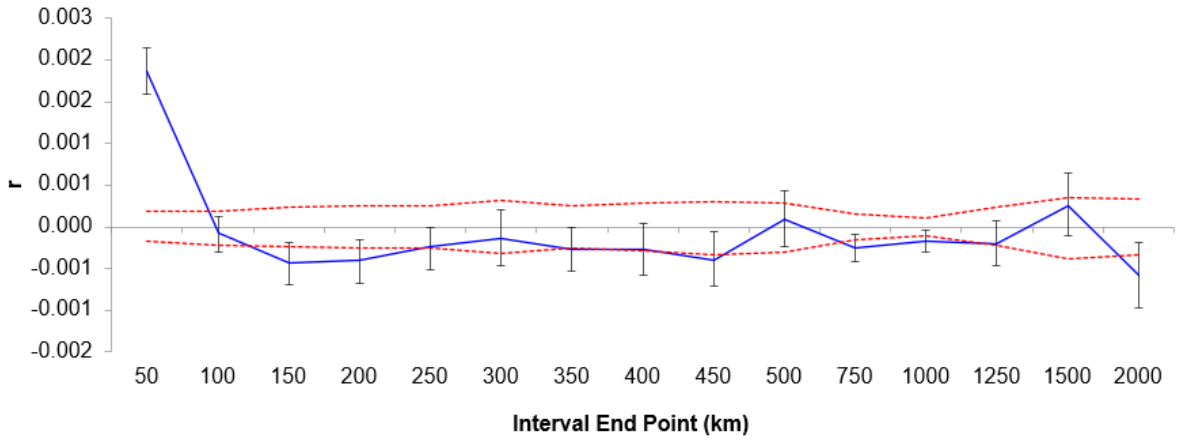

Figure 7. Genetic spatial autocorrelation analysis for *Carcharhinus coatesi*, showing significant positive spatial genetic structure among individuals sampled within 50 km of each other.

The PCoA on the individual *C. coatesi* data (Figure 8) revealed no major genetic clustering among samples corresponding to geographic origin. The first two principal coordinates explained less than 1% of the variation among samples, which is very low. Similar to the results for *R. acutus*, this is in agreement with the analysis of genetic differentiation among sampling regions, which identified very low F_{ST} values (range: 0.0004–0.0047; Table 6), indicating no genetic population structure. However, the PCoA conducted on genetic distances among populations revealed greater similarity among regional groups of samples from the NT relative to samples from the other regions.

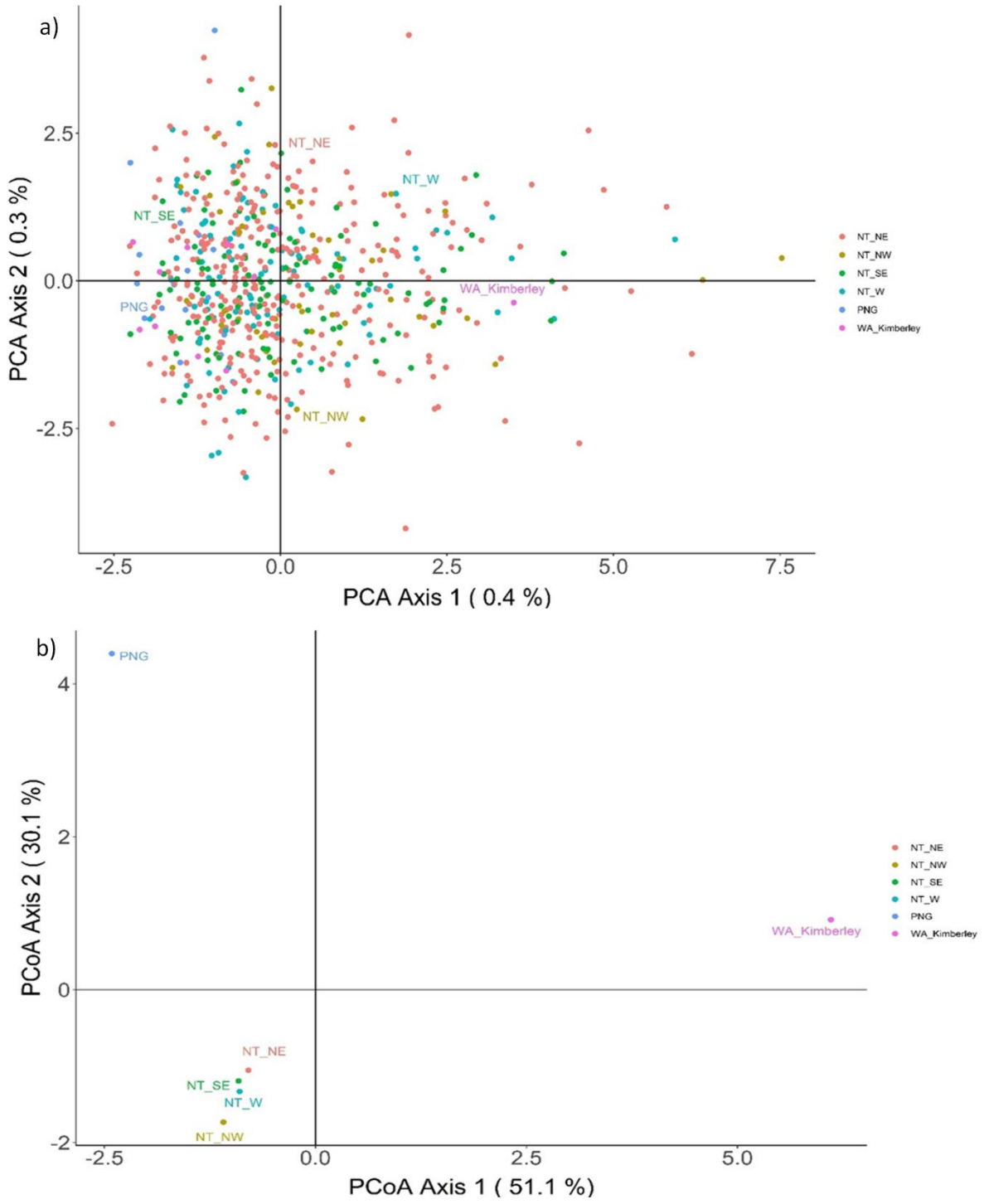

Figure 8. Principal coordinate (PCoA) analysis for *Carcharhinus coatesi*, showing individual PCoA (top panel) and regional-level PCoA (bottom panel).

The discriminant analysis of principal components for *C. coatesi* identified no discrete clusters of individuals, with a model featuring a single group of individuals receiving the lowest (best) BIC value (Table 8).

Table 8. Bayesian Information Criterion (BIC) scores for discriminant analysis of principal components models for *Carcharhinus coatesi,* showing greatest support (lowest BIC) for a scenario of a single genetic group.

K	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C. coatesi	1153.5	1157.3	1161.3	1164.9	1168.8	1172.8	1176.8	1180.8

Results from STRUCTURE revealed K = 2 (two groups) as the most likely number of genetic clusters (Figure 9). However, the ancestry membership plot shows that all samples were nearly 100% assigned to one group, showing the same pattern found for *R. acutus* (Figure 10).

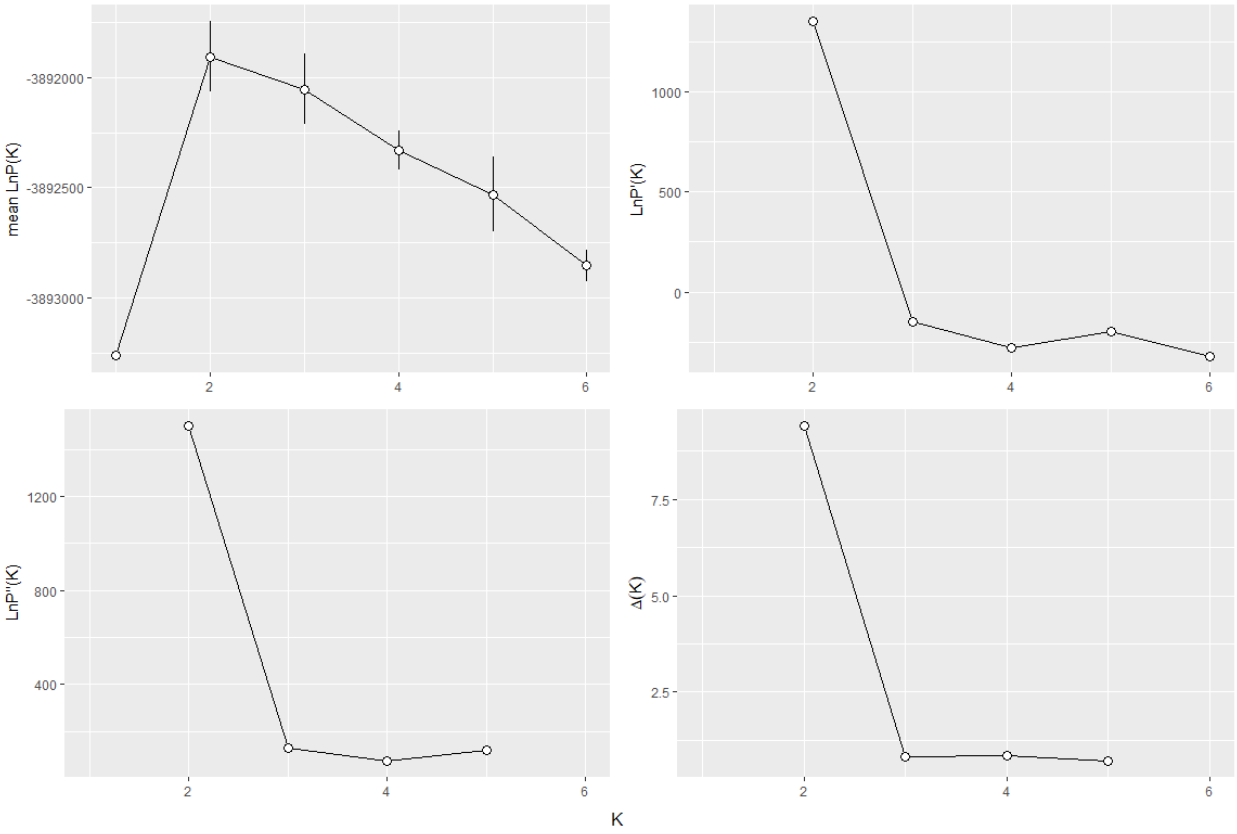

Figure 9. Diagnostic plots showing first-order and second-order changes in the likelihood for K (number of groups) for STRUCTURE analysis following Evanno (2005).

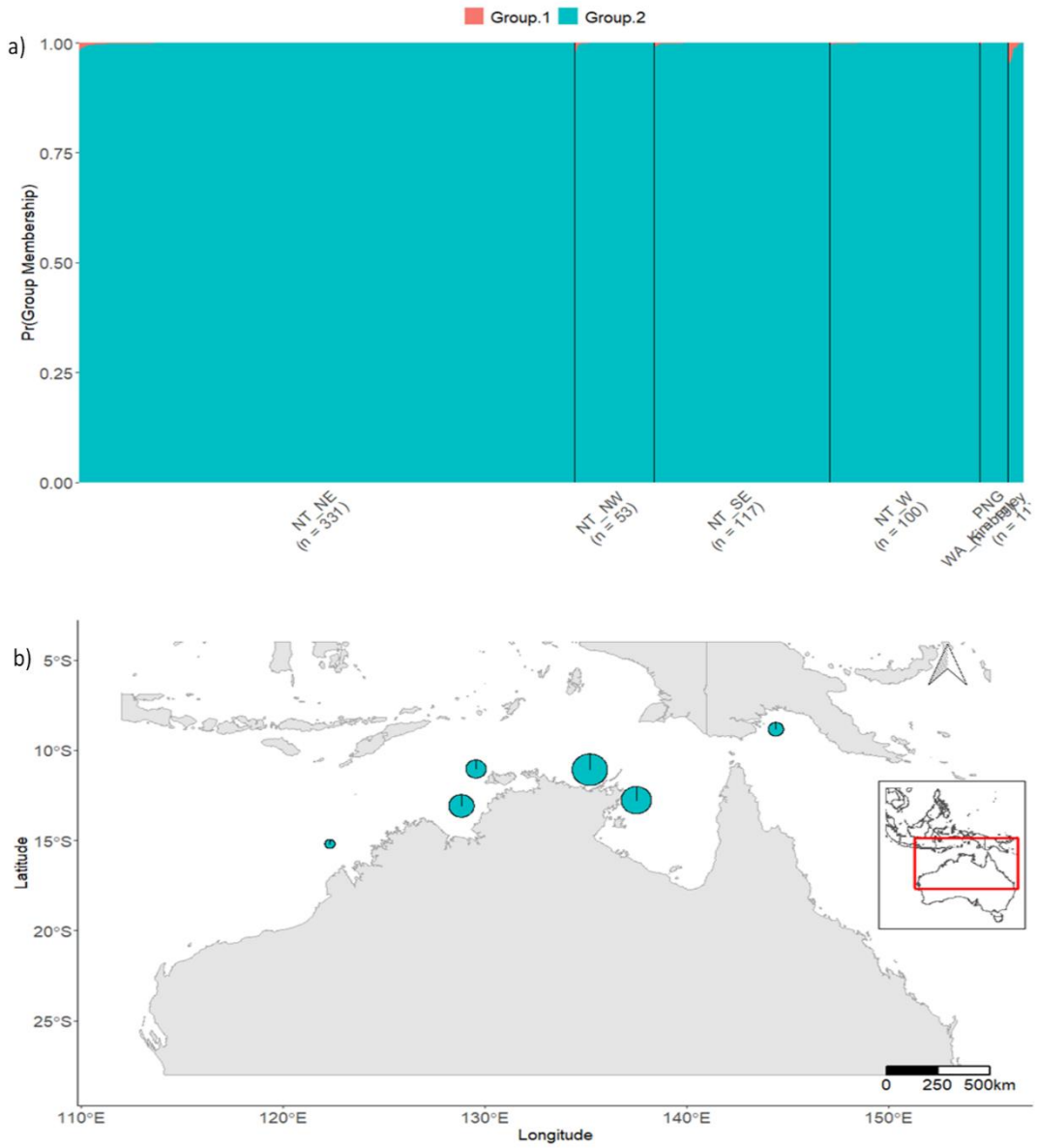

Figure 10. Results of STRUCTURE for *Carcharhinus coatesi*, showing distribution of the probability of group membership for individuals in each population (a). Numbers in brackets represent sample sizes. Each pie chart (b) represents a sampled location, showing no evidence of multiple genetic groups in this species over the sampled region.

4.2.3 Effective population size (N_e)

We grouped all individuals into one population to estimate effective population size, given that the results across the different population structure analyses did not show evidence of population structure. For *C. coatesi*, we estimated a genetically effective population size (N_e) of 6,757 for P_{crit} = 0.05 and 7,051 for P_{crit} = 0.02. The model was unable to estimate confidence intervals.

5 Discussion

5.1 No evidence for genetic population structure of *R. acutus* or *C. coatesi* in NT waters

Our results overwhelmingly suggest there is no substantial genetic population structure within the sampled range of *R. acutus* or *C. coatesi* in NT waters. Genetic clustering analyses for both species consistently identified the sampled sharks as belonging to a single genetic group, and patterns of genetic differentiation among the sampling regions were very low. The PCoAs indicated that, unsurprisingly, samples from locations within NT waters were more genetically similar to one another than they were to samples from locations outside NT waters (PNG and WA). However, this is in the context of incredibly low genetic differentiation between the regions sampled in NT and the WA and PNG samples. Considering the aim of our project was to assess the presence of genetic population structure in the two bycatch shark species in NT waters, our clear result is that there is no substantial genetic structure within the NT region, and this is consistent with the broader patterns in these species from WA and PNG. To help interpret these results, the alternative finding of strong genetic structure among sampling regions would have indicated the presence of discrete populations with limited exchange of breeding individuals. The genetic data generated and analysed in this project provide no evidence for that scenario in either species.

From a qualitative and quantitative angle, the results for the two species were remarkably similar. Genetic diversity levels were similar across sampling units within each species, and genetic differentiation (as measured by F_{ST}) was very low among the NT sampling units and across the full set of samples for both species. While genetic clustering methods can struggle to resolve statistical support for simple population structure scenarios such as those involving one or two genetic groups (Cullingham et al., 2020), the best-supported STRUCTURE model for each species assigned all individuals to a single genetic group within each species across the sampled range.

Some minor genetic structure was apparent in both datasets, with evidence of significant (although very weak) spatial clustering of similar genotypes over fine scales (< 50 km) from the spatial autocorrelation analyses. Weak patterns of inter-individual spatial autocorrelation have been observed in other shark species such as Shortfin Makos (*Isurus oxyrhynchus*) (Corrigan et al., 2018) and Grey Reef Sharks (*Carcharhinus amblyrhynchos*) (Momigliano et al., 2017). Similar patterns observed in the species studied here likely indicate very weak aggregation of genetically similar individuals. Further analysis of whether these spatial genetic patterns are associated with particular age or sex categories would be informative.

The PCoA on grouped sampling units indicated that, despite weak overall genetic structure, there was a spatial pattern where samples of *C. coatesi* from NT waters were more similar to one another than to those from WA or PNG waters. Similarly, *R. acutus* samples from the NT, when grouped into the regional sampling units, grouped with one another and with the PNG samples but showed some distinction from the WA sampling unit. While this pattern occurred in the context of very low overall genetic population structure, additional future sampling over a broader geographic area may prove informative. However, given that the aim of our study was to quantify structure within NT fisheries,

the consistent result across all analyses is one of essentially no genetic structure in this region of the species' distributions.

Analyses of parasite assemblages and vertebral chemistry from a PhD thesis (Kirke, 2024) identified significant differences in *C. coatesi* (but not *R. acutus*) samples from eastern and western NT waters. Similar to the genetic analyses, this indicates that some *C. coatesi* individuals may remain resident in certain locations for extended periods, but that populations are likely to be connected over a large geographic scale on inter-generational timeframes.

5.2 Comparisons to other species and previous research in northern Australia

Our results are broadly consistent with observations of negligible genetic population structure in *R. acutus* sampled in north-eastern Australian waters (Ovenden et al., 2011). Genetic analyses of other small to medium-sized carcharhinids in the region have commonly identified discrete genetic stocks on either side of Cape York, with little genetic structure within either of those regions. For example, little genetic structure occurs in Silvertip Sharks (*C. albimarginatus*) between PNG and eastern Australia (Green et al., 2019). The larger Spinner Shark (*C. brevipinna*) shows little genetic structure across northern Australia, including across Torres Strait (Geraghty et al., 2013), and the much larger Tiger Shark (*Galeocerdo cuvier*) demonstrates effectively no genetic structure across its Indo-Pacific range (Holmes et al., 2017).

In Australia, many of the commercially targeted shark species occur over multiple jurisdictions within Australia and across the world (Benavides et al., 2011; Devloo-Delva et al., 2019; Geraghty et al., 2014; Ovenden et al., 2009). The majority of these species are large, highly mobile, pelagic species, and this presents issues for fisheries management**.** The dispersal of sharks is entirely reliant on their movement, as they lack a larval stage, unlike other fishes. Genetics can aid fisheries managers to understand the scale at which stocks can be managed (Benavides et al., 2011). Stocks may be managed within a single jurisdiction or over multiple jurisdictions and, as sharks tend to be highly mobile, arrangements between jurisdictions are usually required for their management (Ovenden et al., 2009). Our results indicate that these two sharks have similarly low levels of genetic structure as other related species. From the perspective of fisheries management within the NT, our genetic findings present no evidence for the need to manage multiple stocks of these two species in the NT. However, the low genetic differentiation between NT populations and those sampled in WA and PNG suggest the potential need for a degree of coordinated monitoring and management of these species across the scale of this project (including international waters).

5.3 Estimating effective population size of *R. acutus* and *C. coatesi* from genetic data

One of our aims was to apply genetic effective population size estimation approaches to the data from the two shark species. Effective population size (N_e) is a concept from population genetics theory that corresponds to the size of an 'Wright-Fisher' population that would yield the observed genetic diversity parameters. A Wright-Fisher population is an idealised population of constant size where all members of the population can reproduce with each other. This is unrealistic, and N_e estimates are typically substantially lower than actual population sizes (sometimes by many orders of magnitude) (Turner et al., 2002), with species' life histories and patterns of population structure

influencing the degree to which N_e estimates the true population size (Palstra & Ruzzante, 2008). This means that N_e is not an estimate of true population size. However, for a given species and population, temporal trends in N_e can be a useful indicator of trends in abundance (Pierson et al., 2018).

Our results from the genetic population structure analyses suggest that N_e estimators are appropriate to apply across the study region, as it can be considered a single, unstructured population for these purposes. In that context, the N_e estimates from the two species may provide a useful baseline from which to use genetic data to monitor changes in abundance of these species in the sampled region. While we do not know the ratio of N_e to true population size of these species in the study region, evidence from previous research on shark species indicate that N_e does not substantially underestimate 'census' population size (Dudgeon & Ovenden, 2015; Ovenden et al., 2016), with estimates from Zebra Sharks (*Stegostoma fasciatum*), White Sharks (*Carcharodon carcharias*) and the Sandbar Shark (*Carcharhinus plumbeus*) approximating capture-mark-recapture abundance estimates. Population size estimates from an alternative source of data (e.g. mark-recapture, biomass trawl surveys) would provide a useful calibration for estimating N_e/N ratios for *R. acutus* and *C. coatesi,* and should be considered in any future biomass survey work (i.e.Knuckey &n Koopman, 2022) Given the wide geographic range of both species and their apparent abundance as bycatch, with 2354 kg's of this species caught in a recent biomass survey alone, the N_e estimates are an underestimate or the N_e/N ratios in these species are substantially lower than in other shark species. Consequently, we suggest further investigation into genetic approaches for estimation of abundance and effective population size in *R. acutus* and *C. coatesi*.

6 Implications

The key implications of this research for the development of a byproduct commercial fishery opportunity for *R. acutus* and *C. coatesi* in the NT are as follows.

- *R. acutus* and *C. coatesi* have high population connectivity within NT coastal and offshore waters, and genetic evidence suggests they each function as a single population on the inter-generational timescale relevant to genetic analyses.
- Management decisions based on genetic data alone would suggest that one biological stock exists for each species in the NT, and it could be monitored and managed as such.
- The population of these species in NT coastal and offshore waters is highly connected to populations in WA and PNG waters. Long-term monitoring and management of future byproduct fisheries may require some degree of coordination across jurisdictions, because harvest of either species in one jurisdiction may have implications for the species in another jurisdiction.
- We know very little about the individual movement and habitat use of *R. acutus* and *C. coatesi* individuals on seasonal or annual timescales. Data from vertebral chemistry and parasite studies are presented in comparison to these genetic data in a PhD thesis that was

conducted in conjunction with this project (Kirke, 2024) and suggests shorter-term patterns of site fidelity (eastern vs western NT) for individual *C. coatesi.*

- Effective population size estimation from genetic data is feasible, but the expected ratio of effective to 'census' population sizes is unknown, and the power of effective population size estimates to indicate trends in census population size would require some temporal sampling in conjunction with other data sources on population trends (e.g. catch per unit effort, biomass estimates).

7 Recommendations

The results of our genetic study have been communicated to the NT Government, the NT Offshore Net and Line Fishery and the Demersal Fishery to inform discussions around development of byproduct commercial fishery opportunities. While the genetic information presented here indicates a highly admixed breeding population within the sampled region for each species, the data should ideally be interpreted in the context of other sources of information, such as vertebral chemistry or parasitology, that can be indicative of other aspects of individual movement, to provide a fuller picture of the spatial and temporal scales of population connectivity and movement behaviour for stock assessment. In summary, the results of this study suggest that these two species can each be managed as a single stock in the NT. However, information on individual seasonal or annual movement patterns and breeding biology may help to refine management strategies for sustainable harvest.

8 Extension and adoption

The results of this genetic study have been communicated to the NT Government and the NT Offshore Net and Line Fishery and Demersal Fishery at meetings involving representatives of our research project, government and the fisheries industry. Charles Darwin University and NT DITT have regular monthly meetings for research updates, and planning around further project communication will occur in this forum.

The PhD candidate involved in this project has completed a PhD thesis that reports on other aspects of the biology of these two shark species and compares the genetic data in this report to other indicators of population connectivity. Dr Amy Kirke will prepare a scientific journal article on identification of population connectivity in *R. acutus* and *C. coatesi* in the NT region, as well as presenting findings at a relevant fisheries management conference.

References

Archer, F. I., Adams, P. E., & Schneiders, B. B. (2017). stratag: An r package for manipulating, summarizing and analysing population genetic data. *Molecular Ecology Resources, 17*(1), 5–11. https://doi.org/10.1111/1755-0998.12559

Barton, D. P., Taillebois, L., Taylor, J., Crook, D. A., Saunders, T., Hearnden, M., Greig, A., Welch, D. J., Newman, S. J., Travers, M. J., Saunders, R. J., Errity, C., Maher, S., Dudgeon, C., & Ovenden, J. (2018). Stock structure of *Lethrinus laticaudis* (Lethrinidae) across northern Australia determined using genetics, otolith microchemistry and parasite assemblage composition. *Marine and Freshwater Research, 69*, 487–501. https://doi.org/10.1071/MF17087

Benavides, M., Horn, R. L., Feldheim, K., Shivji, M., Shelley, C., Wintner, S., Natanson, L., Braccini, M., Boomer, J., Gulak, S., & Chapman, D. (2011). Global phylogeography of the dusky shark *Carcharhinus obscurus*: Implications for fisheries management and monitoring the shark fin trade. *Endangered Species Research, 14*, 13–22. https://doi.org/10.3354/esr00337

Bravington, M. V., Skaug, H. J., & Anderson, E. C. (2016). Close-kin mark-recapture. *Statistical Science, 31*(2), 259–274. https://doi.org/10.1214/16-STS552

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. *Frontiers in Ecology and Evolution, 6*. https://doi.org/10.3389/fevo.2018.00187

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science, 1*, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

Cullingham, C. I., Miller, J. M., Peery, R. M., Dupuis, J. R., Malenfant, R. M., Gorrell, J. C., & Janes, J. K. (2020). Confidently identifying the correct K value using the ΔK method: When does K = 2? *Molecular Ecology, 29(*5), 862–869. https://doi.org/10.1111/mec.15374

Devloo-Delva, F., Mcallister, J. D., Thomson, R. B., Maes, G. E., Hernández, S. I., Grewe, P. M., & Feutry, P. (2019). Accounting for kin sampling reveals genetic connectivity in Tasmanian and New Zealand school sharks, *Galeorhinus galeus*. *Ecology and Evolution, 9*, 4465–4472. https://doi.org/10.1002/ece3.5012

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). N_eEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources, 14*(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Domingues, R. R., Hilsdorf, A. W. S., & Gadig, O. B. F. (2018). The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics, 19*, 501–525. https://doi.org/10.1007/s10592-017-1038-3

Dudgeon, C. L., & Ovenden, J. R. (2015). The relationship between abundance and genetic effective population size in elasmobranchs: An example from the globally threatened zebra shark *Stegostoma fasciatum* within its protected range. *Conservation Genetics, 16*(6), 1443–1454. https://doi.org/10.1007/s10592-015-0752-y

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology, 14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Geraghty, P. T., Williamson, J. E., Macbeth, W. G., Wintner, S. P., Harry, A. V., Ovenden, J. R., & Gillings, M. R. (2013). Population expansion and genetic structure in *Carcharhinus brevipinna* in the southern Indo-Pacific. *PLOS ONE, 8*(9), e75169. https://doi.org/10.1371/journal.pone.0075169

Geraghty, P. T., Williamson, J. E., Macbeth, W. G., Blower, D. C., Morgan, J. A. T., Johnson, G., Ovenden, J. R., & Gillings, M. R. (2014). Genetic structure and diversity of two highly vulnerable carcharhinids in Australian waters. *Endangered Species Research, 24*, 45–60. https://doi.org/10.3354/esr00580

Green, M.E., Appleyard, S.A., White, W., Tracey, S., Devloo-Delva, F., Ovenden, J.R. (2019). Novel multimarker comparisons address the genetic population structure of silvertip sharks (*Carcharhinus albimarginatus*). *Marine and Freshwater Research 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. *Molecular Ecology Resources, 18*(3), 691–699. https://doi.org/10.1111/1755-0998.12745

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. *Royal Society Open Science, 4*(7), 170309. https://doi.org/10.1098/rsos.170309

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics, 23*(14), 1801–1806. https://doi.org/10.1093/bioinformatics/btm233

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics, 27*(21), 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Caig, V., Heller-Uszynska, K., Jaccoud, D., & Hopper, C. (2012). Diversity arrays technology: A generic genome profiling technology on open platforms. In *Data production and analysis in population genomics* (pp. 67–89). Springer.

Kirke, A. (2024). *Life history of two bycatch shark species in Northern Territory commercial trawl fisheries* [PhD]. Charles Darwin University.

Knuckey, I., Koopman, M., (2022). Survey of Tropical Snapper in Northern Territory Fisheries — 2021. Fishwell Consulting, Queenscliff.

Laikre, L., Palm, S., & Ryman, N. (2005). Genetic population structure of fishes: Implications for coastal zone management. *Ambio, 34*(2), 111–119. https://doi.org/10.1579/0044-7447-34.2.111

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, 119*(3), 142–153. https://doi.org/10.1038/hdy.2017.21

Northern Territory Government. (2018). *Offshore net and line fishery*. Northern Territory Government. https://nt.gov.au/marine/commercial-fishing/offshore-net-and-line-fishery

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 Evolutionary Biology, 9*(1), 1–15. https://doi.org/10.1186/1471-2148-9-40

Ovenden, J. R., Morgan, J. A. T., Street, R., Tobin, A., Simpfendorfer, C., Macbeth, W., & Welch, D. (2011). Negligible evidence for regional genetic population structure for two shark species *Rhizoprionodon acutus* (Rüppell, 1837) and *Sphyrna lewini* (Griffith & Smith, 1834) with contrasting biology. *Marine Biology, 158*(7), 1497–1509. https://doi.org/10.1007/s00227-011-1666-y

Ovenden, J. R., Leigh, G. M., Blower, D. C., Jones, A. T., Moore, A., Bustamante, C., Buckworth, R. C., Bennett, M. B., & Dudgeon, C. L. (2016). Can estimates of genetic effective population size contribute to fisheries stock assessments? *Journal of Fish Biology, 89*(6), 2505–2518. https://doi.org/10.1111/jfb.13129

Palstra, F. P., & Ruzzante, D. E. (2008). Genetic estimates of contemporary effective population size: What can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology, 17*(15), 3428–3447. https://doi.org/10.1111/j.1365-294X.2008.03842.x

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics, 28*(19), 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pearman, W. S., Urban, L., & Alexander, A. (2022). Commonly used Hardy–Weinberg equilibrium filtering schemes impact population structure inferences using RADseq data. *Molecular Ecology Resources, 22*(7), 2599–2613. https://doi.org/10.1111/1755-0998.13646

Pierson, J. C., Graves, T. A., Banks, S. C., Kendall, K. C., & Lindenmayer, D. B. (2018). Relationship between effective and demographic population size in continuously distributed populations. *Evolutionary Applications, 11*(7), 1162–1175. https://doi.org/10.1111/eva.12636

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

R Core Team. (2024). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*. https://www.R-project.org

Smouse, P. E., & Peakall, R. (1999). Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity, 82*(5), 561–573). https://doi.org/10.1038/sj.hdy.6885180

Turner, T. F., Wares, J. P., & Gold, J. R. (2002). Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). *Genetics, 162*(3), 1329–1339. https://doi.org/10.1093/genetics/162.3.1329

Walker, T.I. (2005). Chondrichthyan reproduction in fisheries science, in: Hamlett, W.C. (Ed.), *Reproductive Biology and Phylogeny of Chondrichthyes*. Science Publishers, Plymouth.

Wang, J. (2017). The computer program structure for assigning individuals to populations: Easy to use but easier to misuse. *Molecular Ecology Resources, 17*(5), 981–990. https://doi.org/10.1111/1755-0998.12650

Wang, J., Santiago, E., & Caballero, A. (2016). Prediction and estimation of effective population size. *Heredity*, 117(4), 193–206. https://doi.org/10.1038/hdy.2016.43

Waples, R. S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci*. *Conservation Genetics, 7*(2), 167–184. https://doi.org/10.1007/s10592-005-9100-y

Waples, R. S., & Do, C. (2010). Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: A largely untapped resource for applied conservation and evolution. *Evolutionary Applications, 3*(3), 244–262. https://doi.org/10.1111/j.1752-4571.2009.00104.x

Zhou, S., & Griffiths, S. P. (2008). Sustainability Assessment for Fishing Effects (SAFE): A new quantitative ecological risk assessment method and its application to elasmobranch bycatch in an Australian trawl fishery. *Fisheries Research, 91*, 56–68. https://doi.org/10.1016/j.fishres.2007.11.007

Project materials developed

PhD thesis: Kirke, A. (2024). Life History of Two Bycatch Shark Species in Northern Territory Commercial Trawl Fisheries [PhD]. Charles Darwin University.

Genotyping methods: The *Rhizoprionodon* DartSeq 1.0 genotyping protocol developed for this project is commercially available through Diversity Arrays Technology Pty Ltd. An existing protocol was available through this supplier for *Carcharhinus.*

Data: Sampling locations of individuals and full unfilltered SNP genotypes are included in the dataset "Single nucleotide polymorphism genotypes for the Australian blackspot shark and the milk shark in northern Australian waters" published online via Data Dryad at doi:10.5061/dryad.7h44j103q.