Understanding the movement and dispersal of saltwater crocodiles (*Crocodylus porosus*) within and around Australia

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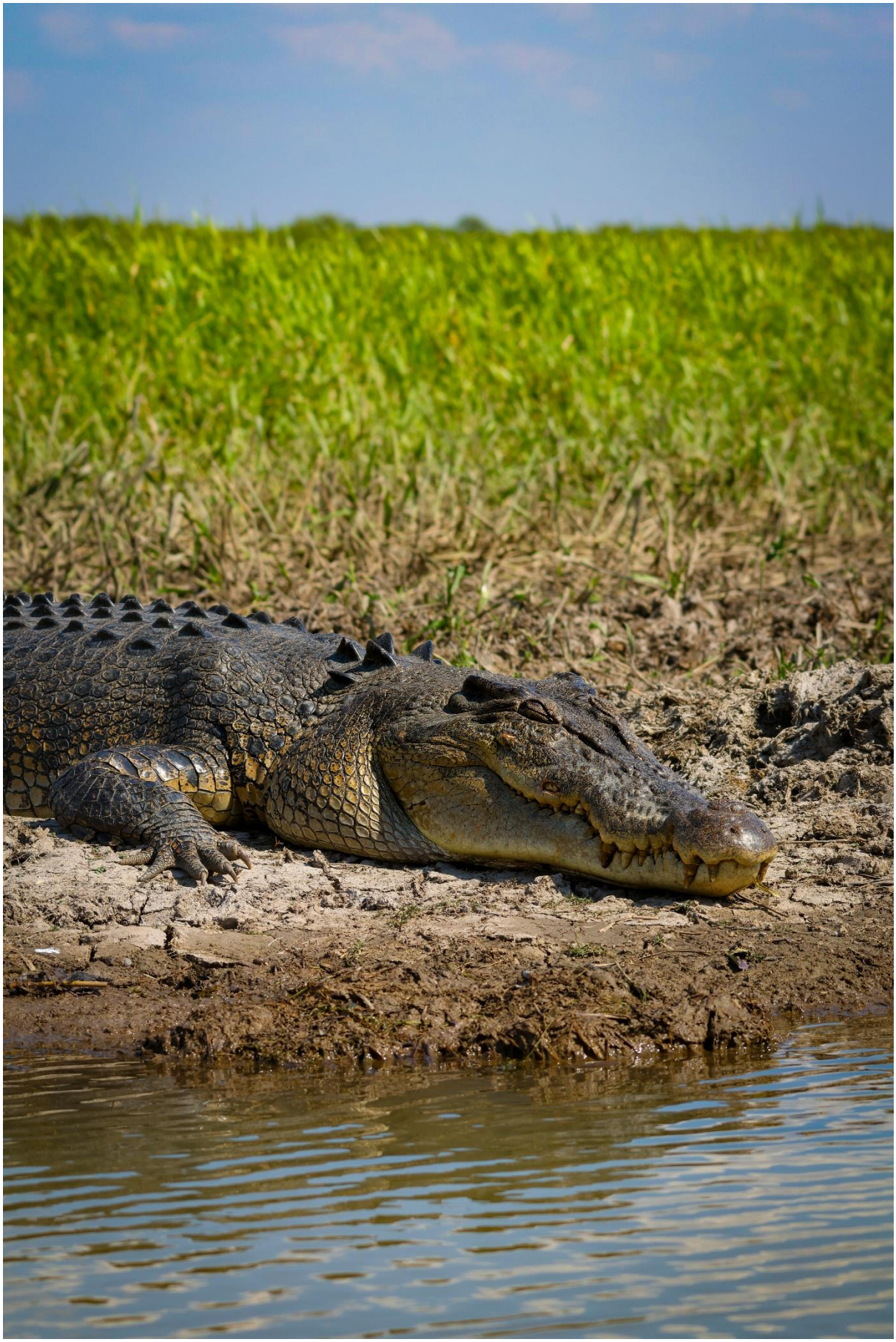

DECLARATION

The research presented in this thesis is my own original work. All the chapters and papers are coauthored with the supervisors and collaborators. No part of this thesis has been submitted for any previous degree. Chapters 2, 3, and 4 have been published in peer-reviewed journals as below. Chapter 5 is being prepared for submission to a journal.

Chapter 2:

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Chapter 3:

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Chapter 4:

Fukuda, Y., Moritz, C., Fitzsimmons, N., Jang, N., Webb, G., Lindner, G., Campbell, H., Christian, K., Leeder, S., and Banks, S. (2024). Natal origin and dispersal of problem saltwater crocodiles in the Darwin Harbour, Australia. Journal of Wildlife Management 88(2), e22525.

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ABSTRACT OVERALL

Human-crocodile conflict (HCC) is becoming a conservation challenge worldwide. The saltwater crocodile (*Crocodylus porosus*) is the largest (>6 m total length, >1000kg) and most aggressive living crocodilian species, being responsible for increasing attacks on people and domestic animals in many countries. This species is highly adapted to both freshwater and saline environments and is widely distributed in the Indo-Pacific region. However, their complex movement and dispersal patterns remain largely unknown. In the first chapter, we examined the spatial events implicated in the homing ability of crocodiles that complicate management interventions aimed at reducing HCC. Five large male crocodiles were shifted and released 100-320 km from their capture sites, and three additional ones were released at their capture sites as controls. Translocated crocodiles were more mobile than the controls and moved at sea in the direction of their original capture site. However, they were unable or unwilling to swim around a geographic structure, the Cobourg Peninsula, which prevented homing from being achieved in all five cases. Genetic analysis of tissue samples from nests demonstrated significant genetic structure across the coast and confirmed that the Cobourg Peninsula contributes to genetic differentiation among populations along the coast. The second chapter demonstrated environmental influences on crocodiles' dispersal, which comprised emigration, movement and settlement. We found that both environmental resistance and properties of the source and destination catchments (i.e. the proportion of breeding habitat which limits carrying capacity) were important factors influencing observed dispersal events. Competition for habitat influences emigration and settlement choices, and environmental resistance to movement occurred, in which high-quality habitat was associated with the greatest environmental permeability. Approximately 42% of crocodiles were migrants from populations other than their sampling locations, and some outstandingly productive populations such as the Goyder River (Arafura Swamp) had a much higher proportion of emigration than immigration. The distance most commonly travelled between source and destination was 150-200 km, although a few travelled much longer distances (600-700 km). The third chapter identified the natal origins of 95 crocodiles caught as problem animals in Darwin Harbour, where habitats suitable for breeding are extremely limited. Population assignments showed most crocodiles came from populations within 200 km, namely the Adelaide and Mary Rivers and Kakadu region as the most significant source (approximately 50%), the Daly, Finniss and Reynolds Rivers as the second (approximately 30%), and Tiwi Islands as the third (approximately 15%). Attributes such as their total length (most commonly 150-180 cm), sex (75% male), and the

year of capture (2015-2017) were not significantly correlated to the distance that they travelled to the harbour. Given that most crocodiles found in Darwin Harbour are migrants from multiple sources, we argue that adaptive measures such as removing crocodiles in the defined management zones may be more efficient at reducing risk to public safety than more proactive approaches, such as interfering with populations at the targeted source. The last chapter measured the population structure of crocodiles in Australia and its neighbouring countries. The analyses showed that populations can be separated at the broadest level into Oceania (Australia and New Guinea) and Southeast Asia (Borneo, Java, Peninsular Malaysia, Mindanao, and Sumatra), broadly aligned with Sunda and Sahul shelves of the Last Glacial Maximum. Results suggested that the genetic structure was affected by barriers such as deep straits and high mountains that effectively isolated the populations. We found no genetic evidence of gene flow between Australia and Timor-Leste, which has been posited as a possible explanation for apparently increasing crocodile attacks in Timor-Leste, probably due to the Timor Sea and the strong Indonesian Throughflow. For a more comprehensive mapping of the genetic connectivity across the range, more extensive sampling will be required. The findings from this study shed light on a better understanding of the movement of crocodiles and provided implications for HCC management in Australia and other countries.

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CHAPTER 1: Introduction overall

1.1 Human-crocodile conflicts

Human-wildlife conflicts are a conservation challenge in many countries in the social-ecological contexts (Dickman 2010a; Pooley *et al.* 2021), especially for large predators, because their carnivorous nature poses a serious risk to the safety of people and stock (Treves and Karanth 2003; Lozano *et al.* 2019). HCC is a good example. There are 27 species of living crocodilians currently recognised (Shirley *et al.* 2018; Stevenson 2019; Murray *et al.* 2019), and although not all of them attack people, Nile crocodiles (*Crocodylus niloticus*), saltwater crocodiles (*C. porosus*), and mugger crocodiles (*C. palustris*) are responsible for at least more than 1600 deaths between 2011 and 2021 (Sideleau 2021). Since many incidents remain unreported in many countries where these species are distributed, this number is likely underestimated (Sideleau and Britton 2016; Brackhane *et al.* 2018; Pooley *et al.* 2018).

1.2 Crocodile movement – why does it matter?

The management of human-wildlife conflicts requires integrated, interdisciplinary approaches such as social, cultural, and scientific measures to promote conservation efforts to mitigate negative interactions (Manfred and Dayer 2004; Dickman 2010b; Wieczorek Hudenko 2012; Castillo-Huitrón *et al.* 2020). While these are all crucial components, the initial step to mitigation, as a prerequisite for effective safety management, is a good understanding of the species' biology.

Understating the patterns of movement and dispersal of a variety of large carnivores, including bears (Bauder *et al.* 2020; Morales-González *et al.* 2020; Gillikin *et al.* 2021), big cats (Bauer and de Iongh 2005; Fattebert *et al.* 2015; Lamichhane *et al.* 2017; Naha *et al.* 2021), and wolves (Bradley *et al.* 2005; Marucco and McIntire 2010; Ditmer *et al.* 2023), provides fundamental implications for the management of conflicts with humans. Crocodilians are no exception - their movement is critical to answering important management questions such as 1) identifying likely sources and sinks of problem crocodiles near settlements and 2) assessing the effectiveness of relocating or removing them. However, their movement and dispersal, which can be extensive and ecologically complicated (Campbell *et al.* 2010; Campbell *et al.* 2013; Baker *et al.* 2019; Baker *et al.* 2022), is one of the most significant knowledge gaps. This is especially true for highly mobile species such as *C. porosus* found in freshwater, brackish, and saltwater (Taplin and Grigg 1981; Webb and Manolis 1989). Moreover, the remarkable recovery of many populations from previous hunting (Fukuda et al. 2011; Taplin et al. 2020),

re-occupying previous habitats where their presence was not known until recently (Webb 2012), is adding another layer of complexity to the difficulty of co-existence of humans and crocodiles in many countries including Australia. This study aims to focus on the movement patterns of *C. porosus*.

1.3 Study species

Crocodylus porosus, commonly known as saltwater or estuarine crocodiles, is the largest extant crocodilian species. Typical males' maximum Total Length (TL) is around 4.6 m (Webb and Manolis 1989), but some individuals may grow over 6 m. A notably large male captured alive in Mindanao, Philippines, in 2011 was measured to be 6.17 m and 1075 kg (Britton *et al.* 2012). Some historical records and associated skulls suggest that a few individuals reached approximately 6.7 m TL in the Northern Territory (NT), Australia (Manolis 2006) and Java, Indonesia (Fukuda *et al.* 2018). Conversely, females are smaller than males and rarely grow over 3.5 m TL (Webb and Manolis 1989).

C. porosus is one of 16 Crocodylidae species within the 27 species of extant crocodilians (Stevenson 2019). *C. porosus* is considered to be a monotypic species (Brazaitis 2001; Brazaitis and Watkins-Colwell 2011), although a few different variant species or subspecies were previously suggested, including *C. pethericki* in Australia (Wells 1985), *C. p. minikanna* in Sri Lanka (Deraniyagala 1939; Deraniyagala 1955), and *C. raninus* in Borneo (Ross 1990; Ross 1992; Cox *et al.* 1993).

Distribution and status

The distribution of *C. porosus* extended throughout Southeast in the Indo-Pacific region before many populations were depleted due to hunting and habitat loss in the last century (Webb *et al.* 2010; Grigg and Kirshner 2015). The historical range included southeast India and Sri Lanka in the west, southern China and the Philippines in the north, northern Australia in the south, and Vanuatu and Fiji in the east (Groombridge 1987; Grigg and Kirshner 2015; Spennemann 2021). Wild populations in southern China, Fiji, Thailand, and Vietnam are considered extinct (Webb *et al.* 2010; Grigg and Kirshner 2015), but itinerants are increasingly sighted in some habitats previously occupied, such as Bali in Indonesia, Caroline Islands in Micronesia, and New Caledonia (Grigg and Kirshner 2015; Van der Ploeg *et al.* 2019; Spennemann 2021).

They are categorised as Least Risk (LR) and Least Concern (LC) under the Red List of Threatened Species by the International Union for Conservation of Nature (IUCN). As of May

2023, populations in Australia, Papua New Guinea, Indonesia, Malaysia (Sarawak), and the Philippines (the Palawan Islands) are listed in Appendix II under the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES 2023; UNEP 2023). All other populations are listed in Appendix I.

Distribution and status in Australia

In Australia, *C. porosus* is commonly found in the coastal areas of the NT, Queensland, and Western Australia. They are distributed across the NT around Gladstone in Queensland (Queensland Department of Environment and Heritage 2017) and Port Hedland in Western Australia (Department of Environment and Conservation 2009). There have been many records of vagrants sighted well outside the range (Mawson 2004; Grigg and Kirshner 2015; Spennemann 2021). However, these animals are unlikely to settle there as the cold climate does not allow them to breed. It is estimated that there are about 100,000 non-hatchlings in the NT, 20-30,000 in Queensland, and 11,000 in Western Australia (Taplin *et al.* 2020; Fukuda *et al.* 2021).

They are a protected species listed as marine and migratory species under Australia's Environment Protection and Biodiversity Conservation Act 1999 (Australian Government 2021). In the NT, they are a protected species listed as least concern under Territory Parks and Wildlife Conservation Act 1976 (NT Government 2022). Likewise, they are listed as vulnerable under the Nature Conservation Act 1992 in Queensland (Queensland Government 2022) and the Biodiversity Conservation Act 2016 in Western Australia (Government of Western Australia 2023).

Habitats

C. porosus is one of few crocodilian species that are adapted to the saline environment as well as freshwater (Taplin and Grigg 1981; Franklin and Grigg 1993; Taylor *et al.* 1995; Cramp *et al.* 2008; Fukuda *et al.* 2021). They are found in diverse habitats, ranging from inland freshwater swamps, lakes, billabongs, floodplains, and rivers to fully saline coastal waterbodies such as coral reefs, sandy beaches, and mangroves (Webb and Manolis 1989; Grigg and Kirshner 2015). The highest density is often found in permanent, brackish water such as tidal rivers (Webb 1991; Fukuda *et al.* 2011). Accordingly, as the apex predators in these various habitats, *C. porosus* preys on numerous items depending on their sizes, such as insects, crustaceans, amphibians (frogs), reptiles (including crocodiles), birds, and mammals although

the most common prey throughout the life is fish (Webb *et al.* 1991; Hanson *et al.* 2015; Grigg and Kirshner 2015; Campbell *et al.* 2022).

Despite their adaptation to saltwater, *C. porosus*, as in the other crocodilian species (Brazaitis and Watanabe 2011), prefers freshwater habitats for nesting (Magnusson 1980; Fukuda and Cuff 2013; Fukuda *et al.* 2022). In the NT of Australia, nesting habitats are characterised by certain freshwater vegetation species such as *Oryza* and *Eleocharis* in tall, closed tussock grassland and *Eucalyptus*, *Melaleuca*, and *Pandanus* in open forests or woodland, being most commonly used for building a mound nest (Magnusson 1980; Webb *et al.* 1983; Fukuda and Cuff 2013). Some nests are found on the floating vegetation mats of several species in freshwater billabongs (Hill and Webb 1982). *C. porosus* nests are vulnerable to flooding during a wet season, and the mortality of eggs tends to be very high (~75% on average) while highly variable over the years (Webb *et al.* 1983; Webb *et al.* 1984).

Known patterns of movement and dispersal

C. porosus is highly territorial throughout life. They show agonistic behaviour and intolerance towards conspecifics shortly after hatching, already establishing dominance hierarchies at an early age (Brien *et al.* 2013a; Brien *et al.* 2013b). Although hatchlings have functional salt glands and are capable of tolerating saline water (Grigg *et al.* 1980; Taplin 1984), they tend to avoid saline environments and stay near nesting sites in a loose group in the first few weeks after hatchling (Webb and Manolis 1989; Fukuda and Saalfeld 2014). Limited observations indicate that, as hatchlings grow larger, they start dispersing from the nesting site. At five weeks after hatching, 91% of hatchlings remained within 1 km from the nesting site while that reduced to 26% after approximately 58 weeks (Webb and Messel 1978). Some male juveniles (60-100 cm TL), estimated around two years old, moved 80 km downstream of the river (Webb and Messel 1978).

It is considered common for juveniles, especially males, to leave their natal habitats in the tidal rivers and move downstream the river along the coast, looking for a new river to settle in (Webb and Messel 1978; Messel *et al.* 1981; Messel and Vorlicek 1986). In Darwin Harbour of the NT where the suitable nesting vegetation is minimal (Fukuda and Cuff 2013), a total of 4910 crocodiles were caught between 1977 and 2013 as a part of the safety program, 83.01% of which were male, with the most common TL being 150-200 cm (Fukuda *et al.* 2014). Earlier studies, based on the mark-recapture monitoring in the 1970-80s in Arnhem Land of the NT,

suggested that dispersed juveniles would attempt to return to the suitable breeding habitats once they reach maturity (Messel *et al.* 1981; Messel and Vorlicek 1986; Messel and Vorlicek 1987).

Males mature sexually at about 3.3 m TL (approximately 17 years old) while females mature at 2.3 m (13 years old) in most cases (Webb and Manolis 1989; Webb and Manolis 1993). Previous tracking studies consistently suggest that males have much larger home ranges with higher movement rates than females (Kay 2004; Brien *et al.* 2008; Campbell *et al.* 2013; Baker *et al.* 2022). In suitable breeding areas, dominant males are more site-attached (higher site fidelity) around females' home ranges than subordinate males that continually roam over a greater extent, probably searching for unguarded females (Campbell *et al.* 2013; Baker *et al.* 2022). These subordinate males may be responsible for multiple paternity (Campbell *et al.* 2013; Baker *et al.* 2022). Given the alpha male's strong territoriality, crocodilians are conventionally considered polygynous (one male mating many females). Still, the large extent of multiple paternity suggests that their mating system is promiscuous (i.e. many males mating many females) as noted by Lewis *et al.* (2013). However, their mating does not seem random, given some mate fidelity shown by females (Lance *et al.* 2009). Females tend to stay in a small home range (e.g. < 1km length of a permanent river), but during the breeding season (November - April in Australia), they migrate to nesting sites that can be more than 50 km away from their normal range (Campbell *et al.* 2013; Baker *et al.* 2019).

As in other crocodilians, *C. porosus* has apparent homing ability (Webb and Messel 1978; Woolard *et al.* 2004; Dominguez-Laso 2008). Webb and Messel (1978) suggested that dispersed subadults abovementioned can return to the natal river with suitable breeding habitats because of their homing instinct (Webb and Messel 1978). Tracking studies showed that both juveniles and adults, male and female crocodiles have strong homing when translocated (Walsh and Whitehead 1993; Read *et al.* 2007; Campbell *et al.* 2010). Campbell *et al.* (2010) reported that a translocated 4.84 m male travelled 411 km over 14 days to return to its capture site, using the water surface currents around Cape York, Australia.

1.4 Aims and objectives

Given its difficult nature to study, little is known about the dispersal behaviour of *C. porosus* despite its importance as an apex predator with the potential to negatively impact humans. The overall aim of this study is to investigate the patterns of the movement and dispersal by *C. porosus* within and around Australia, with a particular focus on the NT where the largest population in the species range occur. The thesis is divided into four main chapters that analyse

different aspects of their movement as below. Based on the results of each chapter, I attempt to derive implications for HCC management.

1. Chapter 2 examines the homing ability of some translocated *C. porosus* being tracked by satellites and the function of complex landscapes as a geographic barrier to their movement. Previous studies showed that *C. porosus* has a strong homing instinct over long distances in the sea. However, historical tracking data in the NT suggest that this may not always be the case if the landscape is intricate with complex coastlines and strong sea currents. To test possible interruption to the movement of crocodiles by a geographic barrier, I analyse: 1) the historical tracking of crocodiles translocated over the Cobourg Peninsula in the NT; and 2) the genetic relationships between populations on the west and east sides of the peninsula. If this landscape interrupts the homing of the crocodiles as implied by the historical tracking, I would expect that the populations in the west and east of the peninsula show significant genetic structuring.
2. Chapter 3 aims to quantify the dispersal of *C. porosus* in the NT and Western Australia and examine a hypothesis that their dispersal is influenced by environmental resistance that is inversely correlated with habitat quality. I predict that environmental resistance estimated from the genetic diversity of crocodiles and environmental properties, especially the availability of suitable breeding habitat, influence dispersal in three different processes (emigration, movement, and settlement). I test the hypothesis by modelling the environmental influences on the movement at each process. Furthermore, the analyses are expected to include information on the population structure, such as the proportions of migrants in each population and the distances that the migrants travel between the populations.
3. Chapter 4 focuses on Darwin Harbour of the NT and aims to identify the natal origins of *C. porosus* caught there as problem crocodiles. Given that suitable nesting habitats are extremely limited within Darwin Harbour and 2) *C. porosus* has high mobility in the saline environment, crocodiles caught in the harbour are most likely born outside the harbour. Given the demonstrated influences of environment on C. porosus dispersal (Chapters 2 and 3), , I anticipate that both distance and the presence of barriers predict natal origins of crocodile in Darwin Harbour. I also examine whether the sex and size of the crocodiles affect the migration distance from their origins.
4. Chapter 5 characterises the population structure of *C. porosus* in Australia and its neighbouring countries by identifying the genetic connectivity and geographic barriers

between the samples collected from different regions. The analysis tests whether crocodiles in the NT migrate to Timor-Leste as strongly believed by local people in the latter country. Given the results from the earlier chapters and previous studies, I predict that: 1) the populations exhibit spatial structure across the range; and 2) the long-distance migration, for example, between Australia and Timor-Leste (approximately 550 km apart), is insignificant. Findings on the large-scale movement from these analyses should provide implications for management, especially intergovernmental.

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CHAPTER 2: Translocation, genetic structure and homing ability confirm geographic barriers disrupt saltwater crocodile movement and dispersal

2.1. Abstract

Translocated saltwater crocodiles (*Crocodylus porosus*) in the Northern Territory (NT) of Australia often return to their original capture sites, which complicates management interventions aimed at reducing human-crocodile conflict. We examined the spatial events implicated in this homing ability, using ARGOS satellite tracking devices. Five large male *C. porosus* (3.03 m to 4.02 m TL) were shifted and released 100-320 km from their capture sites, and 3 additional individuals (3.67 m to 4.23 m TL) were released at their site of capture as controls. Translocated crocodiles were more mobile than the controls, and moved at sea in the direction of their original capture site. However, they did not swim around a geographic structure, Cobourg Peninsula, which prevented homing being achieved in all five cases. Two control crocodiles remained near their capture sites, but one, after the first year, made a 900km journey for six months, before returning to its original capture and release site. Genetic analysis of tissue samples from nests across the NT coast demonstrated significant genetic structure across the coast, and confirmed that Cobourg Peninsula contributes to genetic differentiation among populations along the NT coast. These results provide new insights into *C. porosus* movements, which have management significance for the maintenance of public safety.

2.2. Introduction

Saltwater crocodiles *Crocodyus porosus* are the most widely distributed of living crocodilians, extending from southern Asia to northern Australia (Webb and Manolis 1989; Webb *et al.* 2010). Throughout this range, wild populations were depleted during the 20th century, but are recovering at different rates in different countries (Webb *et al.* 2010). Saltwater crocodiles occupy coastal seas, rivers, and a diversity of saline and freshwater wetlands associated with them. They move between rivers at sea (Webb 1991; Campbell *et al.* 2013), and are known to make long-distance sea journeys beyond the coastal fringe (Webb *et al.* 2010; Brackhane *et al.* 2018). The coastal movements of *C. porosus* create management problems throughout their range, because *C. porosus* are large predators that can prey on humans (Kar and Bustard 1983; Caldicott *et al.* 2005; Fukuda *et al.* 2014; Brackhane *et al.* 2018). Their arrival in coastal areas inhabited by people often requires management interventions. The capture and translocation of "problem" *C. porosus* has had mixed success because of their homing ability (Walsh and Whitehead 1993).

Homing following translocation from distant sites has been reported for a range of crocodilian species, but only a few such movements have been reconstructed through use of telemetry techniques (e.g. *Alligator missisippiensis* tracked by radiotelemetry (Rodda 1984; Woolard *et al.* 2004), *C. porosus* by satellites (Read *et al.* 2007; Campbell *et al.* 2010) and *C. niloticus* by radio (Hocutt *et al.* 1992) and by satellites (Combrink 2015)).

When *C. porosus* were protected in the Northern Territory (NT) of Australia in 1971, the recovery of the wild population was monitored intensively (Messel *et al.* 1981; Webb *et al.* 2000; Fukuda *et al.* 2011), as was the concomitant increase in HCC (Nichols and Letnic 2008; Fukuda *et al.* 2014). "Problem" crocodile removal from Darwin Harbour is an ongoing management intervention aimed at reducing HCC (Saalfeld *et al.* 2016). That 250-300 *C. porosus* per year are removed from the harbour (Fukuda *et al.* 2014), where there is limited local breeding, indicates significant immigration via the coast, assumed to be from rivers to the east, west and north of Darwin, but potentially including immigrants from outside Australia. High mobility and random mating would favour a genetically homogenous population, but none of these assumptions about movement and genetic homogeneity have been tested, despite their importance to HCC management. Here we use satellite tracking and DNA analyses to test two null hypotheses that:

1. movements around the coast of the NT are not confounded by natural barriers; and
2. there are no genetically distinct subpopulations of *C. porosus* across the NT.

Given the high mobility and homing capability of *C. porosus* (Read *et al.* 2007; Campbell *et al.* 2010), satellite tracking focused on translocated individuals, but included some control animals released at their site of capture. The satellite tracking data provided detailed movement information about a small sample of translocated and control individuals. This generated a specific hypothesis about a potential movement barrier (the Cobourg Peninsula), which we tested with a genetic dataset on a larger number of individuals sampled from nests in different locations across the NT coastline.

2.3. Materials and methods

Study area and context

The "Top End" of the Northern Territory (approximately 400,000 km^2, Figure 2.1), contains many coastal rivers and creeks, lined with mangroves and under tidal influence near the sea, extending to a diversity of freshwater, non-tidal floodplain wetlands and sand and rock-lime

watercourses upstream. All are occupied by *C. porosus* at different densities (Webb 1991; Fukuda *et al.* 2007). The species roams along the coastline at sea between rivers, and occupies near-coastal islands. The eastern NT coastline is somewhat separated from the western coastline by a land mass associated with Coburg Peninsula (Figure 2.1).

The Top End climate is the tropical monsoon with a distinct wet season (November to April) and dry season (May to October). Available wetlands expand during the wet season and contract during the dry season (Webb 1991; Fukuda and Cuff 2013), when many temporary waterbodies dry out if early rains are delayed. Crocodiles tend to move back into permanent water areas during the dry season, but are sometimes forced to aestivate in drying mud. In the NT, there is limited intensive agriculture and most coastal and riparian habitats remain intact (Fukuda *et al.* 2007).

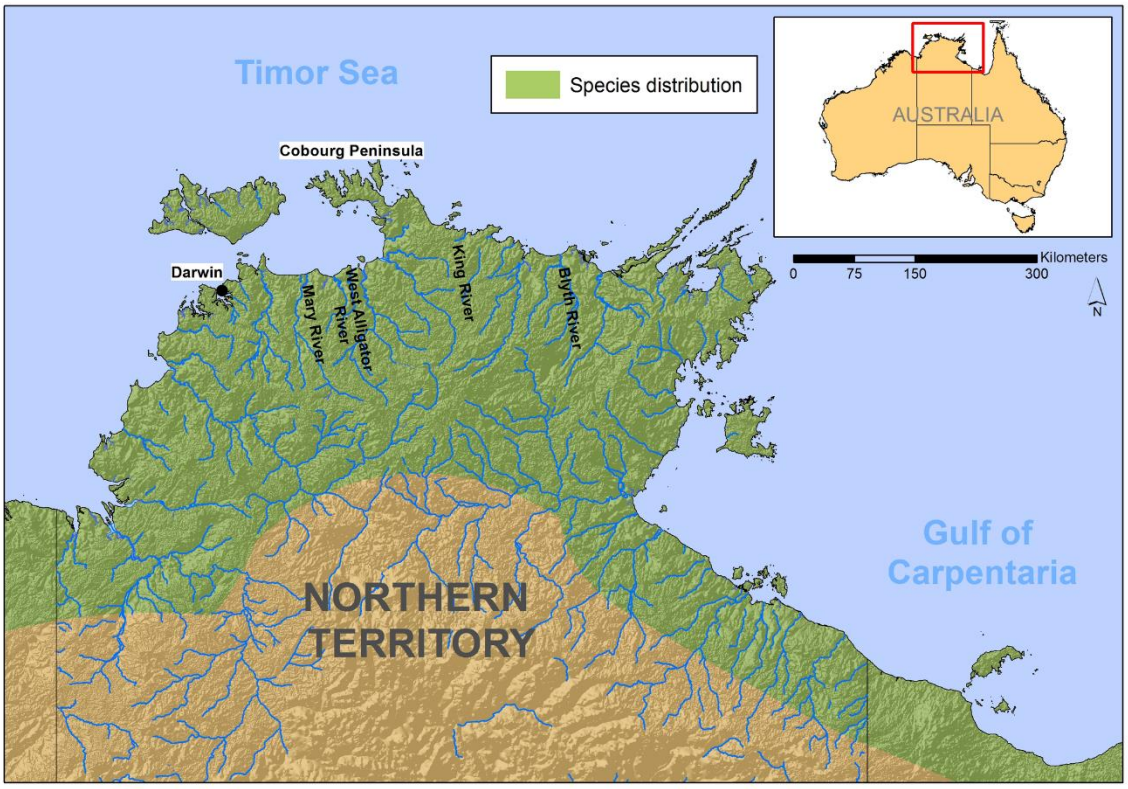

Figure 2.1. Study area in the Northern Territory of Australia. Distribution of *C. porosus* is approximate.

Satellite tracking

To examine movements of crocodiles relative to the Cobourg Peninsula, we used KiwiSat 101 satellite transmitters with specifications following Read et al. (Read *et al.* 2007). Transmitters were attached to the nuchal crest of the crocodiles (Brien *et al.* 2010) and data were retrieved with the ARGOS system. The results for 8 crocodiles are examined here. All were caught in the wild with traps and skin harpoons (Webb and Messel 1977). Based on total length (TL), six were mature (3.67 m to 4.23 m TL), one immature (3.03 m), and one possibly mature (3.27 m TL). On the western side of the Cobourg Peninsula, five crocodiles were caught in the Mary River, and either released near their site of capture (N = 3), transported 100 km further west and released at sea (N = 1), or moved 320 km to the eastern side of Cobourg Peninsula and released in the Blyth River (N = 2) (Figure 2.1). Conversely, crocodiles caught east of Cobourg Peninsula in the Blyth River (N = 1) and King River (N = 1), were translocated west and released in the Mary and West Alligator Rivers, respectively.

We filtered the ARGOS readings, using the R package argosfilter (Freitas 2012). The AROGS locations filter reduces noises in ARGOS data, based on ARGOS Location Class, maximum swimming speed of a tracked animal (threshold 2 meters per second), distance between successive locations, and turning angles (spikes with angles smaller than 15 and 25 degrees with extension higher than 2500 m and 5000 m to be removed) (Freitas *et al.* 2008). We sorted the extracted ARGOS data in chronological order based on the date and time of the signal reception, and mapped them for each of the tracked crocodile using ArcGIS.

Genetic analysis

We collected 192 *C. porosus* tissue samples from 19 locations across the Top End (Figure 2.2). All were pieces of skin from hatchlings that failed to develop to term during incubation. We sampled one hatchling only per clutch (nest). Eggs were collected at different locations as part of the NT's commercial egg-ranching program (Saalfeld *et al.* 2015; Saalfeld *et al.* 2016).

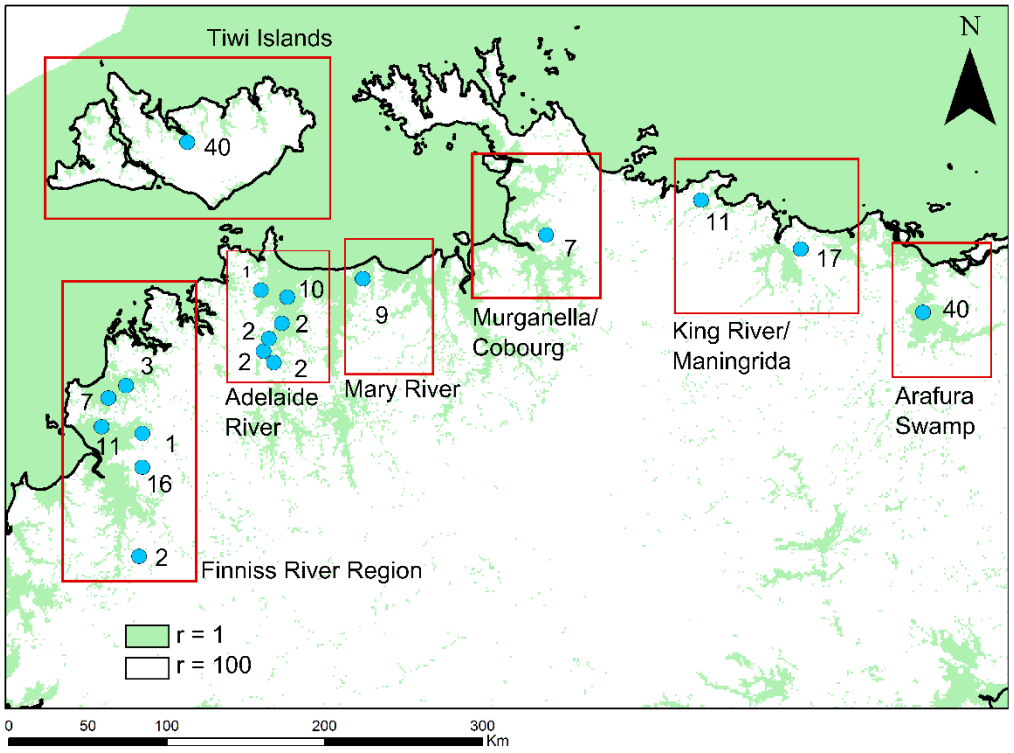

Figure 2.2. Nest locations (blue dots) and skin samples from each nest (numbers) used in the genetic analysis. Red boxes indicate site groupings used for population genetic diversity analysis. Green indicates actual and potential crocodile habitat during the wet season, coded as resistance = 1 in the analyses, and white areas of dry land, coded as resistance = 100 (see text).

DNA extraction and genotyping was conducted at Diversity Arrays Technology (Canberra, Australia) using the DArTseq approach (Kilian *et al.* 2012). DArTseq uses genome complexity reduction and NGS approaches conceptually similar to RADseq, in this case based around a *SphI* enzyme digestion of genomic DNA and Illumina HiSeq 2500 sequencing. Initial sequence processing and SNP-calling by DArT yielded 11,497 single-nucleotide polymorphism (SNP) loci. We used the dartR package in R 3.4.0 (Gruber *et al.* 2017) to filter SNPs according to call rate (CallRate > 0.95), repeatability (RepAvg > 0.95) and dropping 'secondary' SNPs (ranked by polymorphic information content) within sequences with more than one SNP present. We then examined a histogram of the F_{IS} values (inbreeding coefficient) at the filtered SNP loci (Figure S2.1). After this exploratory analysis, we did not filter our SNPs further. As an exploratory population genetics analysis, we estimated observed and expected heterozygosities within the major sampled regions (Figure 2.2) in dartR, as well as by pairwise F_{ST} using StAMPP (Pembleton *et al.* 2013) with 100 bootstraps.

To investigate whether the Cobourg Peninsula acts as a barrier to mixing of subpopulations, we fitted linear mixed-effects models to pairwise genetic similarity data among individuals, to compare models featuring fixed effects representing least-cost distance along watercourses and a putative additional barrier corresponding to the distinction between populations on either side of the Cobourg Peninsula. The genetic similarity metric that we used was a simple Identity-by-State (IBS) measure calculated in the R package SNPrelate (Zheng *et al.* 2012) on the filtered set of allele frequencies, imposing an extra minor allele frequency filter (retaining SNPs with MAF > 0.05) with linkage R^2 threshold criterion of 0.1, yielding 5,675 SNPs. Because multiple individuals came from most nests sampled (Figure. 2), our data points were the average pairwise IBS values among individuals sampled within the 19 sampling locations.

The basic ecological distance estimates among sampling locations were least-cost distances calculated in the R package gdistance (Etten 2017), using a landscape resistance surface with a resistance value of 1 for marine and freshwater areas corresponding to the northern Australian wet season. Areas unsuitable for crocodile movement (i.e. dry land areas not classified as suitable wet season habitat) were coded with an arbitrary resistance value of 100, indicating 100+ times as difficult to move through non-habitat than habitat. The effect of selecting these resistance values was that our ecological-distance matrix linked sampled crocodiles by aquatic movement pathways, excluding 'shortcuts' across land. We added a second candidate explanatory variable corresponding to binary 1/0 distances representing the putative Cobourg Peninsula barrier. We fitted linear mixed-effects models in the nlme R package (Pinheiro *et al.* 2013) using the maximum likelihood population effects (MLPE) correlation structure to account for multiple pairwise comparisons among a set of populations. We constructed the MLPE correlation structure using the R script available at https://github.com/nspope/corMLPE_unsupported/blob/master/corMLPE_unsupported.R and provide our own R scripts to analyze our genetic data (File S2). We fitted models featuring effects of: (1) ecological distance and the putative Cobourg Peninsula barrier; (2) ecological distance alone; (3) the putative Cobourg Peninsula barrier alone; and, (4) the intercept alone. We compared models with the AIC and evaluated significance of our candidate exploratory variables within each model.

As a supporting analysis, we used a genetic assignment test approach to estimate the ancestry of each sampled individual in a set of pre-defined geographic regions, using the R package assignPOP (Chen *et al.* 2018). Given our samples were from harvested eggs, our interest was in detecting recent ancestry from outside the sampling region. To scale up the population

assignment for our purpose of finding out differences in the population structure between the east and west of the Cobourg Peninsula, we re-defined the Darwin/Kakadu region by combining the Adelaide River, Mary River and Murganella Creek, and the northern Arnhem Land region by combining the King River/Maningrida and Arafura Swamp sampling areas (Figure 2.10). We kept the regions of the Tiwi Islands and Finniss River unchanged as these were distinct from the adjunct regions. We applied the MAF <0.05 filtering and used the assign.kfold function of the assignPOP package to estimate ancestry coefficients of each individual in each of the geographic 'reference populations'. For the analysis, we used the 50% of SNPs with the highest F_{ST} for assignment. We iteratively removed 10% of individuals from each population for assignment and used the remaining 90% of individuals for the reference assignment population, until all individuals had been analysed using the support vector machine (SVM) classification model (Chen *et al.* 2018).

2.4. Results

Satellite tracking

The tracking results of the four animals caught and released on the western side of the Cobourg Peninsula, are summarized on Figure 2.3. Crocodiles 60682 (3.85 m TL; 268 days of tracking) and 60683 (3.67 m TL; 48 days of tracking) remained in and around their capture site, and when they did venture to the coast, they stayed within 15 km of the mouth of the Mary River and showed no strong directional movement. Crocodile 34011 (4.23 m TL; 930 days of tracking) was caught 170 km upstream in the Mary River. During its first year, it remained at the capture and release site, but in October 2009, following heavy rains, it travelled downstream to the coast, then journeyed east around 120 km to the East Alligator River, before returning to the Mary River and travelling back upstream to its capture site. The journey involved some 900 km in six months. Crocodile 68551 (4.02 m TL; tracked 180 days) was caught in the downstream Mary River, translocated 100 km further west and released at sea near the Vernon Islands. Within 25 days, it had returned to its capture site in the Mary River, but it then travelled back out to the coast and eastward, moving between two specific inland coastal wetlands, 25 km and 90 km east of the Mary River. Details of each individual's movement are provided as Supporting Information (S3 File online).

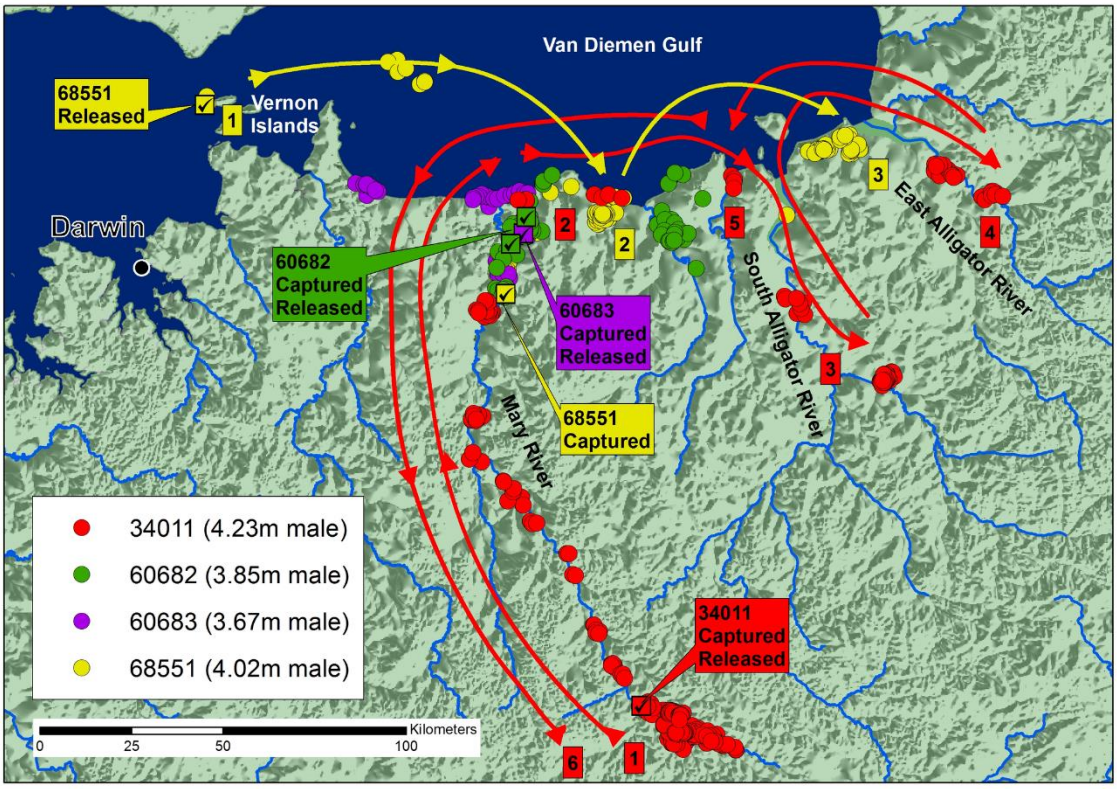

Figure 2.3. Locations and likely paths of movement of four crocodiles caught in the Mary River, three of which were released near the site of capture (60682, 60683, 34011) and one (68551) translocated 100km west and released at sea.

The crocodiles on the western side of the Cobourg Peninsula showed one or more activity centers within and between adjoining rivers, moving east or west, or upstream (south) and downstream (north), between them (Figure 2.3). Some form of refined navigational ability exists, which reflects itself in the rapid homing of the translocated individual. None of the crocodiles followed the coastline beyond the East Alligator River up towards Cobourg Peninsula.

Unlike the non-translocated animals, the crocodiles translocated between the western and eastern sides of Cobourg Peninsula showed different mobility patterns. Crocodile 60686 (3.81 m TL; 396 days tracking) was translocated from the Blyth River on the eastern side of Cobourg Peninsula, 320 km west to the Mary River (Figure 2.4). It was highly mobile around the release site, moving back and forth along the coast from the Adelaide River to the East Alligator River (160 km), and entering Sampan Creek in the Mary River and the South Alligator River. The closest point to the original capture site it reached, upstream in the East Alligator River, was approximately 200 km from the Blyth River capture site, but separated from it by land. No

seasonal trend in movement was detected, although the tracking period encompassed complete wet and dry seasons. By the last transmission, the crocodile had returned to the release site in the Mary River.

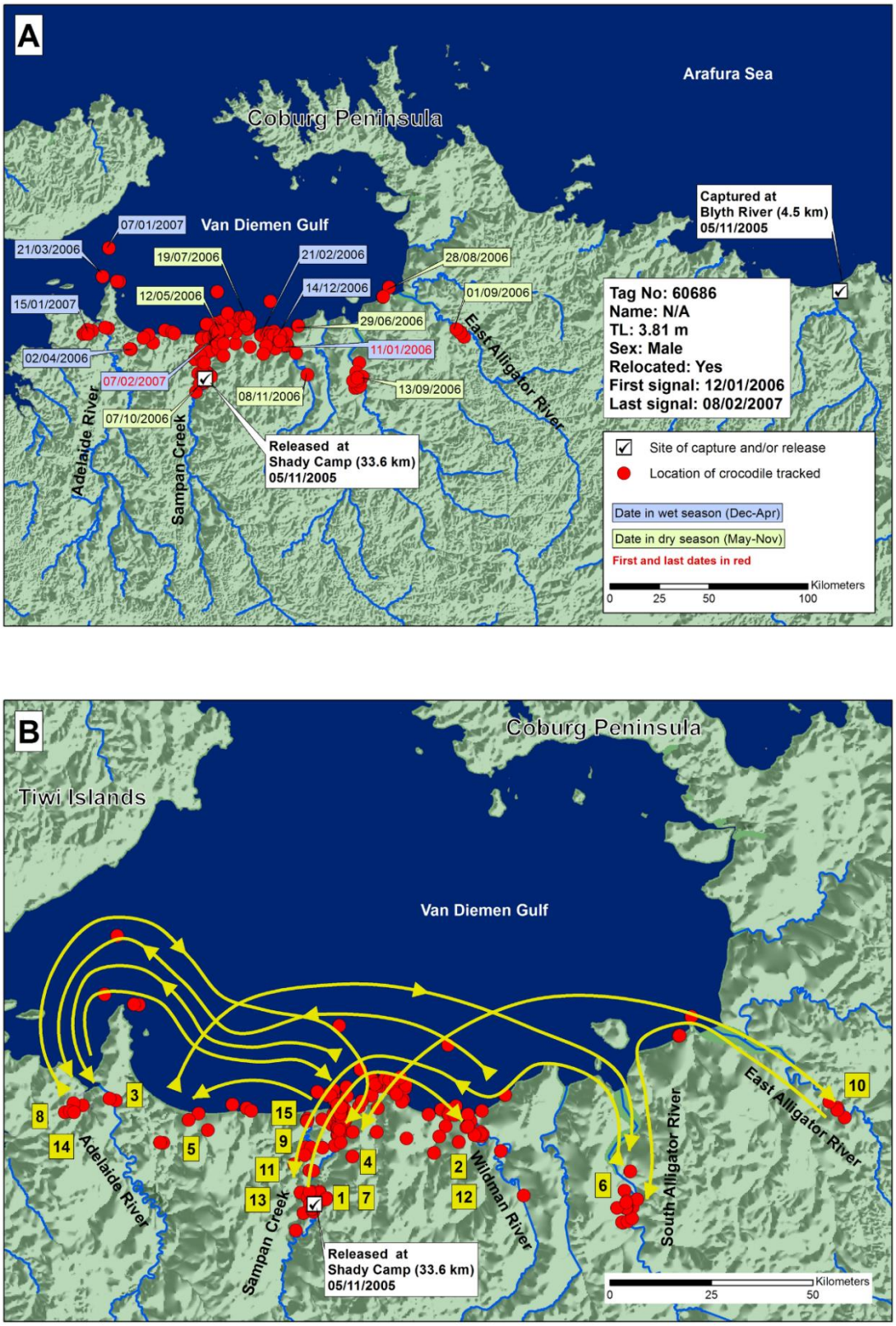

Figure 2.4. A) Locations and dates for crocodile 60686 translocated from the Blyth River east of Cobourg Peninsula to the Mary River, and B) likely paths chronologically numbered of crocodile travel.

Crocodile 61678 (3.03 m TL; tracked 708 days) was the smallest male in the study, and was assumed to be immature. It also was caught in the Blyth River and released 320 km to the west in the Mary River (Figure 2.5). It roamed east and west along 100 km of coastline in the first two months after release, then returned and stayed in a single area near the release site in Sampan Creek, Mary River. Almost two complete cycles of wet and dry seasons were included, with no seasonal movements obvious.

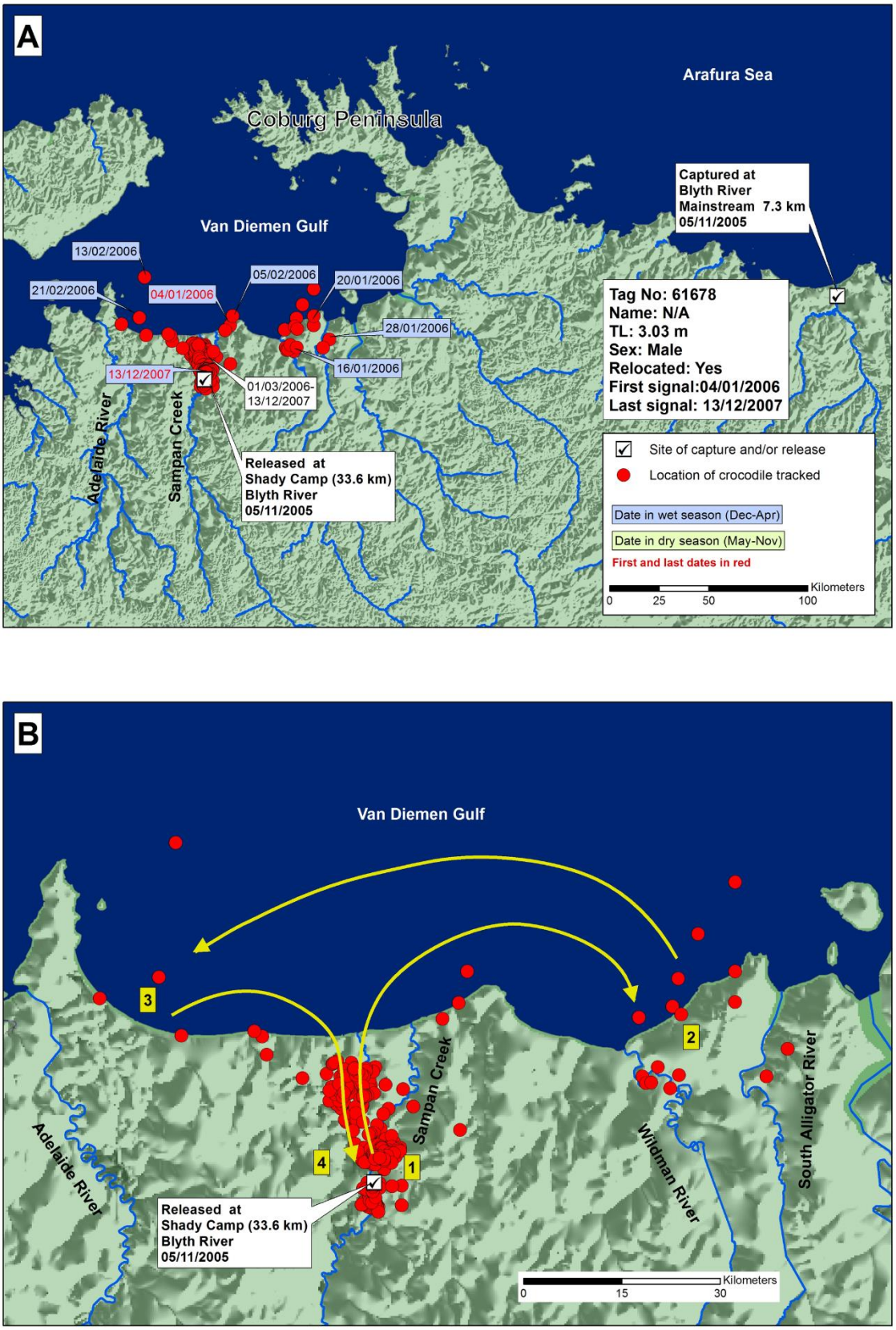

Figure 2.5. A) Locations and dates for translocated crocodile 61678, and B) likely paths chronologically numbered of crocodile travel.

Crocodile 61679 (3.67 m TL; tracked 316 days) was translocated from west to east of Cobourg Peninsula. It was captured in the downstream Mary River, and translocated and released in the Blyth River (Figure 2.6), 320 km east. In the 316 days of tracking, it roamed back and forth over 300 km of coastline between Elcho Island in the east and Brogden Point in the west, entering every major river in-between, including the Blyth, Glyde, Goomadeer and King, and Liverpool Rivers, without going far upstream in any of them. The crocodile continued to move until transmissions ceased at the mouth of the Glyde River. There was no apparent seasonal pattern in the movements. It did not move out onto Cobourg Peninsula when it reached the full extent of its westward journey, but rather returned to the east.

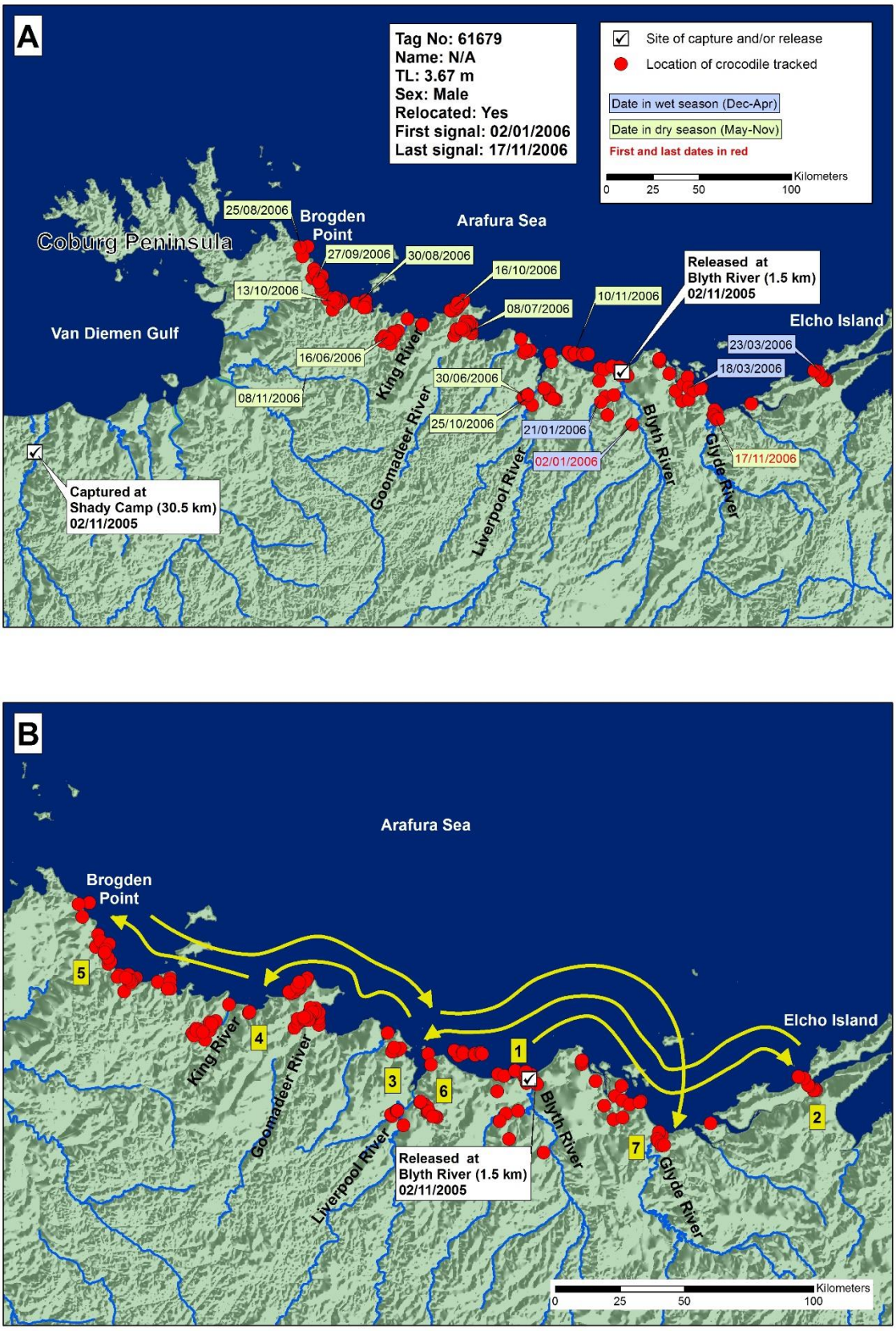

Figure 2.6. A) Locations and dates for translocated crocodile 61679, and B) likely paths chronologically numbered of crocodile travel.

Crocodile 68549 (3.21 m TL; perhaps still immature; tracked 268 days) was captured upstream in the King River to the east of Cobourg Peninsula, and released near the West Alligator River mouth (West Alligator Head, Figure 2.7), west of Cobourg Peninsula. It moved east, in the direction of its capture site, backtracking when it started to head north. It entered all major rivers between the West Alligator River and Minimini Creek. After visiting the West Alligator River, South Alligator River, Minimini Creek, and Murgenella Creek, the crocodile travelled upstream in the East Alligator River, to a point where it began heading away from its capture site. It then backtracked and moved out onto a floodplain, to a position 90 km from its release site and 25 km from its capture site, but separated from it by dry land.

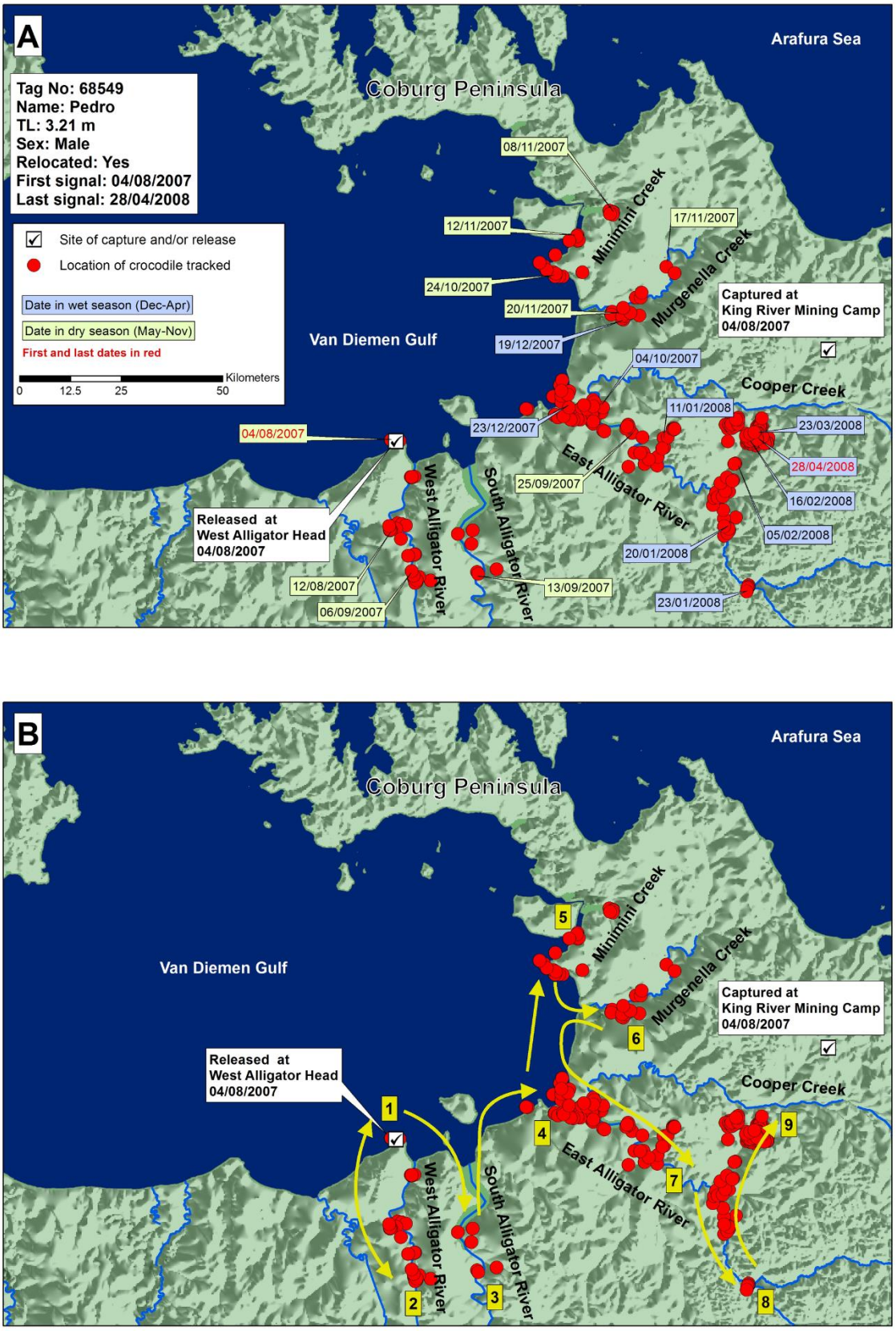

Figure 2.7. A) Locations and dates for translocated crocodile 68549, and B) likely paths chronologically numbered of crocodile travel.

None of the four crocodiles translocated followed the coastline out along the Cobourg Peninsula despite an apparent navigational ability demonstrated.

Genetic analysis

Basic genetic diversity metrics from the 8,312 SNPs that were retained post-filtering, including the distribution of minor allele frequencies and observed heterozygosities within sampling regions (average H_o = 0.203), are shown in Figure 2.8. A PCOA ordination of individual genotypes (Figure 2.8) shows that individuals were broadly grouped by sampling region. Relative to the Cobourg Peninsula, the first PCOA axis separated the eastern regions (Maningrida, King River, Arafura Swamp) from the western regions, while the second PCOA axis was largely a north-south divide on the west, separating the Finniss River samples from those between Darwin and the Cobourg Peninsula. The Tiwi Islands samples clustered closely with those from the nearby Adelaide and Mary Rivers. Mean pairwise F_{ST} among regions was 0.037, with all pairwise values being significant (Table 2.1).

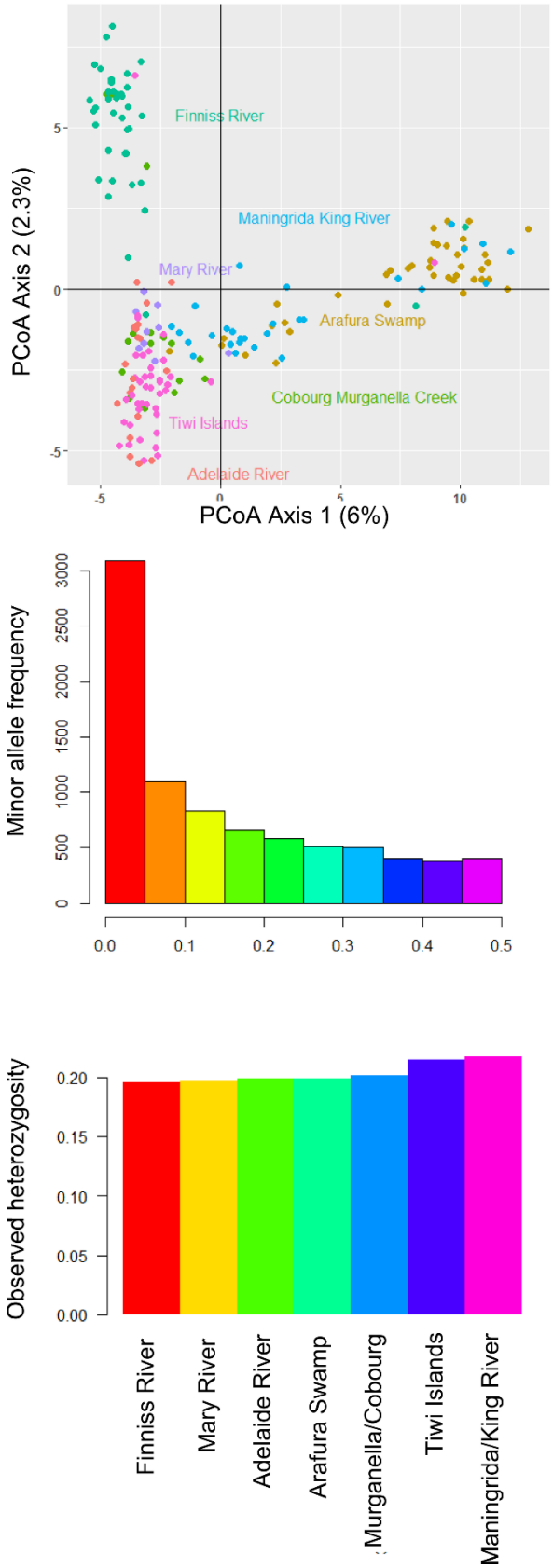

Figure 2.8. Principal coordinates analysis of individual SNP genotypes, minor allele frequencies across post-filtering SNP loci and observed heterozygosities in each region.

Table 2.1. F_{ST} estimates between saltwater crocodile sampling regions. Pairwise F_{ST} estimates in bold represent sampling locations separated by the Cobourg Peninsula. See S4 for bootstrap F_{ST} estimates with 95% confidence intervals, none of which included zero.

	Cobourg Murganella Creek	Adelaide River	Finniss River	Mary River	Arafura Swamp	Maningrida King River
Adelaide River	0.019					
Finniss River	0.023	0.038				
Mary River	0.018	0.029	0.037			
Arafura Swamp	**0.062**	**0.076**	**0.075**	**0.069**		
Maningrida King River	**0.027**	**0.041**	**0.044**	**0.034**	**0.015**	
Tiwi Islands	0.013	0.023	0.032	0.023	**0.061**	**0.031**

After MAF filtering, we used 5,291 loci to calculate IBS among samples. The scatterplot of IBS and ecological distance (Figure 2.9) with a narrow range of ecological distance values (~400 – 750 km) allowed comparison of samples from east and west of Cobourg Peninsula. All pairwise distances less than 400 km were among sampling sites on the same side of the Cobourg Peninsula and all pairwise distances greater than 750 km involved across-Cobourg comparisons. We fitted models to the full dataset and then repeated them with distances restricted to this geographic window. For the full dataset, the ecological distance-only model was highest-ranked, and featured a significant decline in IBS with increasing ecological distance among crocodiles (Table 2.2). The ecological distance and Cobourg barrier model was second-ranked and featured a significant effect of ecological distance, but not the peninsular barrier. We considered the more appropriate models to be those fitted to the restricted dataset, as the two candidate explanatory variables were highly correlated (-0.734) across the full dataset but only weakly correlated (-0.11) in the distance-restricted dataset. Essentially, the distance and putative barrier variables were confounded outside of the restricted dataset. Of these models, the model featuring an effect of the putative peninsular barrier was best supported according to AIC (Table 2.2) and featured a significant negative effect of the putative barrier

on IBS. Ecological distance was not a significant explanatory variable in any of the models fitted to the reduced dataset (Table 2.2).

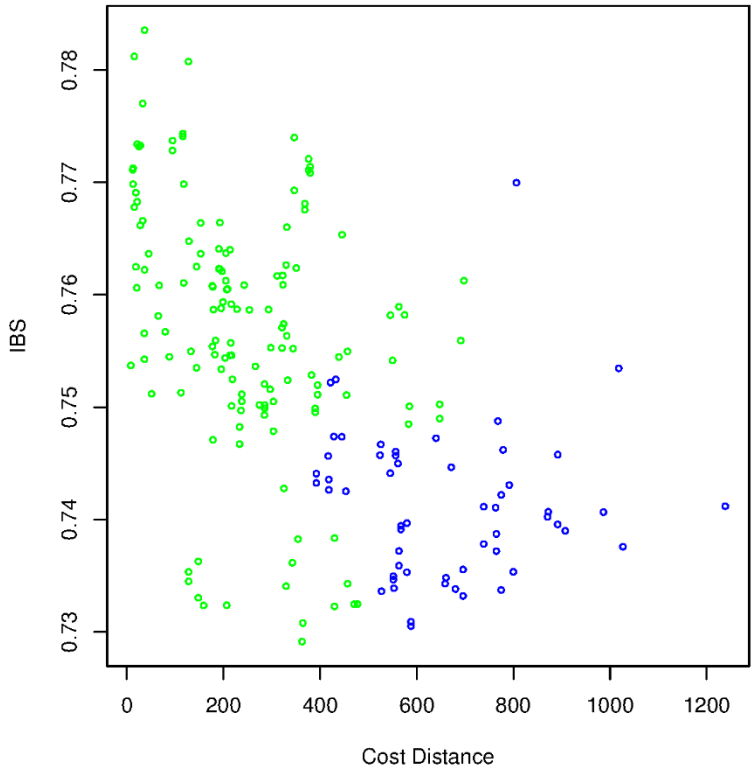

Figure 2.9. Scatterplot of mean Identity-by-State (IBS) among individuals in each of the 19 sampling locations in relation to ecological distance between each point. Pairwise comparisons spanning the Cobourg Peninsula are colour-coded blue and those on the same side of the peninsula are coded green.

Table 2.2. Comparison of linear mixed-effects models with maximum likelihood population effects (MLPE) correlation structure to investigate relationships between mean Identity-by-State (IBS) in saltwater crocodile genotypes among locations, ecological distance, and the effect of a putative barrier to gene flow at the Cobourg Peninsula.

Model		Estimate	SE	t	P	AIC	ΔAIC	MLPE Rho
Full dataset								
1	Intercept	0.762	0.002	316.161	0	-1300.232	11.655	0.199
	Ecol distance	-0.0003	3.13E-06	-5.54	0			
	Cobourg	-0.002	0.002	-0.696	0.487			
2	Intercept	0.763	2.38E-03	321.222	0	-1311.887	0	0.205
	Ecol distance	-0.0003	3.17E-06	-8.923	0			
3	Intercept	0.756	2.04E-03	369.939	0	-1297.136	14.751	0.173
	Cobourg	-0.012	1.85E-03	-6.7073	0			
4	Intercept	0.753	2.40E-03	314.346	0	-1270.18	41.707	0.198
Reduced dataset								
1	Intercept	0.751	0.004	189.614	0	-357.435	24.046	0.337
	Ecol distance	0	0	-0.659	0.513			
	Cobourg	-0.009	0.002	-4.239	0			
2	Intercept	0.75	0.005	152.735	0	-355.809	25.672	0.313
	Ecol distance	0	0	-1.377	0.174			
3	Intercept	0.749	0.002	312.816	0	-381.481	0	0.335
	Cobourg	-0.009	0.002	-4.35	0			
4	Intercept	0.745	0.002	304.914	0	-377.738	3.743	0.322

The genetic assignment analysis showed relatively clear separation among the sampled regions, especially between those in the west of the Cobourg Peninsula and Arnhem Land (Figure 2.10). Of the samples collected from the northern Arnhem Land region, only 3% of individuals had an ancestry probability of greater than 0.2 in the sampled regions west of the Cobourg Peninsula (S5 File). Of the samples collected in the three grouped regions west of the Cobourg Peninsula, only 6% of individuals had an ancestry probability of greater than 0.2 in the northern Arnhem Land region east of the Cobourg Peninsula. This contrasted with 17% of individuals in the regions west of the Cobourg Peninsula having ancestry probabilities greater than 0.2 in neighbouring regions on the same side of the Cobourg Peninsula. These data indicate some gene flow occurs around the Cobourg Peninsula but suggests that it occurs less commonly than gene flow among neighbouring populations either side of this coastal feature.

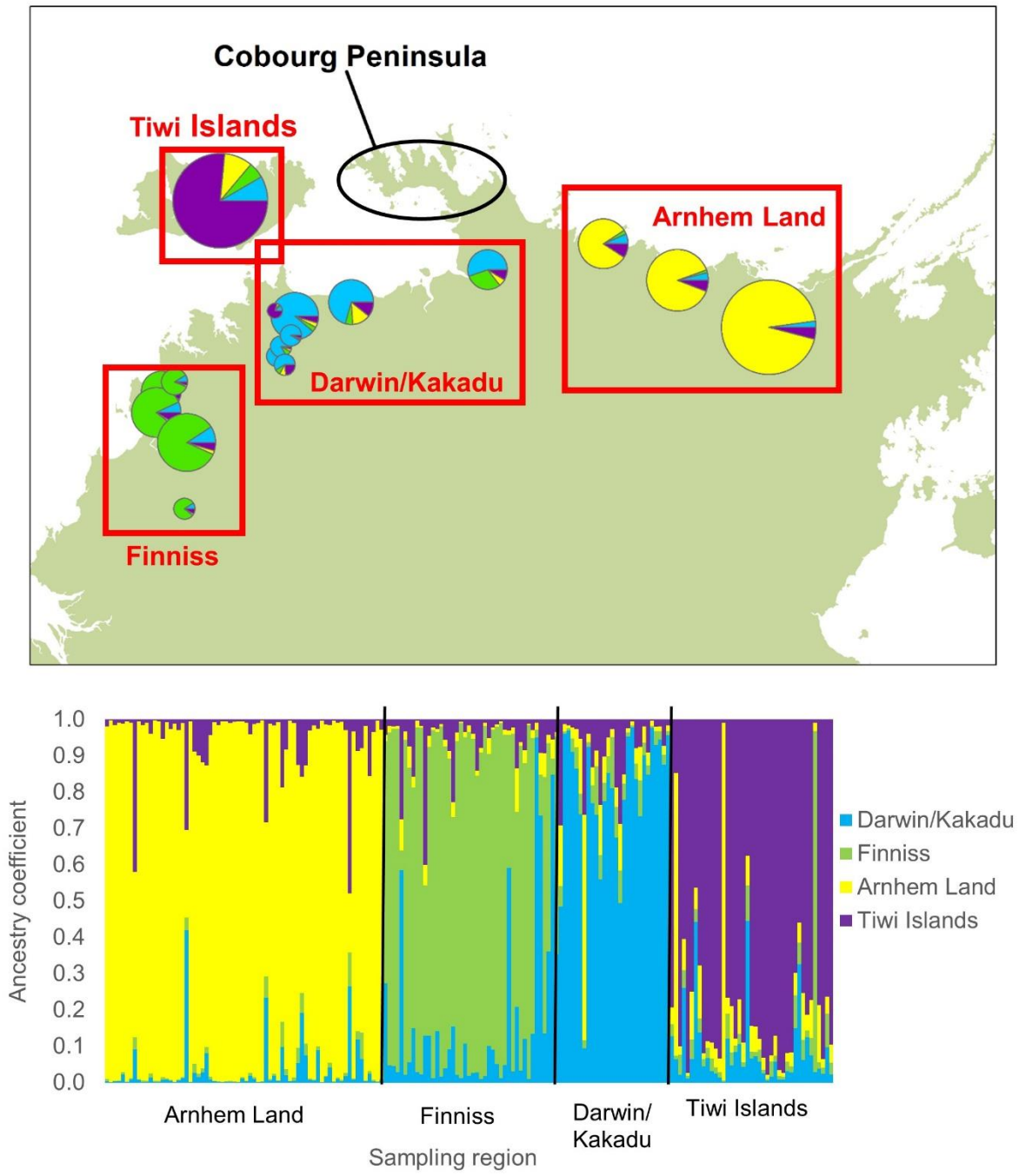

Figure 2.10. Genetic assignment of each individual to the pre-defined regions of the Arnhem Land, Darwin/Kakadu, Finniss River, and Tiwi Islands. The size of pie-charts is relative to the sample size of each population as shown in Figure 2.2. The calculated ancestry coefficients for each individual are presented in S5 File.

2.5. Discussion

Translocated *C. porosus* have a high probability of returning to their site of capture (Walsh and Whitehead 1993), but few results demonstrate how strongly this homing is achieved except for those from Queensland, Australia (Read *et al.* 2007; Campbell *et al.* 2010). The 4.02 m crocodile (68551) on the western side of Cobourg that we released 100 km west of its capture site returned within 25 days (Figure 2.3). This result is consistent with that of a previous study in which 3.1-m and 3.8-m males captured on the eastern and western sides of Cape York Peninsula in Queensland, respectively, and released 56 km and 99 km away on the same side, returned to their capture sites within a month [12].

However, unlike the NT crocodiles we studied, a 4.5-m *C. porosus* caught on the western side of Cape York Peninsula, and released on the eastern side, stayed near the release site for three months then returned 411 km to its capture site within a month, possibly exploiting local surface currents around Cape York (Read *et al.* 2007; Campbell *et al.* 2010). Cape York was clearly not an effective barrier to such homing movements, at least for large individuals. In contrast, Cobourg Peninsula was not crossed by any of the five translocated crocodiles in our study, nor by the three control crocodiles released at the capture site.

How Cobourg Peninsula interferes with east-west and west-east movements of *C. porosus* is unknown. Ocean currents could be involved as in Queensland (Campbell *et al.* 2010), but the coastal oceanography around Cobourg Peninsula is complex, and not well understood. There are some significant deep channels on the eastern side (<164 m), and large tides (<8 m) (Australian Hydrographic Office 2018) combined with seasonal monsoons, in which wind strength and direction change (Bureau of Meteorology 2020), which may interfere with animal movements, perhaps through the avoidance of rough surface currents flowing against them when travelling at sea. Satellite tracking of false killer whales (*Pseudorca crassidens*) in the Cobourg Peninsula area has shown that they rarely entered the western side of the Cobourg Peninsula, into Van Diemen Gulf, despite occupying shallow (<10 m depth) waters within 10km of the coast, on the eastern side (Palmer *et al.* 2017), which could also be related to currents.

Given the unidirectional movements associated with homing in the *C. porosus* studied here, their ability to follow the shoreline and negotiate Cobourg Peninsula could create a navigational conflict, because they would need to detour by swimming north in parts, away from the direction of their capture location. To return to a location like the Blyth River, east of the Mary

or Alligator Rivers (68549, 60686), crocodiles would need to go north, west, north, and then south around Cobourg Peninsula to get back on their eastward trajectory, which may be too sophisticated a navigational challenge for them, although such navigational detours have been observed in pigeons and other animals (Kiepenheuer 1978; Tögell and Wiltschko 1992; Schmidt *et al.* 1992).

Crocodiles would also need to leave the highly productive Alligator Rivers region, which supports a large population of crocodiles and abundant nesting (Fukuda *et al.* 2007; Fukuda *et al.* 2011; Fukuda and Cuff 2013), for a northward journey into an area without major rivers, swamps or nesting habitats. The long-distance journey by the large control animal (34011) during the wet season coincided with the breeding season (Webb *et al.* 1977; Webb 1991). The movement is more consistent with some form of biological need, such as a breeding migration rather than random movements, but indicates a very precise ability to home to its original capture site.

Not all relocated crocodiles sustained movements consistent with homing. The smallest male in this study, caught in the Blyth River and released in the Mary River (61678), settled at its new release site for almost two years. It made only one brief coastal journey, two months after release, travelling 60 km east and then west of the mouth of the Mary River, before returning upstream to the place where it had settled at the release site. This contrast may indicate random differences between individual crocodiles, or differences in the desire to home between immature and mature males, with presumably different histories of individual territoriality at their original capture site. Although *C. porosus* shows apparent territoriality at an early age (Brien *et al.* 2013a; Brien *et al.* 2013b), territoriality in immature crocodiles may not be as developed as in mature individuals.

Many animals, including migratory birds and marine turtles, sense the earth's magnetic field to collect navigational information (Lohmann and Lohmann 1993; Walker *et al.* 2002; Lohmann *et al.* 2004; Wiltschko and Wiltschko 2005). There is anecdotal evidence that crocodilians also use the magnetic field for navigation (Dominguez-Laso 2008). Twenty individuals of *C. acutus*, *C. crocodilus*, and *C. moreletii*, in different sizes ranging from 1.4 and 4.0 m, were attached magnets on the head before being translocated (3-120 km) and released in Mexico. Despite the high probability of those without magnets returning to capture sites, none of them returned by July 2008 (the earliest release was in September 2004 and latest in December 2007). Similar observations on *C. acutus* using a magnet to disrupt their homing behavior have been reported

in popular media in the USA (Morgan 2009). It has been suggested that crocodilians develop homing ability with a magnetic map to detect their position and orientation in relation to a target (Rodda 1984; Combrink 2015). They may also use other cues, such as landscape, astronomy or scents, possibly in combination (Combrink 2015), although the celestial and olfactory cues were not requirements for some *A. mississipiensis* juveniles to reach home from unfamiliar sites (Rodda 1984). In our study, it is difficult to identify which cues the translocated crocodiles relied upon.

Combrink (2015) documented the homing ability of a relocated 2.7m female crocodile (*C. niloticus*) that travelled 178.3 km to its capture site over 136 days. Although the homing track included approximately 18 km of travelling on land, geographical barrier that prevented the animal from travelling was not present. Similar overland homing over a much shorter distance by translocated *A. mississippiensis* has also been reported (Woolard *et al.* 2004). None of our tracked crocodiles, however, showed overland movement between waterbodies. This may be a reflection of the biological differences between the species (e.g. terrestrial locomotion, dehydration tolerance, etc), or a consequence of the small sample size of this study.

None of the eight crocodiles that were tracked, from either east or west of the Cobourg Peninsula, ventured along the Peninsula itself, despite the apparently strong need of some individuals to return to their capture site for their territoriality. This observation is consistent with the peninsula being barrier to dispersal of larger males, for reasons still unknown. If such a barrier exists, there is the potential for genetically distinct subpopulations on either side of the Cobourg Peninsula. Even though the number of the crocodiles tracked by satellites was rather small (N = 8), the genetic analysis that sampled 192 crocodiles across the study area identified greater differentiation across the Cobourg Peninsula than over equivalent distances elsewhere (Figure 2.9), allowing us to reject the possibility of genetic homegeneity across the NT coastline.

By restricting the spatial genetic analyses to a smaller geographic scale, where we had adequate sample sizes for pairwise genetic comparisons either side of the Peninsula versus the same side of the Peninsula, the results were clear that crocodiles on the same side of the Peninsula were more genetically similar than those across the Peninsula. This result that Cobourg Peninsula is a barrier to the coastal movement of crocodiles is further supported by the assignment analysis that shows lower migrant ancestry across the peninsula compared to among regions on the western side of the peninsula. It should be noted that the one individual that was sampled in

the Darwin/Kakadu region but showed high probability of ancestry in the Arnhem Land region also had some ancestry from the Tiwi Islands, suggesting that this crocodile is unlikely to be the first generation of migrants from Arnhem Land.

The approach that we used to analyze the genetic data can estimate the effects of environmental features on relative rates of dispersal (Manel *et al.* 2005; Landguth *et al.* 2010), but does not tell us the absolute migration rates across the Peninsula or elsewhere in the study area. Increased sampling in the immediate vicinity of Cobourg Peninsula could improve our ability to define where and how the barrier operates. Our results also suggested apparent genetic separation other than across the peninsula (e.g. the Finniss River and Tiwi Islands – see Figure 2.3). Other genetic methods can demonstrate individual dispersal events and rates (Landguth *et al.* 2010). The following chapters will explore their movement and dispersal across northern Australia in more detail with more samples and methods available.

From a management perspective aimed at reducing HCC, the practice of translocating "problem crocodiles" needs to be re-evaluated. Not only are they likely to return to their capture site, unless moved from one side of a movement barrier to another, but their active movements in new areas may create HCC within other communities of people. That genetically distinct subpopulations do exist, and can be identified from tissue samples, potentially allows the origins of problem animals in a particular context to be identified. This in turn could guide efforts to reduce the number of potential problem crocodiles roaming into urban centers, where the risk of HCC is considered unacceptable, by increasing intervention such as removal or harvest pressure based on new understanding of movement barriers and sources.

This study presented an example of how telemetry and genetic analysis can aid each other in revealing the movement patterns of animals with high and complex mobility like *C. porosus*. Although satellite tracking can provide detailed information on movement over a short period, it is often difficult with limited resources to deploy on many individuals. On the other hand, the genetic technique can be applied to much larger sample sizes, but its spatial and temporal resolution may be coarser, reflecting the effects accumulated over many generations.

2.6. Ethics and acknowledgement statement

This study was conducted in accordance with the Code of Practice on the Humane Treatment of Wild and Farmed Australian Crocodiles (Department of the Environment and Energy 2013). The protocol was approved by the Animal Ethics Committee of the Australian University (Animal Ethics Protocol Number A2017/11) and the Parks and Wildlife Commission of the

Northern Territory (Research Permit Number 7902). It is a contribution to the Northern Territory Government's crocodile management programs (Leach *et al.* 2009; Saalfeld *et al.* 2016).

The satellite tracking of crocodiles originated as a collaborative project between Parks Australia, Wildlife Management International Pty. Limited, Charles Darwin University, and the Parks and Wildlife Commission of the Northern Territory. A number of staff from these organizations contributed to the project. The genetic analyses presented in this study were financially supported by the Australian National University, Northern Territory Government, National Geographic Foundation for Science and Exploration – Asia, Holsworth Wildlife Research Endowment, IUCN-SSC Crocodile Specialist Group Student Research Assistance Scheme, and ACT Herpetological Association. The authors are grateful to the Northern Territory Crocodile Farmers Association and Northern Territory Government for providing tissue samples for the DNA analysis. Ruth Patterson, Neil Smit, Carol Palmer, Alan Withers, Tim Clancy and Glenn Edwards provided valuable input or comments on this study. Nam Chul Jang provided laboratory assistance in processing the tissue samples. We are grateful to the traditional owners of the Kakadu National Park for letting us conduct the study.

2.7. Data Accessibility

All the relevant data used in this study and supporting information material are publicly available at https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205862#sec011.

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CHAPTER 3: Environmental resistance and habitat quality influence dispersal of the saltwater crocodile

3.1. Abstract

Landscape genetics commonly focusses on the effects of environmental resistance on animal dispersal patterns but there is an emerging focus on testing environmental effects on emigration and settlement choices. In this study, we used landscape genetics approaches to quantify dispersal patterns in the world's largest crocodilian, the saltwater crocodile (*Crocodylus porosus*) and demonstrated environmental influences on three processes that comprise dispersal; emigration, movement, and settlement. We found that both environmental resistance and properties of the source and destination catchments (proportion of breeding habitat) were important factors influencing observed dispersal events. Our habitat quality variables related to hypotheses about resource competition and represented the ratio of breeding habitat (which limits carrying capacity), suggesting that competition for habitat influences emigration and settlement choices, together with the strong effect of environmental resistance to movement (where high-quality habitat was associated with greatest environmental permeability). Approximately 42% of crocodiles on average were migrants from populations other than their natal origins and some outstandingly productive populations had much higher proportion of emigration rather than immigration. The distance most commonly travelled between source and destination was 150-200 km although a few travelled much longer distances, up to 600-700 km. Given the extensive dispersal range, individual catchments or hydrographic regions that combine two or three adjacent catchments are an appropriate scale for population management.

3.2. Introduction

Animal dispersal influences the dynamics and distribution of populations and an understanding of dispersal patterns has direct application to population management for some species (Banks & Lindenmayer, 2014; Bowler & Benton, 2005; Morales et al., 2010; Nathan, 2001). Genetic data can identify dispersal events and estimate rates of effective migration, thereby enabling researchers to model the effects of environmental conditions on dispersal within and among populations. Landscape genetics has provided a wealth of tools to explore associations between environmental variation and population connectivity, yet the focus of research in this field has been predominantly on environmental influences on the 'movement' phase of dispersal (such as land cover variables that cause resistance to movement). However, dispersal is the outcome

of several interacting factors (Saastamoinen *et al.* 2018). Bowler and Benton (2005) considered that animal dispersal is not a single process but comprises three interdependent phases: emigration (decision to leave), inter-patch movement, and finally immigration (decision to settle). Emigration and immigration may be influenced by a number of factors such as competition, habitat saturation, breeding opportunity and inbreeding risk while movement may be influenced by factors that include the existence of geographic barriers, landscape 'permeability' to movement and the mobility of an individual (Banks and Lindenmayer 2014; Fraser *et al.* 2015; Robertson *et al.* 2019). Different environmental or demographic conditions may influence these phases of dispersal individually (Travis *et al.* 2012; Pflüger and Balkenhol 2014) and understanding these relationships will improve our capacity to model dispersal and population connectivity for wildlife population management.

When managing wildlife, understanding the role of dispersal in population growth is particularly important (Pulliam 1988; Pease *et al.* 1989). Quantifying the dependence of recruitment on local reproduction *versus* immigration, and mapping the locations and environmental properties of net sources and sinks of dispersers across population networks can help to understand population dynamics and prioritise actions to achieve management goals (Fletcher & Westcott, 2013; Hampton et al., 2004; Hörnell-Willebrand, Willebrand, & Smith, 2014). Landscape genetics studies can map dispersal across the landscape and model the properties of dispersal sources and sinks. To do this, such studies need to focus not only on landscape resistance to movement (i.e. matrix quality among populations) but also on the local environmental properties of dispersal sources and destinations that influence dispersal events (Pflüger and Balkenhol 2014; Fraser *et al.* 2015; Parsley *et al.* 2020). Here, we apply landscape genetics approaches to provide dispersal data to model movement by a large, top order predator, the saltwater crocodile (*Crocodylus porosus*). The motivation of the study is to provide biological data to inform population management and to investigate the relative importance of landscape resistance (i.e. inter-site environmental characteristics) and site-level environmental characteristics on dispersal patterns.

The saltwater crocodile, *Crocodylus porosus,* is an iconic species subject to intensive management in northern Australia because they are of conservation importance and represent a sustainable natural resource yet are dangerous predators of people and domestic animals (Leach, Delaney, & Fukuda, 2009; Saalfeld, Fukuda, Duldig, & Fisher, 2016). Populations were seriously depleted between 1946 and 1971, and subsequently received protection (Webb, Manolis, Whitehead, & Letts, 1984), which was followed by the introduction of sustainable

use programs aimed at increasing the value of these dangerous predators to landowners and the public (Hutton, Ross, & Webb, 2002; Webb & Manolis, 1993). Wild populations have been closely monitored since protection in in the 1970s (Fukuda et al., 2011; Messel, Vorlicek, Wells, & Green, 1981; Webb et al., 1984). Their ability to travel a long distance to occupy habitat in the sea, rivers, lakes, floodplains and swamps has been well established (Read *et al.* 2007; Campbell *et al.* 2010; Campbell *et al.* 2013). However, dispersal rates among populations and the environmental influences on dispersal are not understood.

In this study, we used landscape genetics to investigate genetic structure, movement patterns and the spatial scale of dispersal by saltwater crocodiles in northern Australia. We also investigate the role of environmental variables (habitat quality in terms of suitability for breeding (Fukuda & Cuff, 2013; Fukuda, Whitehead, & Boggs, 2007) in influencing dispersal. We focus on three phases of dispersal to test two major hypotheses about environmental processes driving dispersal patterns:

1. Resistance to movement hypothesis: Dispersal will be influenced by environmental resistance to movement between source and destination, with resistance inversely correlated with habitat quality. A common assumption is that habitat quality is correlated with habitat permeability, yet this is rarely tested empirically (Spear *et al.* 2010). We estimated environmental resistance to movement via landscape genetics approaches (Peterman 2018).

2. Habitat availability hypothesis: The availability of habitat in dispersal sources and destinations will influence both emigration and settlement phases of dispersal. We predicted that emigration will be more likely from river systems with high proportion of breeding habitat relative to total habitat as mapped by previous studies (Fukuda & Cuff, 2013; Fukuda et al., 2007), corresponding to predicted high resource competition for habitat (Pflüger and Balkenhol 2014). Immigration rates will be higher in river systems with a low proportion of breeding habitat relative to total habitat. This relates to the priority effects hypothesis, where high population density and local reproduction precludes settlement by dispersing individuals (Fraser *et al.* 2015).

Using landscape genetic information collected from contemporary populations of saltwater crocodiles, we quantify the relative importance of environmental influences on the emigration, resistance and settlement phases of dispersal by large and highly mobile predators. This

information can potentially advance the application of landscape genetics to wildlife population management through informing spatial population dynamics.

3.3. Materials and methods

Study area and context

The Top End of Northern Territory (approximately 400,000 km^2) and East Kimberley (117,5100 km^2, Figure 3.1) contain many coastal rivers and creeks, lined with mangroves and under tidal influence near the sea, extending to a diversity of freshwater, non-tidal floodplain wetlands and sand and rock-lined water courses upstream. All are occupied by *C. porosus* at different densities, as indicated by sighting densities ranging from 0.1 to 15 crocodiles sighted per kilometer of river (Fukuda et al., 2007; Fukuda et al., 2011; Webb, 1991). The species also occurs along the coastline between rivers, and occupies near coastal islands (Webb, 1991; Webb & Manolis, 1989).

The climate in the study region is monsoonal, with a distinct wet season (November to April) and dry season (May to October). Available wetlands expand during the wet season, during which crocodiles nest, and contract during the dry season (Fukuda & Cuff, 2013; Webb, 1991), when many temporary water bodies dry out if early rains are delayed. Crocodiles tend to move back into permanent water areas during the dry season, but are sometimes forced to aestivate in drying mud (Webb & Manolis, 1989). In the NT, there is limited intensive agriculture and most coastal and riparian habitats remain intact (Fukuda et al., 2007).

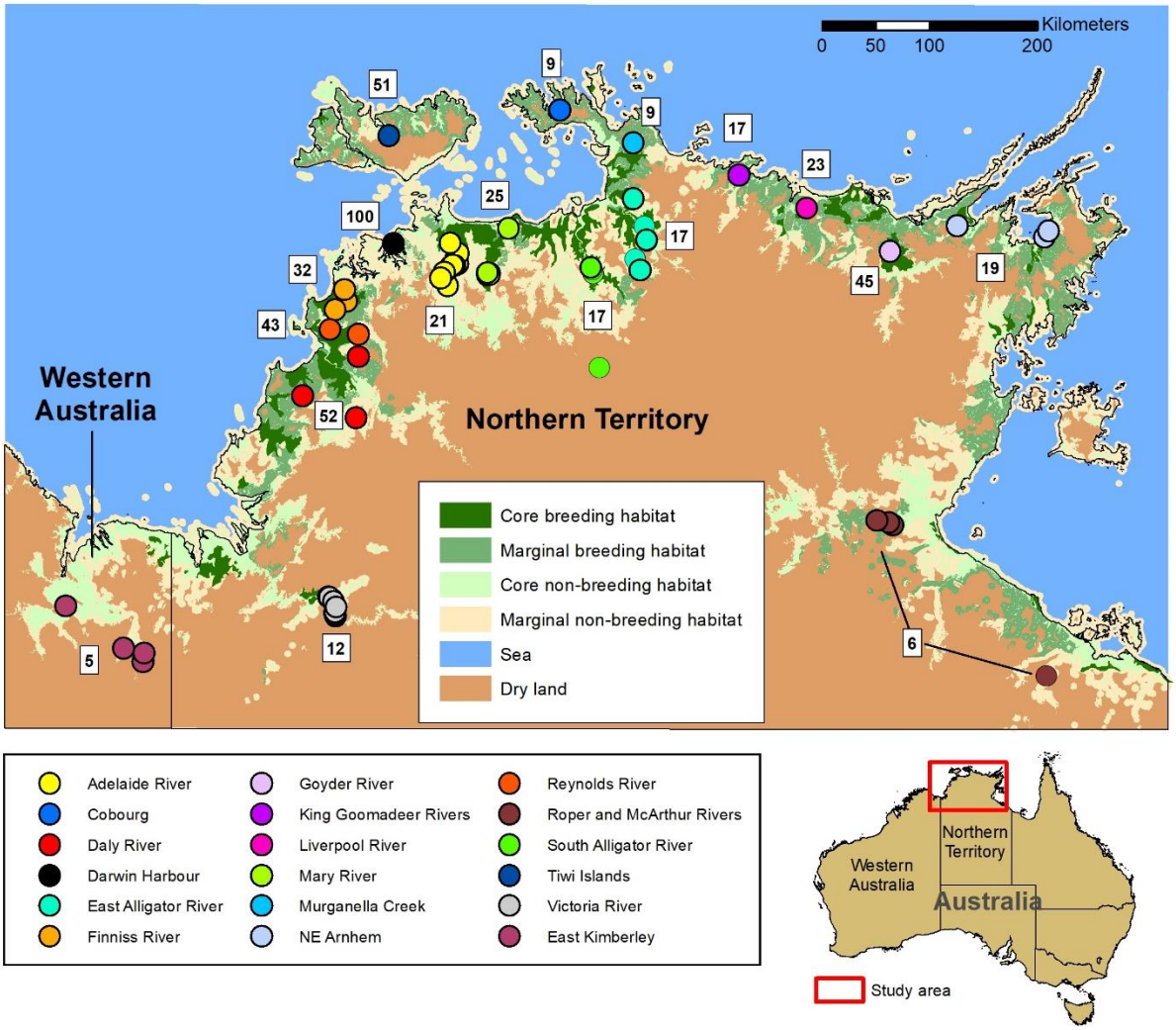


Figure 3.1. Catchment-based populations of *C. porosus* sampled within the study area in the Northern Territory and Western Australia. The number of samples collected for each population is shown on the map.

SNP genotyping and filtering

From a sample of 714 geo-referenced *C. porosus* tissue samples collected across northern Australia and nearby countries, we analysed 515 samples from across the focal area for this study in northern Australia (Figure 3.1). The remaining 199 samples outside the study area were included in the single-nucleotide polymorphism (SNP) filtering process but the samples were excluded from population analyses. For analyses where population allele frequencies were required, we grouped individuals according to the 18 river systems in which sampling was conducted. The tissue samples were pieces of skin collected randomly from either 1) free-ranging crocodiles, using a biopsy pole (Barrow and Halford 2019) or by hands ($N = 60$), 2) crocodiles captured as part of the public safety program (Fukuda, Manolis, & Appel, 2014; Saalfeld et al., 2016) ($N = 117$), or 3) embryos that failed to develop to term during incubation

(*N* = 338). These embryos came from eggs collected from wild populations as part of the NT's commercial crocodile farming program and we used one egg per clutch collected from a geo-referenced nest location (Leach et al., 2009; Saalfeld et al., 2016). The length of the sampled (free-ranging) crocodiles ranged from 0.3 to 5.1 m. We assumed that it was unlikely that these sampled crocodiles were related to each other because only a fractional number of samples (e.g. minimum of two at the Adelaide River and maximum of 21 at the East Alligator River) were collected along long transects (135.7 km at the Adelaide River and 49.0 km at the East Alligator River) set as a part of population monitoring programs (Fukuda *et al.* 2011; Fukuda *et al.* 2013). These sampled numbers represented only 0.3-6.0% of the relative abundance (number of crocodiles sighted per kilometre of river) estimated from these surveys (see Table 3.1) (Saalfeld *et al.* 2016). The tissue samples used for the analyses were all collected between 2015 and 2019.

DNA extraction and genotyping were conducted at Diversity Arrays Technology (Canberra, Australia) using the DArTseq approach (Kilian *et al.* 2012). DArTseq uses genome complexity reduction and NGS approaches conceptually similar to RADseq, in this case based around a *SphI* enzyme digestion of genomic DNA and Illumina HiSeq 2500 sequencing. We ran 30% of samples twice as technical replicates to assess the repeatability of SNP calls. Initial sequence processing and SNP calling by DArT yielded 17,791 SNP loci across 16,637 contigs. As an initial data exploration step, we plotted basic SNP metrics on sequence depth and other 'quality' metrics, and explored relationships between these metrics and basic population genetics statistics, including observed and expected heterozygosity (S3.1-3.3). The graphical analysis was used to evaluate whether genotype properties (e.g. observed heterozygosity) were associated with SNP quality metrics such as mean sequencing depth.

We used the dartR package in R 3.5.3 (Gruber *et al.* 2017) to filter SNPs according to call rate (CallRate >0.90) and repeatability (RepAvg >0.98) and added custom filtering criteria to drop SNPs with a mean sequence depth of less than 8 and a mean ratio of sequence depth between alleles of greater than 2 (to remove loci in which one allele was preferentially detected, indicating potential susceptibility to null alleles through PCR bias). We also dropped SNPs (ranked by polymorphic information content) within sequences with more than one SNP present, SNPs that did not align to a reference genome (ICGWG 2014) with e-value threshold of -20 or lower, and SNPs that mapped to more than one location on the reference genome. We dropped genotypes of individuals for which greater than 10% of SNPs were not genotyped. Following these filtering steps, we retained 4703 SNPs in 515 individuals with a minor allele

frequency of greater than 0.02, corresponding to ≥ 20 allele copies for analysis. We graphed the distribution of the inbreeding coefficient (F_{IS}), observed heterozygosity (H_O), expected heterozygosity within populations (H_S), expected heterozygosity overall (H_T) and genetic differentiation among populations (F_{ST}) to visualize any unexpected patterns in regard to Hardy-Weinberg expectations within populations (Figure 3.2) but did not filter any further based on this graphical analysis.

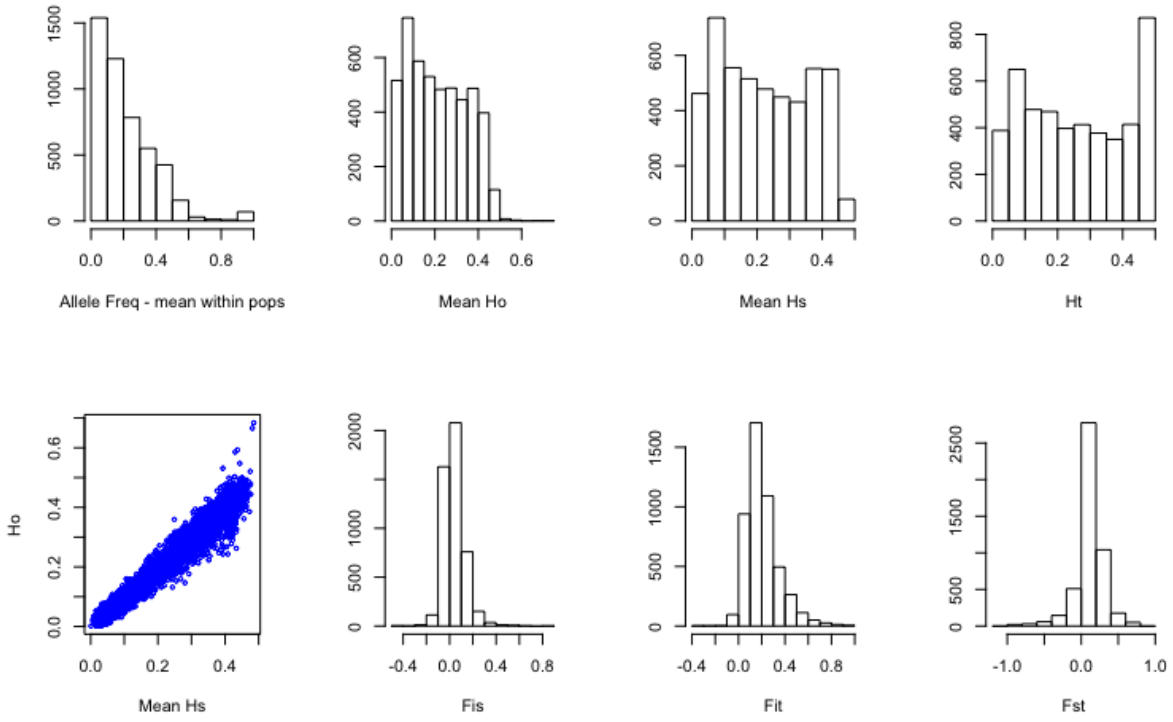


Figure 3.2. Histograms of the population genetics statistics after the filtering (see S3.2 for those before the filtering). Y axis is the number of SNPs unless labeled differently.

Genetic diversity and population structure

We calculated the basic genetic diversity statistics, including H_S, H_O, H_T and F_{IS} of the dataset before and after filtering. We then estimated Smouse and Peakall's (1999) genetic distance metric among individuals and performed a Principal Coordinates Analysis (PCOA) on the genetic distances to visualize patterns of genetic similarity among individuals and populations.

To quantify fine-scale patterns of genetic structure among individuals, we calculated a multilocus spatial correlogram from genetic and geographic distances among individuals, following Smouse and Peakall (1999) in GenAlEx 6.503 (Peakall and Smouse 2012; Peakall and Smouse 2017). We used distance intervals with upper bounds of 10, 50, 100, 200, 400, 600,

800 and 1000 km for the analysis and calculated multilocus spatial autocorrelation *r* values with 1000 bootstraps and 1000 permutations of the data to evaluate significance. We did not include populations with a small number of samples (WA East Kimberley with *N*= 5, and Roper and NT MacArthur Rivers with *N* = 6) in the population-level PCOA and the genetic-geographic distance analysis.

Landscape resistance analysis

We used the ResistanceGA R package (Peterman, 2018) to evaluate models of environmental resistance to between-population movement, represented by individual pairwise genetic distances among individuals. ResistanceGA models pairwise genetic distances in response to pairwise 'ecological distances' using linear mixed effects models with a maximum-likelihood population effects (MLPE) random effects structure (Clarke *et al.* 2002), represented by individual ID in our models. We used Smouse and Peakall (1999) pairwise genetic distance as the response variable for this purpose. We constructed landscape layers representing the major habitat-related environmental categories that we predicted would be relevant to crocodile movement.

We created a categorical resistance surface layer using a 3 km x 3 km cell size raster (with 325 x 202 cells) with cells classified as sea, dry land, and the different types of habitats (Figure 3.1). We classified habitats into 'core breeding habitat', 'marginal breeding habitat', 'core non-breeding habitat' or 'marginal non-breeding habitat', based on the previous habitat suitability models (Fukuda & Cuff, 2013; Fukuda et al., 2007; Webb, 1991). Breeding of *C. porosus* is highly seasonal during the wet season (November-April) and constrained to temporarily flooded, freshwater waterbodies which are not necessarily the most suitable habitat for saltwater crocodiles outside the breeding period (Campbell et al., 2013; Fukuda & Cuff, 2013; Fukuda et al., 2007; Webb, 1991). The core breeding habitats are the most favourable nesting areas represented by particular vegetation types such as *Melaleuca*, *Eucalyptus* and *Pandanus* open forests in perennial, freshwater wetland and swamp with mixed grasses and sedges (*Chrysopogon*, *Xerochloa*, *Oryza* and *Eleocharis* species) derived from the Vegetation Communities of the Tropical Savanna (CRC, 2004) as described by Fukuda et al. (2007), while the marginal breeding habitats were identified by broader vegetation communities occasionally used for nesting, derived from National Vegetation Information System (NVIS) version 4.1 (DSEWPC, 2012; Fukuda & Cuff, 2013). The core non-breeding habitats are the permanent waterbodies as defined being suitable for residence (Fukuda & Cuff, 2013), but persisting

outside the breeding areas defined above. These GIS data were derived from Geoscience Australia (2006). We defined the marginal non-breeding habitats by buffering the core non-breeding habitats by 3 km so that these habitats would include temporary waterbodies that may dry up during the dry season (May-October) or coastal areas with salinity levels similar to seawater (typically 35 parts per thousand). Although *C. porosus* is highly adapted to the saline environment (Cramp, Meyer, Sparks, & Franklin, 2008; Grigg, Taplin, Harlow, & Wright, 1980; Taplin, 1985), the species occurs in much higher density in brackish or fresh water (Fukuda et al., 2011; Webb & Manolis, 1989) and nesting females and embryos require access to freshwater (Webb, Manolis, Buckworth, & Sack, 1983; Webb, Messel, & Magnusson, 1977). Although some individuals access sea, especially when moving between the rivers (Campbell et al., 2010; Fukuda, Webb, Manolis, Lindner, & Banks, 2019), it is considered less favoured than brackish or freshwater habitats, and dry land is almost inaccessible to crocodiles as suggested by the previous tracking by satellites (Fukuda et al., 2019). Because these habitats for *C. porosus* have largely remained intact over years (Webb and Manolis 1989), we assumed that bias from asynchronicity in the timeframe between the parameterisation of the environmental variables and the collection of the genetic samples was unlikely.

We used ResistanceGA to estimate resistance surfaces that optimised random-walk commute distances (Etten 2017) among the locations of sampled individuals as an explanatory variable in models of pairwise genetic distances among individuals. We ran a single surface optimisation in ResistanceGA (Peterman 2018) to estimate resistance values for the six environmental cover categories and stopped each model after 25 consecutive generations of no improvement in log-likelihood (see S5 for R script).

We visualized predicted flow using the optimized resistance surface layer in Omniscape (Landau *et al.* 2021), with source strength set to a value of 2 for core breeding habitat and 1 for marginal breeding habitat, with a maximum radius corresponding to 900 km, which was the largest individual crocodile movement inferred from assignment tests (see below). Ominiscape calculated omnidirectional connectivity between every possible pair of start and end points across the landscape by applying the circuit theory principles iteratively to the input resistance (Landau *et al.* 2021).

Population assignment to identify movement events

We used a genetic assignment test approach to estimate the origin of each individual in the sampled river systems across the study, using the R package rubias (Moran and Anderson 2018).

We excluded the Darwin Harbour region as a candidate source population for assignment because there is no breeding population in Darwin Harbour (Fukuda & Cuff, 2013). As above, we did not include WA East Kimberley and NT Roper and MacArthur Rivers in the assignment. We used the self_assign function of the rubias package that implements Bayesian inference to estimate the posterior probability of assigning each individual to the geographic 'reference populations' given equal prior on every population in the reference (Moran and Anderson 2018).

Modelling environmental effects on crocodile population connectivity

We used the individual movements (dispersal destinations) identified in the genetic population assignment analysis as a response variable in a multinomial logit model (Croissant 2020a; Croissant 2020b) to identify the influence of site-level habitat properties and between-site landscape permeability (within vs between-site variables) on dispersal patterns by saltwater crocodiles. The dataset comprised all free-ranging crocodiles over one metre in total length (i.e. free-living crocodiles sampled in the field) assigned to a single population with greater than 90% probability. An exploratory analysis found that all crocodiles under one metre in length had not yet dispersed (all assigned to the 'home' population) so we did not include such individuals in the analysis of dispersal patterns. We did not include the size of crocodiles as an explanatory variable because there was not a significant difference in the mean between the individuals that migrated (249.27 cm) and those did not (244.70 cm) [$F(1,51) = 0.036$, $p = 0.85$].

We used the multinomial (discrete choice) modelling function (R package mlogit version 1.1-1 (Croissant 2020b)) to model environmental influences on crocodile movement. The (true or false) response variable was a matrix for each possible dispersal destination and natal population (river system). The data for each crocodile was represented as a 'True' for the dispersal choice and 'False' for all the other populations.

For each dispersal choice from the assigned natal location to the sampled destination, we modelled the influences of the estimated resistance distance (from the best ResistanceGA model) between the source and the destination (relating to the movement phase of dispersal) and the environmental properties of the 'destination' population where the crocodile was sampled (relating to the immigration or settlement stage of dispersal). The candidate explanatory variables were: (1) resistance calculated as the pairwise commute distance calculated in *gdistance* (Peterman, 2018) from a resistance landscape parameterised to match the best ResistanceGA model; (2) ratio of area suitable for breeding to total habitat area at

destination as described by Fukuda and Cuff (2013) and Fukuda et al. (2007); and (3) interaction between these two variables. For this analysis, we combined the 'core' and 'marginal' breeding habitat categories to represent estimated total breeding area available in each catchment. Likewise, to examine the influence of the environmental properties of the assigned 'source' population (relating to the emigration stage of dispersal), we ran another set of models for each natal assignment (response variables) with resistances, ratios of breeding area to total habitat at source, and the interaction between these two (explanatory variables).

We ranked the models using Akaike Information Criteria (AIC) and examined visually the effects of the environmental variables on the dispersal probability, generating heatmaps using the levelplot function in the lattice R package (Sarkar *et al.* 2021)..

3.4. Results

Genetic diversity and population structure

From an initial 17,791 SNPs in 679 individuals (11 were excluded from the filtering process due to SNP coverage) with mean H_T, H_S, H_O and F_{IS} of 0.19, 0.15, 0.15, and 0.01, respectively, we filtered the dataset to 4807 SNPs in 503 individuals (Figure 3.4), with mean H_T, H_S, H_O and F_{IS} of 0.29, 0.23, 0.25 and -0.08, respectively (Figs 2 and 4). Population-specific genetic diversity metrics for the 18 sampling locations are shown in Table 3.1.

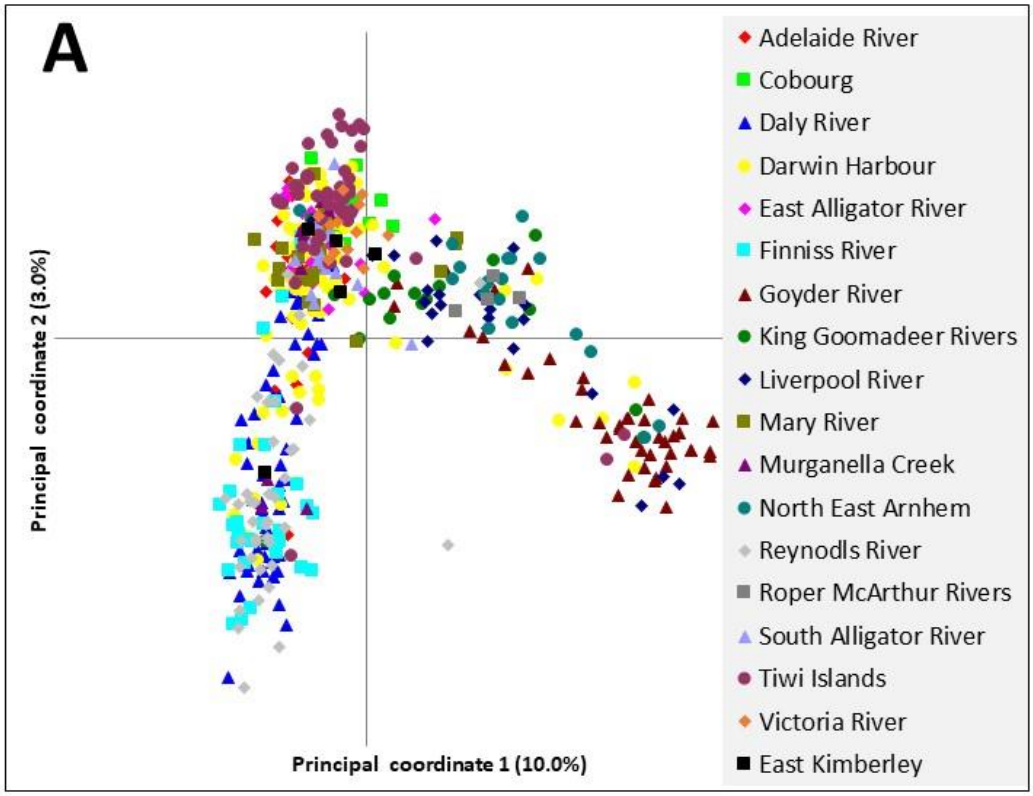
Table 3.1. Basic genetic diversity for the individual populations (individuals grouped by river system) and areas of quality habitats. n = number of samples analyzed, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, and F_{IS} = inbreeding coefficient. Numbers in brackets are standard errors.

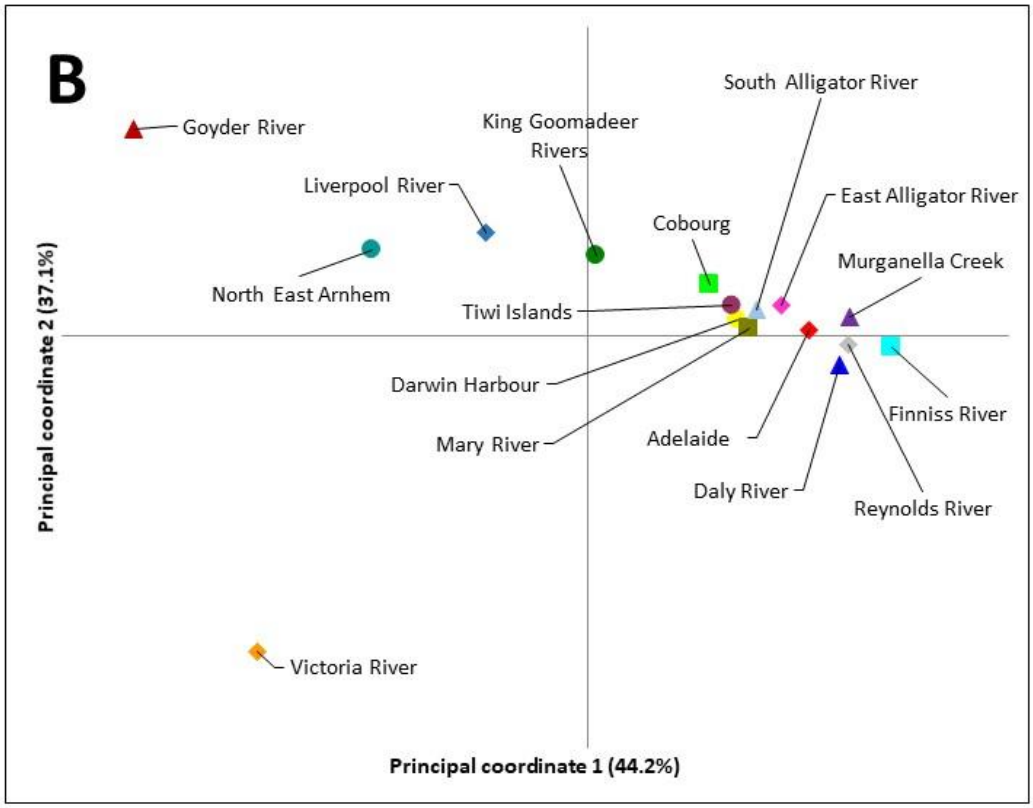
Population	Centroid latitude	Centroid longitude	n	Effective number of alleles	Ho	He	F_{IS}	Core breeding (km^2)	Core non-breeding (km^2)	Marginal breeding (km^2)	Marginal non-breeding (km^2)	Density (sighting/km)	Average of
Adelaide River	-12.66	131.34	21	1.370 (0.005)	0.223 (0.003)	0.226 (0.003)	0.018 (0.003)	1039	2830	1051	1957	4.98	2016, 2017, 2018
Cobourg	-11.34	132.24	9	1.363 (0.005)	0.212 (0.003)	0.219 (0.003)	0.024 (0.005)	29	737	1326	1512		
Daly River	-13.48	130.55	52	1.365 (0.005)	0.207 (0.002)	0.223 (0.003)	0.069 (0.003)	1818	4378	4584	5670	7.13	2013, 2016, 2018
Darwin Harbour	-12.46	130.85	100	1.38 (0.005)	0.215 (0.002)	0.235 (0.002)	0.085 (0.002)	<10	813	<10	1275		
East Alligator River	-12.43	132.97	17	1.36 (0.005)	0.216 (0.003)	0.22 (0.003)	0.017 (0.004)	1029	1579	1009	1945	7.17	2014, 2016, 2017
Finniss River	-12.96	130.43	32	1.354 (0.005)	0.205 (0.003)	0.216 (0.003)	0.042 (0.003)	709	1581	1152	1479		
Goyder River	-12.52	135.00	45	1.376 (0.005)	0.204 (0.002)	0.228 (0.003)	0.098 (0.003)	634	1251	934	1072	5.63	2012, 2016
King and Goomadeer Rivers	-11.88	133.74	17	1.39 (0.005)	0.226 (0.003)	0.238 (0.003)	0.04 (0.004)	13	718	1264	2131		
Liverpool River	-12.16	134.30	23	1.396 (0.005)	0.228 (0.003)	0.242 (0.003)	0.05 (0.003)	817	2190	2652	3210	3.4	2012, 2016, 2018
Mary River	-12.33	131.81	25	1.372 (0.005)	0.209 (0.003)	0.228 (0.003)	0.075 (0.004)	915	2179	683	1156	9.81	2013, 2015, 2017
Roper and McArthur Rivers	-14.78	134.95	6	1.38 (0.005)	0.234 (0.003)	0.228 (0.003)	-0.037 (0.005)	39	3618	1578	6014	2.77	2017

Murganella Creek	-11.62	132.85	9	1.344 (0.005)	0.202 (0.003)	0.206 (0.003)	0.01 (0.006)	415	997	1705	1548		
North East Arnhem	-12.38	136.31	19	1.393 (0.005)	0.227 (0.003)	0.24 (0.003)	0.047 (0.004)	244	2096	3473	4259		
Reynolds River	-13.18	130.36	43	1.362 (0.005)	0.207 (0.002)	0.222 (0.003)	0.059 (0.003)	682	929	745	661		
South Alligator River	-12.663	132.507	17	1.37 (0.005)	0.228 (0.003)	0.225 (0.003)	-0.01 (0.003)	949	1916	373	3068	5.28	2015, 2016, 2017
Tiwi Islands	-11.5599	130.8163	51	1.39 (0.005)	0.22 (0.002)	0.241 (0.002)	0.082 (0.003)	49	1863	3214	3321		
Victoria River	-15.487	130.3555	12	1.354 (0.005)	0.207 (0.003)	0.212 (0.003)	0.015 (0.004)	421	1593	169	2298	1.92	2017
East Kimberley	-12.3533	136.3312	5	1.346 (0.005)	0.215 (0.003)	0.208 (0.003)	-0.049 (0.006)	<10	1299	<10	1129		

Overall F_{ST} among populations was 0.032 with mean pairwise F_{ST} of 0.034. Pairwise genetic F_{ST} among the 18 populations ranged from 0.001 (between the Reynolds River and Daly River) to 0.110 (between the Victoria River and Goyder River) as shown in S6.

The first three PCOA axes explained 7.0%, 3.0% and 2.1% of variation in genetic distances among individuals and loosely clustered individuals into geographic groupings corresponding to spatial proximity of sampling locations (Figure 3.3A). The population-level PCOA (based on pairwise F_{ST}) grouped populations broadly according to geographic proximity (Figure 3.3B). Genetic differentiation among populations was consistent with geographic distances as indicated by the isolation by distance plot (Figure 3.3C). The spatial autocorrelation analysis showed significant positive spatial correlation in distance classes up to 200 km (Figure 3.3D).


A
Principal coordinate 2 (3.0%)
Principal coordinate 1 (10.0%)
Adelaide River
Cobourg
Daly River
Darwin Harbour
East Alligator River
Finniss River
Goyder River
King Goomadeer Rivers
Liverpool River
Mary River
Murganella Creek
North East Arnhem
Reynodls River
Roper McArthur Rivers
South Alligator River
Tiwi Islands
Victoria River
East Kimberley


B
Principal coordinate 2 (37.1%)
Principal coordinate 1 (44.2%)
Goyder River
King Goomadeer Rivers
South Alligator River
Liverpool River
East Alligator River
Cobourg
Murganella Creek
North East Arnhem
Tiwi Islands
Darwin Harbour
Mary River
Adelaide
Finniss River
Daly River
Reynolds River
Victoria River

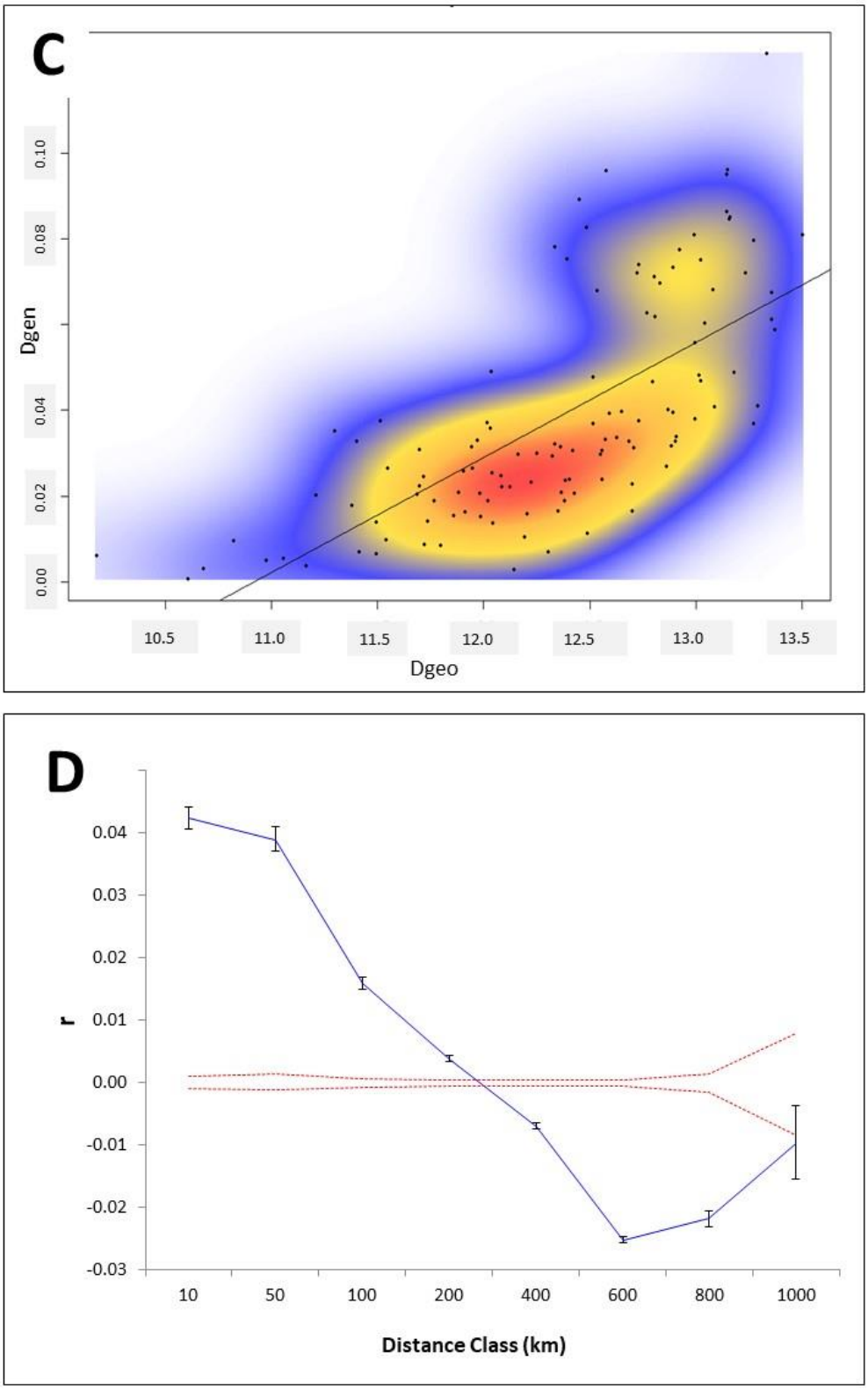


Figure 3.3. A) PCOA of the individual crocodiles based on the genetic distances (F_{ST}) (Smouse and Peakall 1999), B) PCOA for the populations based on a non-standardised distance metric among the populations, C) Isolation By Distance (IBD) plot between the genetic and geographic distances (Dgen and Dgeo, respectively), and D) spatial autocorrelation at the

distance of 10, 50, 100, 200, 400, 600, 800 and 1000 km with r = autocorrelation coefficient. The density of data points is indicated by colours (red = high, medium = yellow, and blue = low) in C.

Landscape resistance analysis

ResistanceGA fitted using individual-level pairwise genetic distance data showed that the model fitted with resistance distances received much greater support (ΔAIC = 0, R^2m = 0.297, R^2C = 0.725) than the null model (ΔAIC = 31819, R^2m = 0.0, R^2C = 0.603) or the unmodified geographic distance model (ΔAIC = 11646, R^2m = 0.090, R^2C = 0.653). Scatterplots of pairwise geographic and fitted resistance distances (random walk commute distance) for the individual-level distance dataset are shown in S3.4.

The selected model identified core breeding habitat as having the lowest resistance (fitted cell value = 1), with increasing resistance attributed to core non-breeding habitat (21.2). Marginal habitats whether breeding (125.7) or non-breeding (122.7) had similar resistances to each other (Figure 3.4B). Fitted resistance values for open sea (707.0) and dry land (2216.9) were much greater than the other environmental cover categories.

Cumulative and normalized current flow maps we created in Omniscape as an exploratory analysis, using the optimized resistance surface from ResistanceGA are shown in Figure 3.4 E and F, respectively.

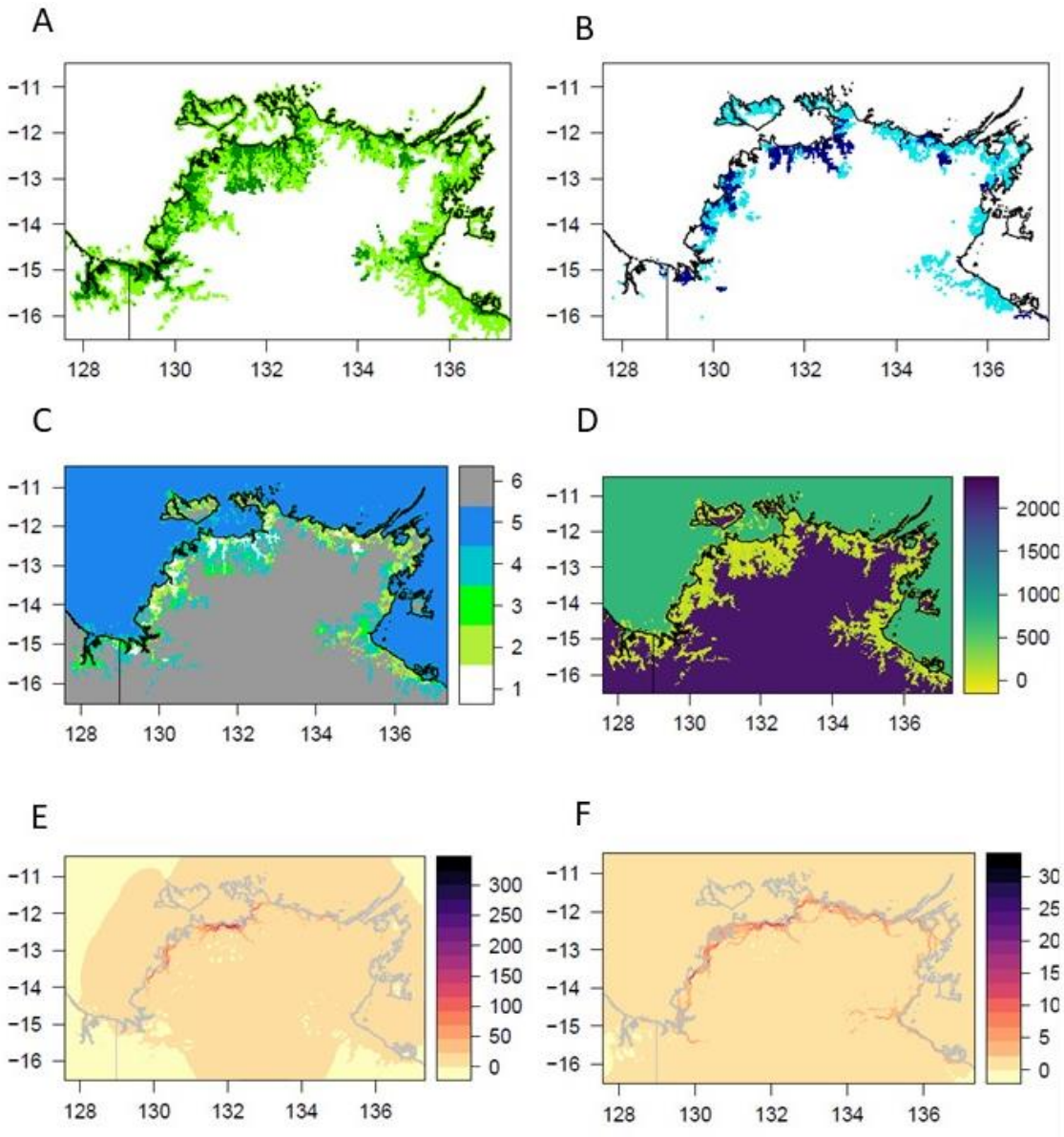


Figure 3.4. Spatial analyses of the crocodile habitats and movement resistances. X and Y axises are longitude and latitude, respectively. A) core (dark green) and marginal (light green) habitats, B) breeding habitats in the core (dark blue) and marginal (light blue) habitats, C) categorical (1 = breeding areas in core habitats, 2 = breeding areas in marginal habitats, 3 = core habitats, 4 = marginal habitats, 5 = sea, and 6 = land), environmental surfaces used for the resistance modelling, D) optimized resistance surface, E) cumulative current flow mapped by Omniscape, and F) normalized current flow mapped by Omniscape.

Population assignment to identify movement events

Population assignment testing suggested that 41.67% of free-ranging crocodiles sampled were most likely to come a different river to where they were sampled, based on an assignment threshold probability of 0.9 for $N = 60$ crocodiles (Figure 3.5). Among the assigned individuals

that moved between the populations, the most common distance travelled between populations, typically between river mouths along coasts, was 150-200 km (N = 48), with fewer than 20% of assigned non-embryo crocodiles moving over 200 km between rivers but some dispersal events over 500 km were detected (Figure 3.6).

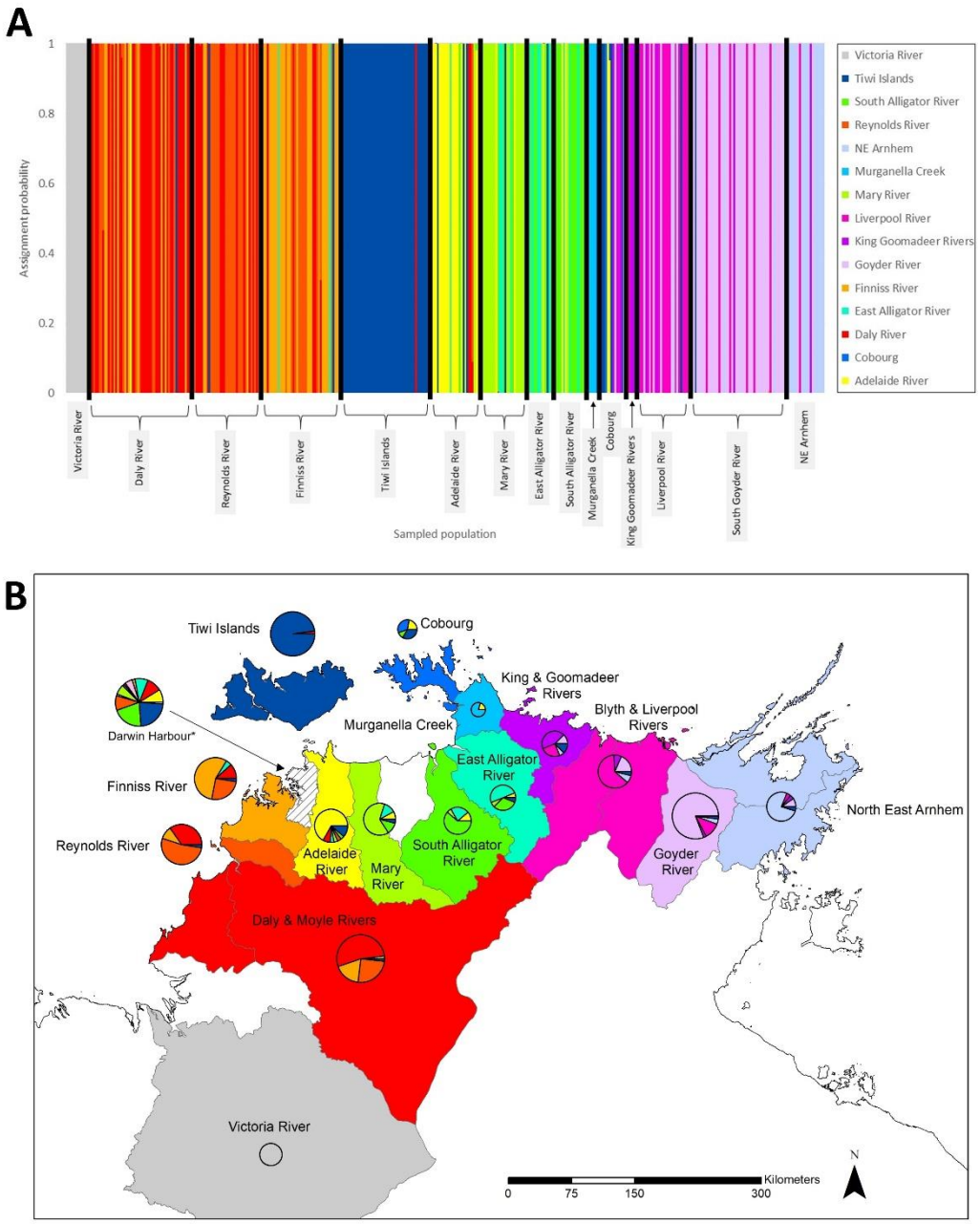


Figure 3.5. Population assignment of sampled crocodiles across the study area. A) The colour of the bars signify the source locations of each crocodile sampled and B) the colour of the catchments shows the source locations. The size of the pie charts is proportional to the sample size (see Table 3.1) and the pie chart in each location shows proportion of the crocodiles

assigned to different sources indicated by the colour coding. Catchments without a colour indicate that no sample was collected.

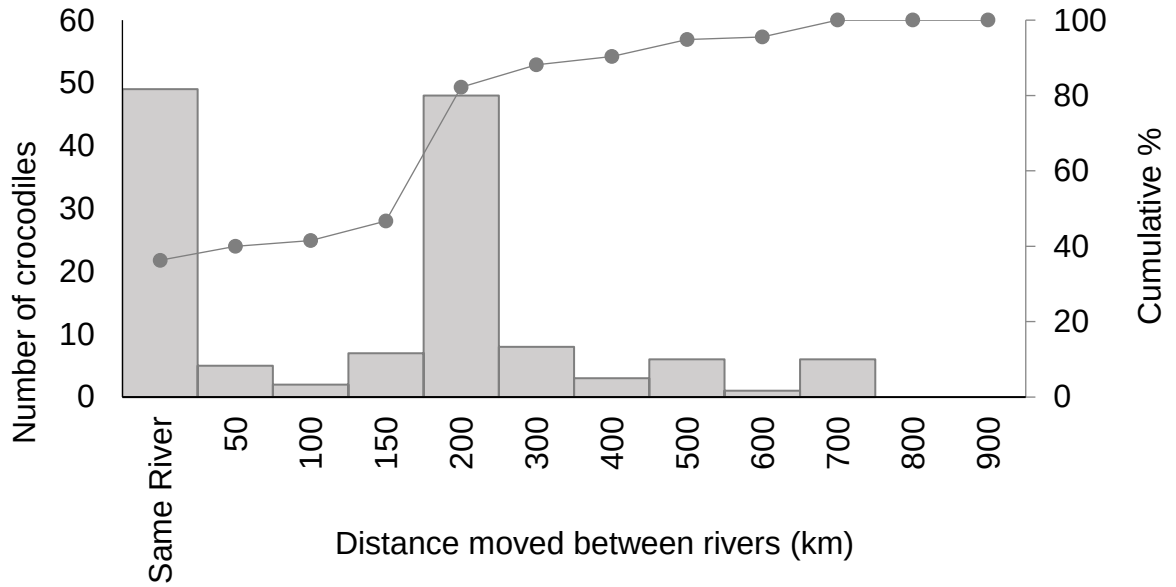


Figure 3.6. The number (bars) and cumulative percentage (line) of non-embryo crocodiles travelling over different distances among river systems as identified by genetic assignment testing. Only crocodiles assigned to a population with a probability greater than 0.9 are included.

Modelling environmental effects on crocodile population connectivity

The best-supported model of dispersal choice from natal locations to destination included negative effects of landscape resistance and the ratio of breeding area to total habitat in the destination river catchment (Figure 3.7, Tables 3.2 and 3.3). The best-supported model of natal assignment from sampled locations included negative effects of landscape resistance and the ratio of breeding area to total habitat in the source river catchment, as well as positive effect of the interaction of these variables. The negative effect of landscape resistance was weaker in cases where source populations have a high ratio of breeding habitat area to total habitat area. Overall, both landscape resistance and habitat characteristics (proportion of breeding habitat) were important in both sets of models, receiving a variable importance of over 0.99 when we summed the Akaike weights of all models containing either variable (Table 3.2).

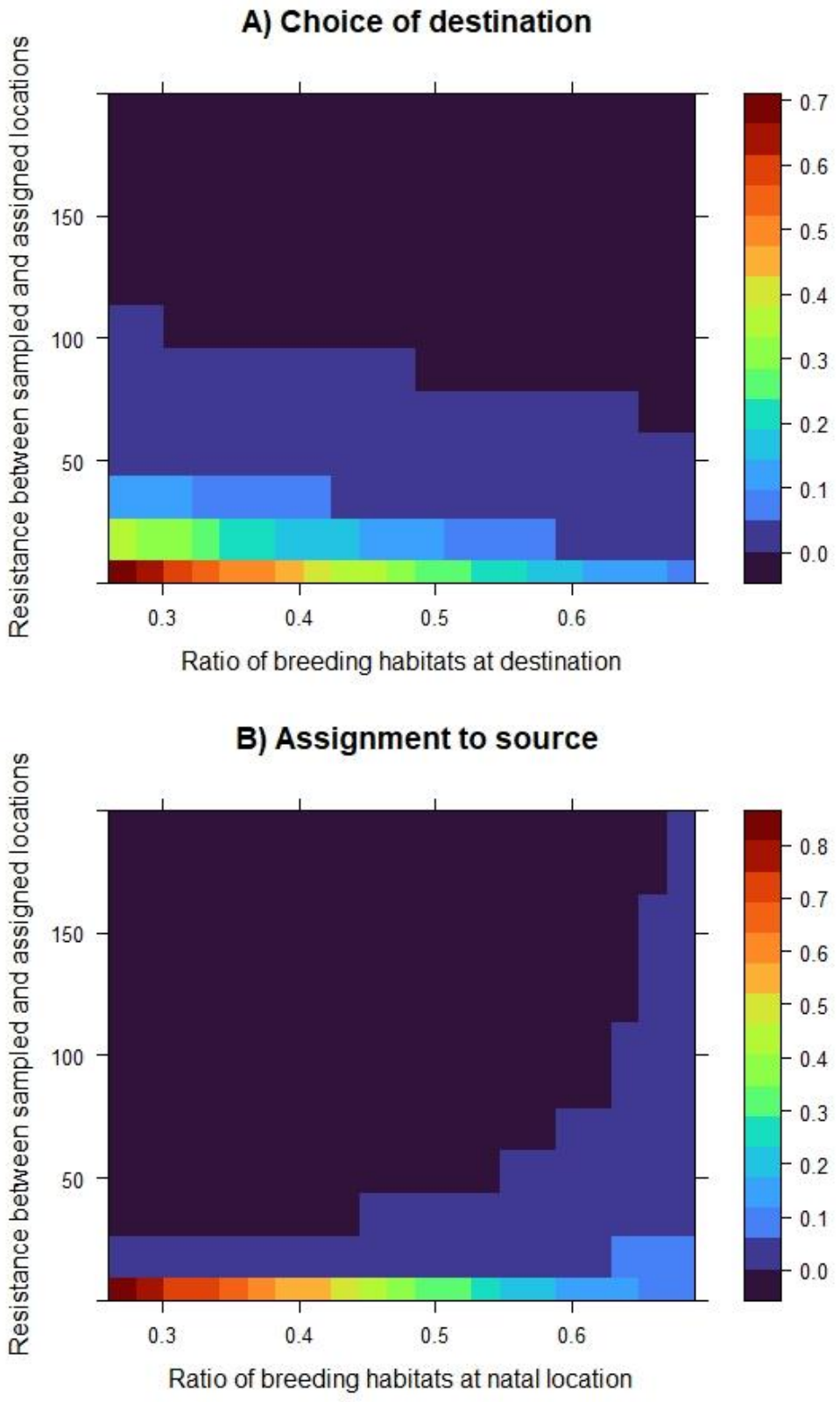


Figure 3.7. Heatmaps of the effects of A) landscape resistance and ratio of breeding area to total habitat in sampled destination and B) landscape resistance and ratio of breeding area to total habitat in assigned source on the probability of movement predicted by the best supported

models (see Table 3.2). The heat colour represents the probability of the dispersal choice (A) and natal assignment (B) of free-ranging crocodiles in the study area.

Table 3.2. The models ranked by AIC to examine the environmental effects on the dispersal choice (A) and natal assignment (B) of free-ranging crocodiles in the study area.

Model set	Model	df	AIC	ΔAIC	wAIC
A	Resistance + Breeding habitat	3	257.45	0	>0.99
	Resistance	2	275.24	17.79	<0.01
	Breeding habitat	2	377.43	119.98	<0.01
	Resistance * Breeding habitat	4	1005.38	747.93	<0.01
B	Resistance * Breeding habitat	7	251.45	0	>0.99
	Resistance + Breeding habitat	3	266.11	14.66	<0.01
	Resistance	2	279.16	27.71	<0.01
	Breeding habitat	2	381.89	130.44	<0.01

Table 3.3. The models most supported by AIC to examine the environmental effects on the dispersal choice (A) and natal assignment (B) of free-ranging crocodiles in the study area.

Model	Variable	Coefficient	SE
A. Resistance + Breeding habitat	Intercept	2.67	0.82
	Resistance	-0.07	0.02
	Breeding habitat	-7.37	1.88
B. Resistance * Breeding habitat	Intercept	3.96	0.88
	Resistance	-0.54	0.12
	Breeding habitat	-9.34	1.83
	Resistance * Breeding habitat	0.77	0.18

3.5. Discussion

Our analyses supported both the resistance to movement hypothesis and the habitat availability hypothesis as influencing dispersal by *C. porosus*. Landscape resistance had slightly higher support than habitat characteristics (proportion of breeding habitat) in our models of assigned movement but the difference was minor and we conclude that these are both important and

interacting drivers of dispersal behaviour. Further, we found that there was an interaction between these variables in their effect on assigned dispersal events, suggesting that populations in catchments with a high proportion of breeding habitat to total habitat can contribute to recruitment via dispersal to populations separated by large resistance distances. This information enabled us to quantify and compare the roles of the environmental influences on *C. porosus* dispersal that has been little known previously.

The resistance to movement hypothesis

We found that the environmental resistance surface, the environmental variable most relevant to the movement phase of dispersal, was the most important variable in our multinomial models of dispersal choices. The optimized resistance surface was inversely correlated with habitat quality, which is commonly assumed to influence connectivity (Spear *et al.* 2010). Similar approaches that incorporate genetic connectivity in parameterizing resistance surfaces have been used for many predatory species at different spatial scales (Cushman and Lewis 2010; Garroway *et al.* 2011; Ruiz-González *et al.* 2014; Warren *et al.* 2016). While we detected effects of source and destination habitat on dispersal, our results are in concordance with recent research suggesting that landscape permeability can be more important than local habitat characteristics in shaping functional connectivity in semi-aquatic species (Parsley *et al.* 2020). The broad environmental variables relating to habitat quality could be used to map likely patterns of population connectivity. However, we note that the effects of resistance were mediated by habitat characteristics in each catchment. Essentially, the negative effect of increasing resistance distance on assigned dispersal events (crocodiles were less likely to be assigned to source populations separated by greater resistance distances to their sampled location) was moderated by the proportion of breeding habitat in the source population.

The large variation in the resistance estimated for *C. porosus*, ranging from 1.0 for core breeding habitat to 2216.9 for dry land is consistent with empirical data that the availability of breeding habitat is the primary influence on population abundance (Fukuda et al., 2007; Webb, 1991) and the species rarely moves over land. While overland movement is more frequently reported for other crocodilian species such as *Alligator mississippiensis* (Woolard *et al.* 2004) and *C. niloticus* (Combrink 2015), such movement is rare for *C. porosus* as shown by tracking of over 100 crocodiles in Australia (Baker, Franklin, Campbell, Irwin, & Dwyer, 2019; Fukuda et al., 2019). It should be noted that the open sea had a relatively high resistance (707.0) compared to the other estuarine or freshwater habitat types (marginal habitats whether breeding

125.7 or non-breeding 122.7). This suggests that, despite their physiological adaptation to the fully saline environment (Grigg et al., 1980; Grigg, 1981; Taplin, 1984; Taplin & Grigg, 1981), brackish or freshwater environments, generally more preferred habitats (Fukuda & Cuff, 2013; Fukuda et al., 2007), are also better for population connectivity.

The habitat availability hypothesis

As well as the importance of landscape resistance to movement, we identified effects of breeding habitat at the dispersal sources and destinations in our modelling and model selection process. This highlights the importance of addressing immigration and emigration as well as movement in considering the dispersal process (Bowler and Benton 2005; Travis *et al.* 2012; Banks and Lindenmayer 2014). Density and resource competition within a population are hypothesized to be important drivers of emigration and settlement choices in animals (Gadgil 1971; Bowler and Benton 2005; Travis *et al.* 2012). A number of studies have shown that dispersal is related to population density at the source location (Amarasekare 2004; Matthysen 2005; Poethke *et al.* 2011) and that population density and resource competition can be negatively correlated with settlement opportunities (Wauters *et al.* 2010; Fraser *et al.* 2015).

Our hypothesis about the ratio of mapped breeding to total habitat is that a high ratio is likely to correspond to high competition from locally-born individuals, leading to fewer opportunities for settlement by immigrants. In our case, the relative availability of breeding habitats at destination sites was included in the best supported model for dispersal choice. The negative coefficient for breeding habitat at the destination site as an important component of settlement is consistent with that habitats become occupied with a net flow of individuals from higher to lower suitability when density is increasing (Carr *et al.* 2007). Given that crocodile density in most rivers in the Northern Territory have been increasing towards an asymptote (Fukuda *et al.* 2011; Saalfeld *et al.* 2016), it is likely that individuals are forced out of favourable, thus saturated sources to habitats less suitable for breeding as indicated by our destination choice models. The interaction between breeding habitat to total habitat ratio and landscape resistance supports this, suggesting that populations with a high proportion of breeding habitat can contribute long-distance dispersers that recruit to distant populations. This may explain increasing sighting of crocodiles in places where they were previously scarce such as distant waterholes and extreme upstream of rivers far inland (Letnic and Connors 2006; Webb 2012; Fukuda *et al.* 2014).

Contrary to our expectation, our models on the assignment to natal sources showed a negative effect of the ratio of breeding habitats on emigration, suggesting that migrants were more likely to come from habitats where less breeding areas were available. As shown by Carr, Bowman, & Wilson (2007) that habitats are filled in order of suitability in an increasing population, if breeding areas are available at a higher proportion, crocodiles may remain within quality habitat. This is not surprising given that our assignment analysis showed that more than 58% of the sampled crocodiles did not leave the natal sources. Crocodiles that did not disperse may be mature individuals, dominating limited breeding opportunities while many of the crocodiles leaving their natal rivers could be subordinate, actively looking for new territories, although we did not find a significant difference in the mean total length between these two groups ($p = 0.85$).

As in many other species (Prugnolle and de Meeus 2002; Cano *et al.* 2008; Liebgold *et al.* 2011), *C. porosus* males are more actively dispersing while females are philopatric (Campbell *et al.* 2013). Although we were not able to identify sex of each sampled individual in the non-invasive biopsy method (Barrow and Halford 2019), the empirical data suggested that nomad individuals captured as problem crocodiles in the Northern Territory were most commonly immature males (Nichols and Letnic 2008; Fukuda *et al.* 2014). Moreover, non-environmental factors that were not considered in this study, such as individual, genetic variation in dispersal-linked traits (DiLeo *et al.* 2018), can also influence population connectivity and dispersal.

Wildlife management applications

How we apply movement data, whether generated through landscape genetics or other approaches, to wildlife population management is determined by the management context. In the case of the saltwater crocodile, sustainable harvest and safety of humans and livestock are major issues around which population management is framed, with conservation as an underlying goal (Saalfeld et al., 2016; Saalfeld, Fukuda, Duldig, & Fisher, 2015; Webb et al., 1984). Currently, the major hydrographic catchments or regions that group two or three adjacent catchments are treated as population units for population monitoring (Fukuda et al., 2011) and egg harvest allocation for the crocodile farming industry (Saalfeld et al., 2016). Our data support this strategy of classifying these catchments or regions as ‘management units’ (Moritz 1994; Waples 1998; Moritz 2002) as the intervening land or saline environments limit movement among river systems. In addition, coastal geographic features or regions of high or complex ocean current flow may exacerbate difficulties in moving among river systems

(Fukuda et al., 2019). However, our data show that populations are linked by extensive dispersal, with approximately 42% of individuals having been born in a different catchment to where they were sampled. The data also show that some river systems are disproportionately important for providing recruits to other populations. For instance, the Goyder River (Arafura Swamp) population can be seen as contributing recruits to many other river systems in either direction along the coast from this productive breeding population (Figure 3.5). In contrast, the Tiwi Islands population appears to draw recruits predominantly from the local population and is the source of immigrants to few areas other than the nearby Cobourg Peninsula, presumably due to the high resistance of the marine environment surrounding the islands.

Our approach can help manage populations by identifying recruitment patterns, including the location of dispersal sources and sinks. As previous studies suggested (Carr *et al.* 2007), dynamic population connectivity and habitat quality which is difficult to map due to some uncertainty in inter-annual variation in environmental conditions (e.g. rainfall known to influence the abundance of hatchlings in the following year (Fukuda & Saalfeld, 2014)) can be inferred from the genetic structure. Different levels of carrying capacity in recovering populations shown by the extensive monitoring (Messel *et al.* 1981; Fukuda *et al.* 2011; Saalfeld *et al.* 2016) suggest that dispersal of individuals is density dependent and such movement patterns are predictable.

Managing large predator populations for human safety can also benefit from the kind of dispersal data generated through landscape genetics. Saltwater crocodiles (and other large predators) are often removed from locations with high usage by humans such as major population centres (Brien et al., 2017; Fukuda et al., 2014; Saalfeld et al., 2016). Understanding the population sources and dispersal distances of problem animals can help identify and evaluate management options (such as increased intervention or harvest at source locations, or identification of likely dispersal destinations). From our data, 200 km appears to be the most common range of *C. porosus* movement between the river mouths along the coast (Figure 3.6), but a few dispersed much long distances (up to 600-700 km). Campbell et al. (2010) showed that such long-distance movement could be assisted by ocean currents and that individuals of the species are likely to be able to move much further from its natal location than other crocodilian species that are constrained to freshwater predominantly, for which much shorter movement events have been reported (Woolard *et al.* 2004; Campos *et al.* 2006; Lance *et al.* 2011; Combrink 2015). In the next chapter, we identify the source of problem crocodiles in

Darwin Harbour, the largest city in the Northern Territory, where our analysis showed the most admixture (Figure 3.5).

In summary,environmental permeability was most important in the environmental models of the observed dispersal events, but properties of the source and sink catchments also influenced patterns of emigration and immigration. Our habitat quality variables representing the availability of wet season breeding habitat, suggested that competition for habitat influences both emigration and settlement choices, together with the strong effect of environmental resistance to movement.

3.6. Ethics and acknowledgement statement

This study was conducted in accordance with the Code of Practice on the Humane Treatment of Wild and Farmed Australian Crocodiles (Environment 2013). The protocol was approved by the Animal Ethics Committee of the Australian National University (Animal Ethics Protocol Number A2017/11), Parks and Wildlife Commission of the Northern Territory (Research Permit Number 7902), Department of Parks and Wildlife of the Western Australian Government (Permit Numbers 08-002527-1 and 08-002527-2), and Parks Australia of the Australian Government (Permit Number AU-COM2017-361).

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3.7. Data Accessibility

The data that support the findings of this study are openly available in dryad at the following doi reference numbers.

[dataset] Fukuda, Y., Banks, S.; 2020; *C. porosus* genotype data;

Dryad; https://doi.org/10.5061/dryad.bcc2fqz94

[dataset] Fukuda, Y., Banks, S.; 2020; Landscape layer for resistance;

Dryad; https://doi.org/10.5061/dryad.q2bvq83gb

[dataset] Fukuda, Y., Banks, S.; 2020; Omniscape files;

Dryad; https://doi.org/10.5061/dryad.2z34tmpj1

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CHAPTER 4: Natal origin and dispersal of problem saltwater crocodiles in the Darwin Harbour, Australia

4.1. Abstract

The management program that successfully recovered wild saltwater crocodile *Crocodylus porosus* populations in the Northern Territory of Australia (since 1971), did so with a commitment to maintaining public safety. One aspect of the program is the ongoing removal of resident and immigrant *C. porosus* within Darwin Harbour (since 1979), the main urban center. We determined the likely sources of crocodiles caught as problem animals by comparing recently developed methods for population assignment. Depending on the assignment model used, we estimated that between 30% and 50% of crocodiles in Darwin Harbour originated from the Adelaide and Mary Rivers, and the Kakadu region, to the immediate east of Darwin, and between 20% and 30% of crocodiles originated from the Finniss, Reynolds and Daly Rivers south west of Darwin. *C. porosus* occur at particularly high densities in these catchments. The remainder came from a mixture of the different sources across the Northern Territory. The most common animals captured were immature (150-180 cm) males that have travelled 100-200 km. No significant relationships between the distance from the inferred origin to the Darwin Harbour and the size and sex of the crocodiles, or the year of capture could be determined. The targeted removal of crocodiles from specific sites such as Darwin Harbour, where most people live, improves public safety in the highest risk areas, without compromising abundant source populations in most areas.

4.2. Introduction

Many large predator populations, including a number of crocodilian species (Groombridge 1987), were depleted historically by direct hunting, but were subsequently rebuilt by conservation action, potentially compromising public safety in various ways (Ferretti et al. 2015, Wallach et al. 2015, Marshall et al. 2016, Pooley et al. 2021). In the Northern Territory (NT) of Australia, the depletion and recovery of saltwater crocodiles (*Crocodylus porosus*), one of the largest and most aggressive of extant crocodilian species, exemplifies this conundrum. The NT now contains the largest population of saltwater crocodiles throughout its range (Webb et al. 2010, Fukuda et al. 2011). While valued highly by some stakeholders for aesthetic, cultural, ecological and economic reasons (Lanhupuy 1987, Ryan 1998, Saalfeld et al. 2015, Campbell et al. 2022), public and political support to sustain large wild populations depends on minimising crocodile attacks on people (Webb 2012, Fukuda et al. 2014).

The management protocols that evolved after protection in 1971 for saltwater crocodiles were initially focussed on preserving a small remnant population (Webb et al. 1984, 1994). But by the end of the 1970's, with crocodile abundance increasing across coastal wetlands, direct action regarding public safety became increasingly important (Webb 2014, 2021). This was the basis of the decision to remove crocodiles from Darwin Harbour in 1979, where the highest human population of the NT occurs in residential and industrial areas that are expanding (Australian Bureau of Statistics 2022). Today some 250 to 300 crocodiles are removed annually for public safety (Fukuda et al. 2014, Saalfeld et al. 2016, Department of Environment, Parks and Water Security (DEPWS) 2021). The removed crocodiles are predominantly sexually immature males (150-200 cm total length [TL]), thought to be excluded by dominant males from core habitats and searching for a potential home territory in marginal habitats along the coastlines (Nichols and Letnic 2008, Fukuda et al. 2014, 2022b). Although saltwater crocodiles are highly adapted for saline environments, and they may travel along coastlines or take long sea voyages (Read et al. 2004, Campbell et al. 2010, Fukuda et al. 2019, Spennemann 2021), they need access to lower salinity waters if remaining in a marine environment for long periods (Grigg et al. 1980, Taplin and Grigg 1981, Taplin 1985). Their breeding is largely confined to freshwater or low salinity parts of rivers and associated wetlands (Webb et al. 1977, Magnusson 1980, Webb 1991, Fukuda and Cuff 2013, Fukuda et al. 2022a). To build nests, females use a reasonably narrow range of vegetation types such as tall, closed tussock grassland with Oryza species and open forest with *Eucalyptus*, *Melaleuca*, and *Pandanus* species with tussock sedgeland ground layers (Magnusson 1980, Fukuda et al. 2007, Fukuda and Cuff 2013). Extensive vegetation mapping across the NT indicates that such suitable nesting habitat is extremely limited in the Darwin Harbour catchment (Fukuda et al. 2007, 2022a, Fukuda and Cuff 2013). Subsequently, very few hatchlings have ever been encountered in Darwin Harbour (Messel et al. 1981, Nichols and Letnic 2008, Fukuda et al. 2014). All these suggest that most crocodiles caught in the Harbour are not locally recruited but are migrants from other populations within variable proximities. In addition, recent molecular analyses suggested fairly high gene flow among crocodile populations in the NT, with approximately 42% of 515 crocodiles sampled across the NT being identified as originating from areas outside where they were sampled (Fukuda et al. 2022b, Chapter 3).

If we can identify the sources and more detailed movement patterns of crocodiles caught in Darwin Harbour, safety programs can be more strategic and effective at mitigating potential conflicts between crocodiles and people. For example, if we find out that a certain population

is the source of most crocodiles in the Harbour, management efforts that often have limited resources, can be focused on that population as a priority. Alternatively, if crocodiles are a mixture from different origins, they may be managed more efficiently within the Harbour.

In this study, we test a hypothesis that crocodiles found in Darwin Harbour, where local recruitment is considered minimal, are sourced from other populations by applying recently developed genetic population assignment methods. After we determine the likely sources of crocodiles in the Harbour, we also examine whether crocodiles from different origins vary in their length, sex, and time of capture to see any patterns between their migration and biological attributes (e.g. more immature males travel longer distances in a particular year).

4.3. Materials and methods

Study area

The study focused on Darwin Harbour, with reference samples collected from a focused region around the Top End of NT and East Kimberley of Western Australia (WA) (Figure 4.1A) and lower density sampling from a broader area across northern Australia and Southeast Asia (Figure 4.1B). The Top End of the NT (approximately 400,000 km2, elevation 0-300 m) and East Kimberley (117,5100 km2, elevation 0-1,200 m) contain numerous coastal rivers and creeks, lined with mangroves and under tidal influence, and extending upstream from the sea to a diversity of freshwater, non-tidal floodplain wetlands and sand and rock-lined water courses (Webb 1991). All are occupied by saltwater crocodiles at different densities, as indicated by spotlight sighting densities, ranging from 0.1 to 15 crocodiles sighted per kilometer of river (Fukuda et al. 2007, 2011, Saalfeld et al. 2016). The species also occurs along the coastline between rivers and occupies near coastal islands (Webb and Manolis 1989, Webb 1991). The climate in the study region is monsoonal, with a distinct wet season with heavy rains from November to April, and a dry season from May until October with little rain. Available wetlands expand during the wet season when crocodiles nest, and contract during the dry season (Webb 1991, Fukuda and Cuff 2013), when many temporary water bodies dry completely if early rains are delayed. Crocodiles tend to remain in permanent water bodies during the dry season, but are sometimes forced to aestivate in drying mud (Webb and Manolis 1989, Lindner 2004). In the NT, there is limited intensive agriculture and most coastal and riparian habitats remain intact (Fukuda et al. 2007). Outside Australia, the species is distributed in many Indo-Pacific countries ranging from the eastern coasts of India in the west, to Vanuatu

in the east, and from the Philippines in the north to around 23°S latitude in Australia in the south.

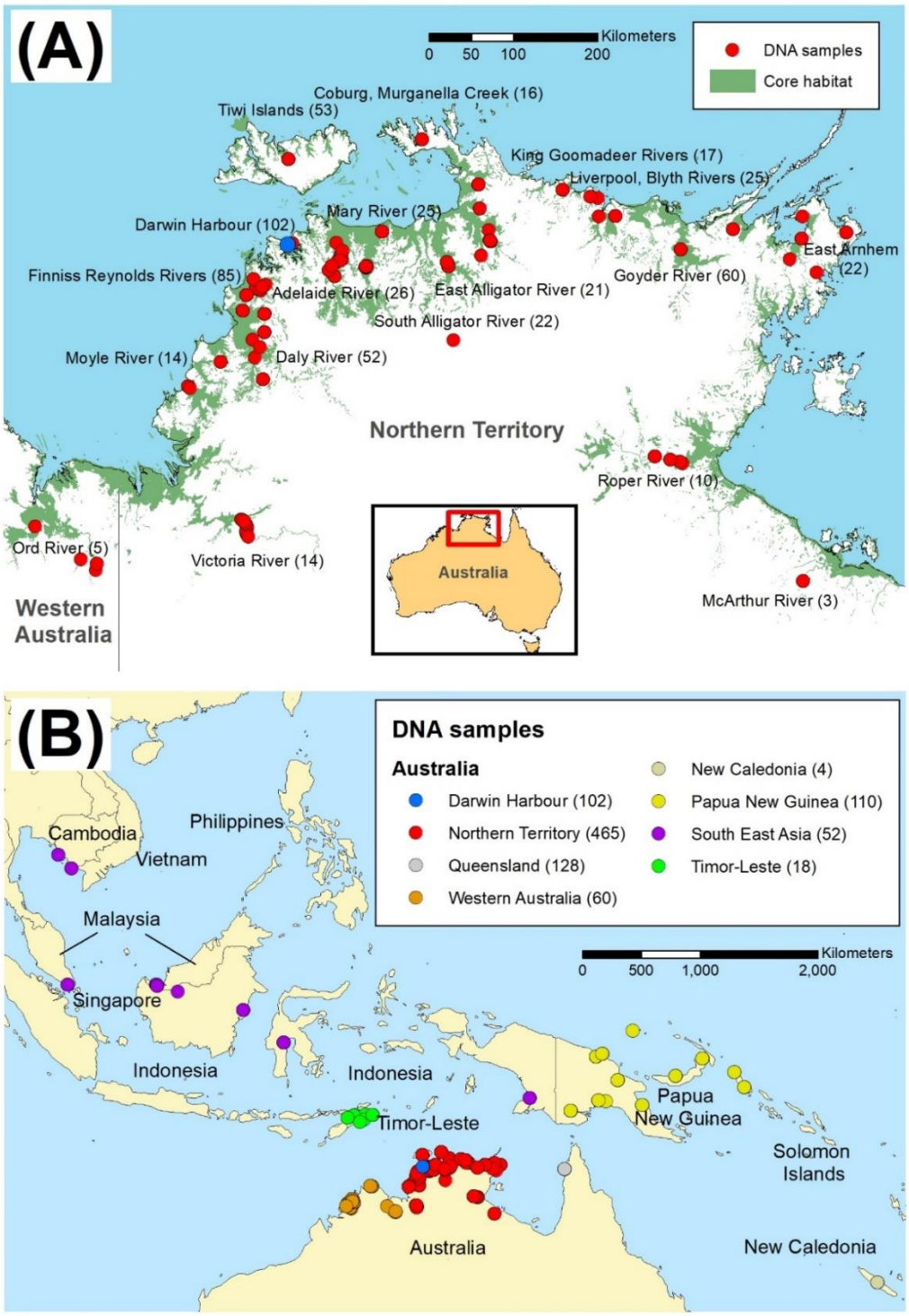

Figure 4.1. Locations of genetic samples of saltwater crocodiles collected in A) the Northern Territory and Western Australia, Australia and B) the neighboring countries between 1997 and 2022. The number in brackets is the number of the samples collected for each location.

Sample collection and genotyping

We collected 939 geo-referenced saltwater crocodile tissue samples from across northern Australia and the nearby countries of Cambodia, Indonesia, Malaysia, New Caledonia, Papua New Guinea (PNG), Singapore, Timor-Leste, and Vietnam (Figure 4.1). The tissue samples were pieces of skin collected randomly from 1) free-ranging crocodiles using a biopsy pole (Barrow and Halford 2019) or by hand capture in 2016-2021, 2) crocodiles captured as part of the public safety programs in 2015-2017 (Fukuda et al. 2014, Saalfeld et al. 2016), 3) embryos that failed to develop to term during incubation in 2015-2020, or 4) museum samples that had been collected for previous studies in 1997-2000 (FitzSimmons et al. 2001, FitzSimmons et al. 2002). The embryos came from eggs collected from wild populations as part of the NT's commercial egg ranching program (Leach et al. 2009, Saalfeld et al. 2016), and we used one egg per clutch collected from a geo-referenced nest location. The free-ranging crocodiles sampled ranged from 0.3 to 5.1 m long.

DNA extraction and genotyping were conducted at Diversity Arrays Technology (Canberra, Australia) using the DArTseq approach (Kilian et al. 2012). DArTseq uses genome complexity reduction and NGS approaches conceptually similar to RADseq, in this case based around a SphI enzyme digestion of genomic DNA and Illumina HiSeq 2500 sequencing. We ran 30% of samples twice as technical replicates to assess the repeatability of single nucleotide polymorphism (SNP) calls. Initial sequence processing and SNP calling by DArT yielded 22,227 SNP loci across 16,637 contigs. The reference genome assemblies were CroPor_comp1 (Mississippi State University 2016) and Cpor_3.0 (ICGWG 2020). We retained 19,630 SNPs as they mapped to either of these assemblies. As an initial data exploration step, we plotted basic SNP metrics on sequence depth and other 'quality' metrics and explored relationships between these metrics and basic population genetics statistics, including observed and expected heterozygosity. The graphical analysis was used to evaluate whether genotype properties (e.g. observed heterozygosity) were associated with SNP quality metrics such as mean sequencing depth.

Population assignment – broad scale

Before assigning crocodiles samples (with unknown, mixed origins) from Darwin Harbour to broad geographic sources, we filtered the SNPs, using the dartR package in R 4.2.2 (Gruber et al. 2017), according to call rate (>0.80), repeatability (>0.95), mean total depth (10-200), and mean ratio of sequence depth (0.5-2). The allele depth ratio criterion was set for removing loci

in which one allele was preferentially detected, indicating potential susceptibility to null alleles through PCR bias. We also dropped 1) SNPs (ranked by polymorphic information content) within sequences with more than one SNP present, 2) SNPs that did not align to the reference genomes (Mississippi State University 2016, ICGWG 2020) with an e-value threshold of -10 or lower, and 3) SNPs that mapped to more than one location on the reference genome. We dropped genotypes of individuals for which greater than 10% of SNPs were not genotyped. Following these filtering steps, we retained 1270 SNPs in 912 of the 939 sampled individuals with a minor allele frequency of greater than 0.02, corresponding to ≥ 20 allele copies for analysis. We graphed the distribution of the genetic diversity statistics to visualize any unexpected patterns with regard to Hardy-Weinberg expectations within populations, but did not filter any further based on this graphical analysis (S4.1).

Depending on the location of the 912 filtered samples, we broadly grouped the Australian samples (N = 732) as being from Darwin Harbour (N = 95), non-Darwin Harbour NT (N = 463), Queensland (QLD; N = 126), and WA (N = 55), and other samples as PNG (N = 100), Southeast Asia (N = 49; Cambodia, Indonesia, Malaysia, Singapore, Vietnam), and Timor-Leste (N = 18) (Figure 4.1B). We excluded New Caledonia from this analysis due to its geographic isolation and small sample size (N = 4). PNG and Timor-Leste were kept separate because of their close proximity to Darwin Harbour. We calculated the observed heterozygosity (HO), expected heterozygosity (HE), inbreeding coefficient (FIS) within populations and genetic differentiation among populations (FST) to assess the genetic differentiation differences between these broad groups, using GenAlEx (Peakall and Smouse 2006, 2012).

For the broad genetic assignment, we used the R package assignPOP (Chen et al. 2018) and the program Structure (Pritchard et al. 2000, Wang 2017). The assignPOP package estimates membership probabilities of each individual with unknown origins in a supervised machine-learning framework (Chen et al. 2018). We used the Support Vector Machines (SVM) with linear kernel and parameter cost = 1 to build the predictive models for estimating probabilities as it generally gives higher accuracies than other models in mixed-stock analyses (Chen et al. 2018, Euclide et al. 2021). In Structure, we set 20,000 for the Length of Burnin Period (LBP) and 80,000 for the number of Markov Chain Monte Carlo (MCMC) repetitions after Burnin with 2 iterations. We used the admixture model with GENSBACK = 2, MIGPRIOR = 0.05, and ALPHA = 0.03 as well as the correlated allele frequency model with 0.01 prior mean, 0.05 prior SD, and 1.0 lambda. We also considered other population assign methods [BONE

(Kuismin et al. 2020) and rubias (Moran and Anderson 2018)], but found these more sensitive to differences in the sample size than assignPOP and Structure (S4.2).

Population assignment – fine scale

As all broad genetic assignments indicated that the crocodiles sampled in the Darwin Harbour were most likely related to the NT group (Figure 4.2), we conducted further analyses at a finer scale within the Northern Territory (Figure 4.1A). We used the same criteria for the SNP filtering but only used the NT samples and Ord River (N = 565). Prior to filtering, we excluded the McArthur River (N = 3) and Roper River (N= 10) from the analysis due to their distance from Darwin Harbour (>2000 km along the coast) and small sample sizes. Analysed sample locations from west to east included Ord River (N = 5), Victoria River (N= 14), Moyle Rivers (N = 14), Daly River (N = 52), Finniss and Reynolds Rivers (N = 85), Adelaide River (N= 26), Mary River (N = 25), Tiwi Islands (N = 52), Kakadu (Cobourg Peninsula and Murganella Creek with N = 16, East Alligator River with N = 18, South Alligator River with N = 21; N = 55), West Arnhem Land (Blyth River with N = 23, King Goomadeer River with N = 17; N = 40), Goyder River (N = 60), East Arnhem Land (N = 22) (Figure 4.1A).

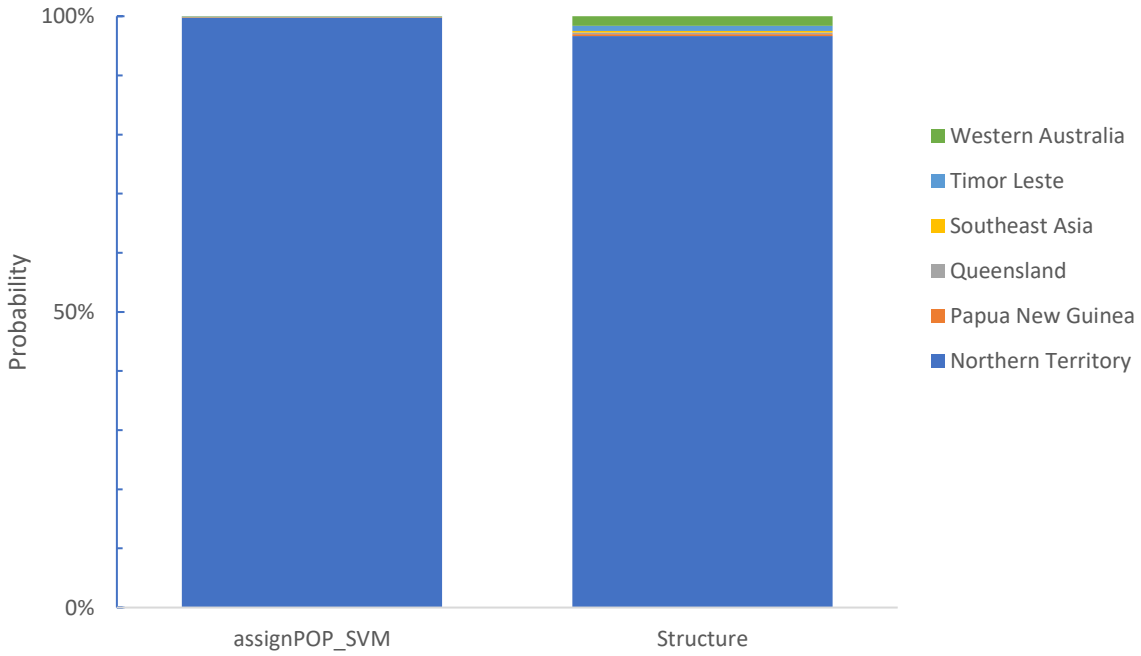

Figure 4.2. Probability of saltwater crocodiles averaged across the individuals sampled in the Darwin Harbour (N = 95) being assigned by the different methods to broad state-wide populations in Australia and neighbouring countries between 1997 and 2022.

We estimated H_O, H_E, F_{IS}, and F_{ST} within the NT as the smallest unit of the collected samples. Because of the generally low genetic differentiation among the catchments (S4.3) and some sample sizes being so low (Table 4.1), we combined the catchments into distinct genetic clusters for the population assignments within the NT. For this, we conducted Discriminant Analysis of Principal Components (DAPC) using the R package adegenet (Jombart 2008, Jombart et al. 2010). We selected the optimized number of clusters with the lowest value of Bayesian Information Criterion (BIC) estimated by DAPC (Figure 4.3A). Based on the a-score analysis (Jombart 2008) (Figure 4.3B), we set the number of retained principal components at 52 and the number of retained discriminant functions at 4. Based on the results of DAPC (Figure 4.3C-F), we separated the NT and Ord River samples into 6 groups of Darwin Harbour (N = 95), Daly River-Finniss and Reynolds Rivers (N = 137), Adelaide River-Mary River-Kakadu (N = 106), West and East Arnhem Land (N = 62), Goyder River (N = 60), Moyle River-Victoria River-Ord River (N = 33), and Tiwi Islands (N = 52). Although DAPC grouped Tiwi Islands with Adelaide-Mary-Kakadu when the number of clusters was set at 5 (Figure 4.3), we separated it as an independent cluster because of its geographic isolation as islands and specific management interest as one of the major sources of eggs for commercial ranching - we thus used 6 clusters in total. As above, we used assignPOP (SVM) and Structure to assign the Darwin Harbour crocodiles to the populations.

Table 4.1. Number of the genetic samples of *Crocodylus porosus* collected from individual catchments in the Northern Territory (NT), Australia, 1997–2022, before and after data filtering and their genetic diversity statistics (H_E, H_O and F_{IS}). H_E = expected heterozygosity, H_O = observed heterozygosity, and F_{IS} = inbreeding coefficient estimated as (mean H_E – mean H_O)/mean H_E. Cluster includes a combination of the individual catchments used for the NT population assignments (Figure 4.4).

Cluster	**Catchment**	**Before filter**	**After filter**	**H_E**	**H_O**	**F_{IS}**
Adelaide River, Mary River, Kakadu	Adelaide River	26	26	0.20	0.19	0.02
	Coburg, Murganella Creek	16	16	0.19	0.19	0.00
	East Alligator River	21	18	0.20	0.20	0.00
	Mary River	25	25	0.20	0.19	0.04
	South Alligator River	22	21	0.20	0.20	0.02
East and West Arnhem Land	Blyth River	23	23	0.21	0.21	0.00
	East Arnhem Land	22	22	0.21	0.21	0.00
	King, Goomadeer Rivers	17	17	0.20	0.20	0.01
Daly River, Finniss River, Reynolds River	Daly River	52	52	0.19	0.19	0.00
	Finniss, Reynolds Rivers	85	85	0.19	0.19	0.04
Goyder River	Goyder River	60	60	0.21	0.20	0.06
Moyle River, Ord River, Victoria River	Moyle River	14	14	0.20	0.18	0.10
	Ord River	5	5	0.17	0.19	-0.11
	Victoria River	14	14	0.19	0.18	0.02
Tiwi Islands	Tiwi Islands	53	52	0.21	0.20	0.02

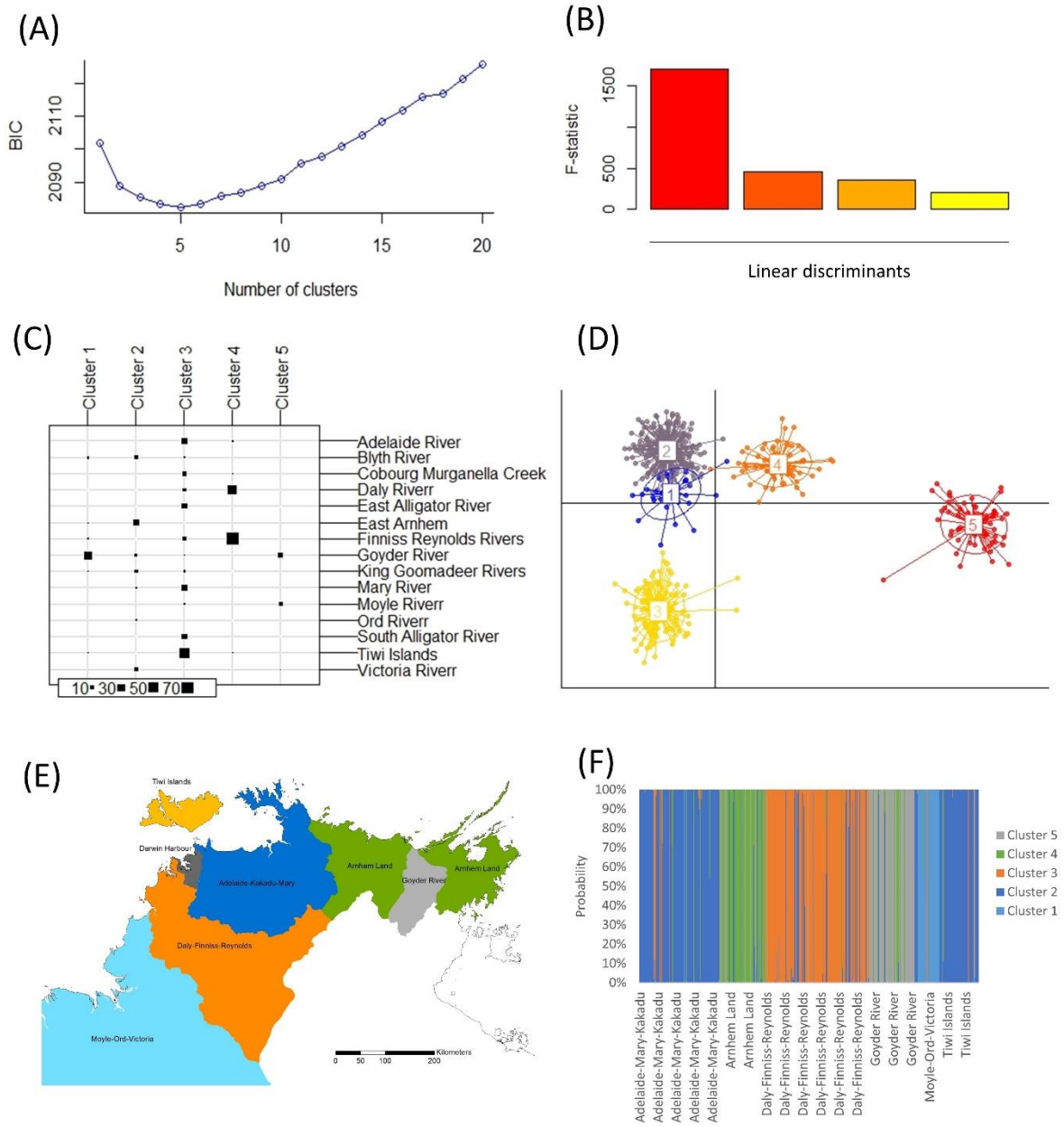

Figure 4.3. Discriminant Analysis of Principal Components (DAPC) of the genetic samples of saltwater crocodiles collected in the Northern Territory and Western Australia, Australia, 1997–2021 with A) Bayesian Information Criterion (BIC) estimated for increasing number of clusters, B) eigenvalues estimated by discriminant analysis, C) cross-validation, D) scatterplot, E) geographic map and F) barchart of the genetic clusters separated by DAPC. Numbers in D indicate the clusters. The cluster numbers are consistent across the panels (C, D, and F).

With the possible sources of the individual samples from the Darwin Harbour inferred by the different methods (with >0.8 probability), we examined whether there was any particular trend

between the inferred origins and their biological attributes including Total Length (TL), sex, and year of capture (2015-2017). We examined these relationships using Generalized Linear Modelling (GLM) and set the distance from the inferred origin to the Darwin Harbour as the response variable and TL, sex, and capture year as the explanatory variables. Because saltwater crocodiles rarely move a long distance over land (Baker et al. 2019, Fukuda et al. 2019), we measured the distance to the Harbour along the coastlines rather than overland, using the Path Distance function in ArcGIS 10.6.1 (esri 2022) with a land cover surface derived from Fukuda et al. (2022) being set as the input cost raster. Because the plot of the response variable against the explanatory variable TL showed a somewhat quadratic relationship (Figure 4.7A), we made the variable a quadratic term in the models (as well as linear to compare). We used the Gaussian link function in both the linear and quadratic models. We ran the models on the results given by each of the population assignment methods. We also used G-test to determine whether the proportions of these variables (TL, sex, traveled distance, and inferred source) varied significantly.

4.4. Results

We filtered SNPs using stringent criteria to retain a set of SNPs consistently genotyped across a large sample size across all populations. From the obtained 19,630 SNPs in 939 individuals, we filtered the dataset to 1270 SNPs (6.47%) in 912 individuals (97.12%) for the broad-scale analysis. The filtering resulted in a change in total expected heterozygosity (H_T), mean subpopulation heterozygosity (H_S), observed heterozygosity (H_O) and F_{IS} from 0.19, 0.13, 0.12, and 0.12, respectively to 0.20, 0.15, 0.16 and -0.02, respectively. For the fine-scale analysis, we retained 1259 SNPs (6.41%) in 545 individuals (58.04%) from the NT and north-eastern WA populations.

The broad scale analyses showed a high overall level of genetic differentiation (F_{ST} = 0.144, SE = 0.004). As expected, animals within the Australian regions showed less genetic differentiation relative to more distant locations (F_{ST} = 0.035 between the NT and WA, 0.042 between the NT and QLD, 0.102 between the NT and Southeast Asia, 0.089 between the NT and Timor-Leste and 0.074 between the NT and PNG). The broad genetic assignment of the Darwin Harbour samples by the different methods consistently showed the highest mean probability for the NT among the above 6 regions and countries (Figure 4.2). assignPOP estimated the probability as 99.9% for the NT while Structure estimated 96.6%. Structure estimated 1.60% probability for WA (<0.01% for PNG, QLD, Southeast Asia, and Timor

Leste). Structure assigned one individual to WA, but assignPOP assigned no individuals to any of the interstate or international groups.

In the fine-scale analyses of the NT and WA, the overall F_{ST} for the individual catchments was 0.065 (SE = 0.003). The highest value of pairwise F_{ST} was 0.062 between the Goyder River (NT) and Ord River (WA). The lowest F_{ST} value was 0.004 between Daly River and the nearby Finniss-Reynolds Rivers (NT). The DAPC suggested 5 clusters based on the lowest BIC when testing with 1 to 20 clusters (Figure 4.3A). Four discriminant functions were retained (Figure 4.3B), cross validation (Figure 4.3C) and scatterplot of principal components (Figure 4.3D), and the bar chart of the probability of the samples being assigned to the clusters (Figure 4.3F) showed distinct genetic separation among the clusters. The genetic diversity statistics (H_E, H_O, and F_{IS}) estimated for the clusters defined by DAPC showed similar values to the catchments (H_O and H_E = 0.18-0.21, F_{IS} = 0.02-0.10; Table 4.2). The overall genetic differentiation (F_{ST}) for the 5 clusters was 0.045 (SE = 0.003). The highest value of pairwise F_{ST} was 0.083 between the Goyder River and Moyle-Ord-Victoria Rivers. The lowest F_{ST} was 0.014 between Adelaide-Mary Rivers-Kakadu and Tiwi Islands (SI-S4).

The probability estimates from the finer-scale genetic assignment of the Darwin Harbour samples varied across the different methods for the 6 clusters in the NT (Figure 4.4). In all cases, the proportions of the clusters as a possible origin varied significantly (G = 116.17 and $p = 2.2\times10^{-16}$ for assignPOP, G = 104.41 and $p = 2.2\times10^{-16}$ for Structure). The combined Adelaide-Mary-Kakadu reference population was the largest source of Darwin Harbour animals and the Goyder River and Moyle-Ord-Victoria the smallest. The combined Finniss-Reynolds-Daly Rivers reference group was identified as the second largest source of Darwin crocodiles. Both assignPOP and Structure showed similar probabilities for most individuals between the clusters (Figure 4.5).

Table 4.2. Genetic diversity statistics for regional clusters of *Crocodylus porosus* samples collected in the Northern Territory (NT), Australia, 1997–2022, including sample size (N), expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) estimated as (mean H_E – mean H_O)/mean H_E.

Cluster	**N**	**H_E**	**H_O**	**F_{IS}**
Adelaide River, Mary River, Kakadu	106	0.20	0.19	0.05
Arnhem Land	62	0.21	0.21	0.03
Daly River, Finniss River, Reynolds River	136	0.19	0.19	0.03
Goyder River	60	0.21	0.20	0.06
Moyle River, Ord River, Victoria River	33	0.20	0.18	0.10
Tiwi Islands	52	0.21	0.20	0.02

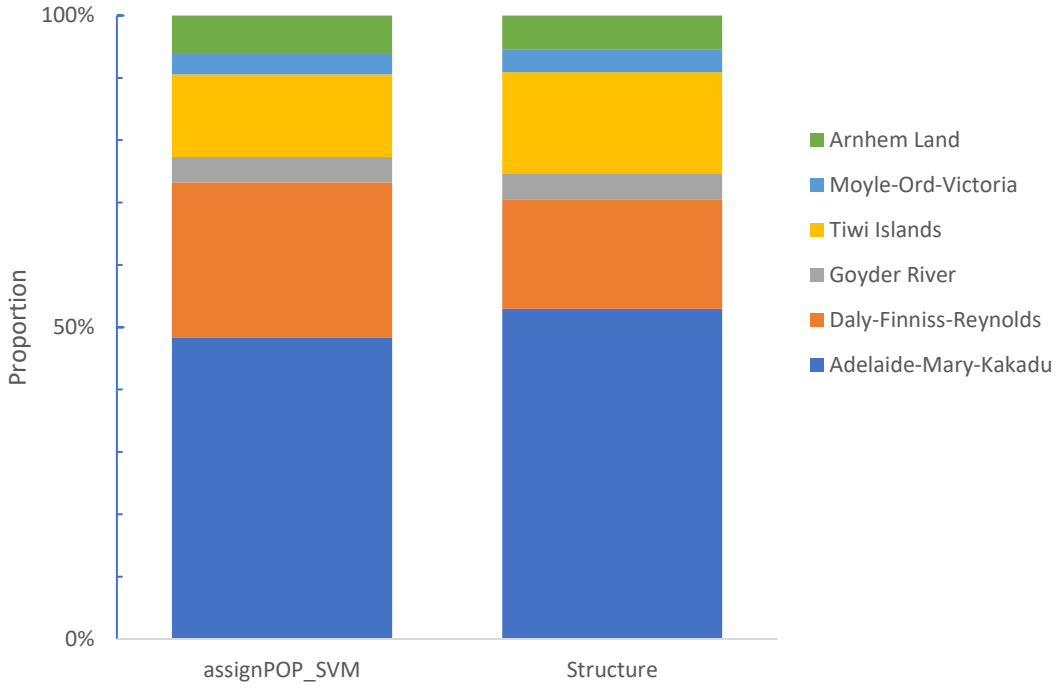

Figure 4.4. Probability of saltwater crocodiles averaged across the individuals sampled in the Darwin Harbour (N = 95) being assigned by assignPOP and Structure to the 6 clusters identified by Discriminant Analysis of Principal Components (Figure 4.3) that grouped rivers in the Northern Territory and Ord River sampled between 1997 and 2022.

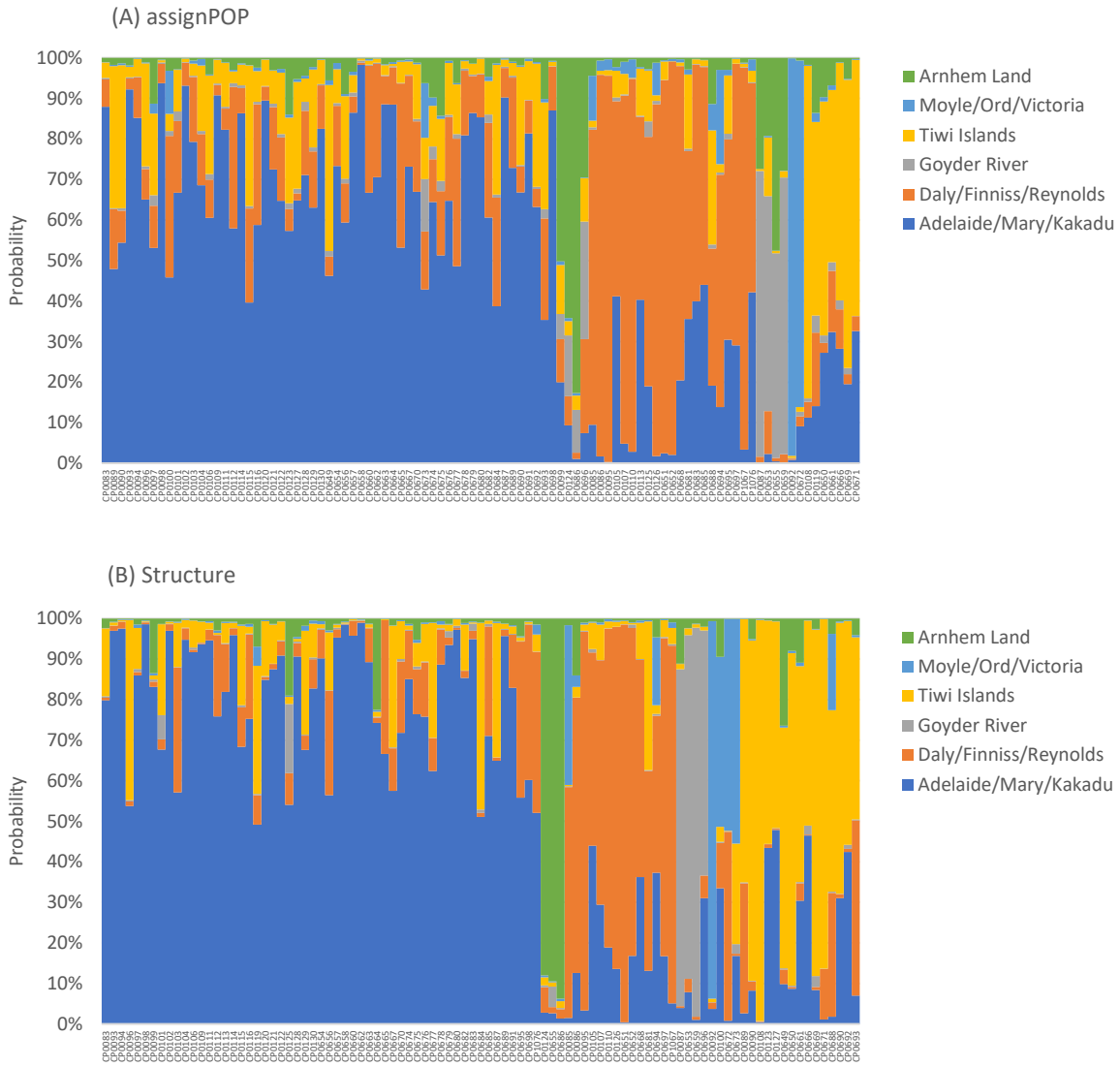

Figure 4.5. Probability of individual saltwater crocodiles sampled in the Darwin Harbour (N = 95) being assigned by A) assignPOP and B) Structure to the 6 clusters identified by Discriminant Analysis of Principal Components (Figure 4.3) in the Northern Territory and Ord River between 1997 and 2022.

The proportion of the sex of the Darwin Harbour crocodiles varied significantly (75.5% male, $G = 16.49$, $p = 4.88\times10^{-5}$) and the population assignments indicated that the distance most commonly travelled to Darwin Harbour was 100-200 km for both females and males ($G = 97.47$, $p = 2.2\times10^{-16}$) (Figure 4.6A). The TL of these crocodiles ranged from 119 to 400 cm for males and from 118 to 276 cm for females, with the most common size class between 150-180 cm for both sexes ($G = 9.96$, $p = 0.002$) (Figure 4.6B).

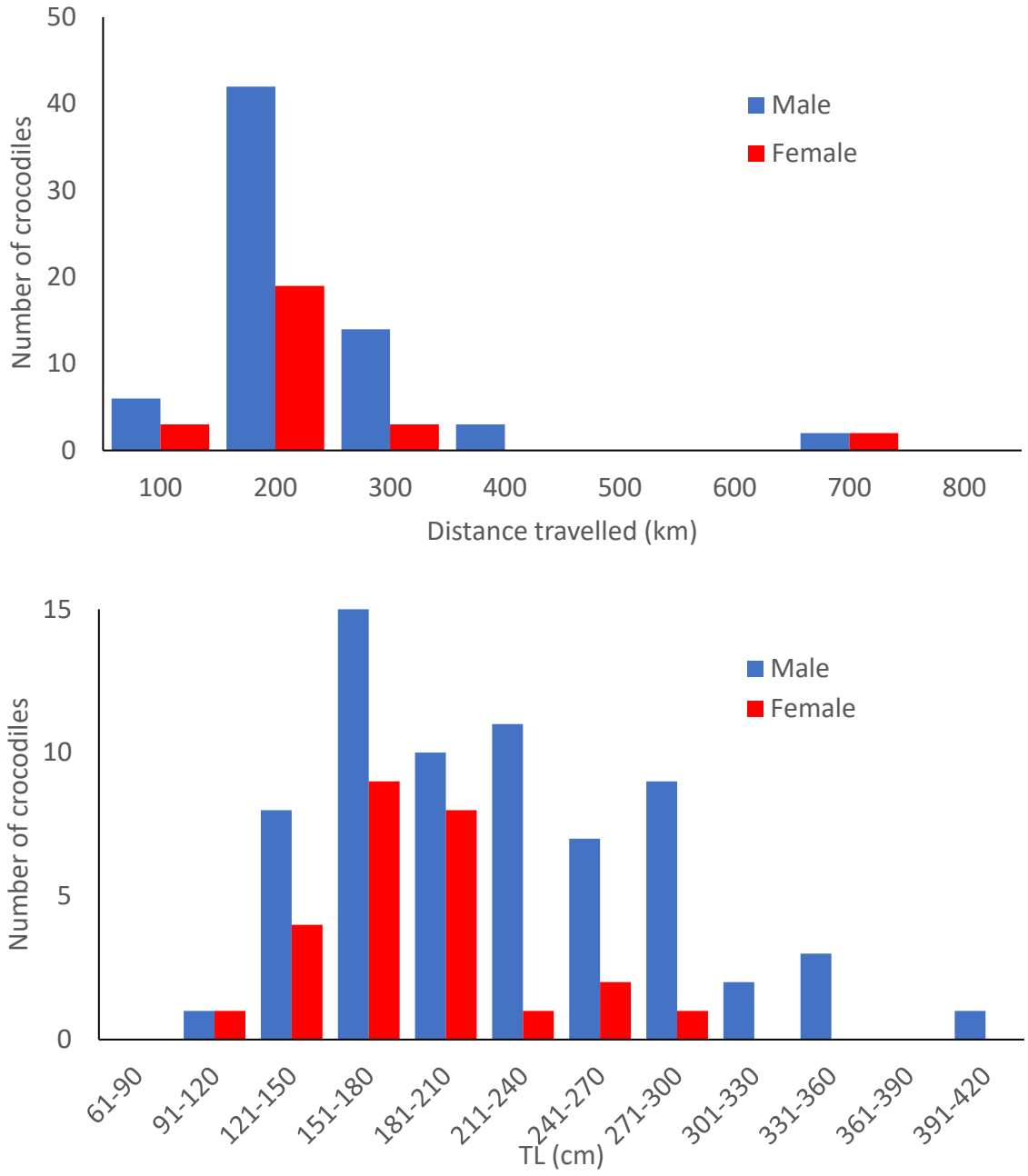

Figure 4.6. Frequencies of A) distances travelled by saltwater crocodiles caught as problem crocodiles in the Darwin Harbour (N = 95) in the Northern Territory, Australia, 2015–2017, and B) their measured Total Length (TL).

The population assignment methods, both the linear and quadratic models, indicated that there were no significant relationships between the response variable (distance from the inferred origin to the Darwin Harbour) and explanatory variables of TL, sex, or year ($p > 0.1$ in all cases). Similarly, we did not find a significant relationship between these variables when females and males were analysed separately ($p > 0.1$ for both female and male).

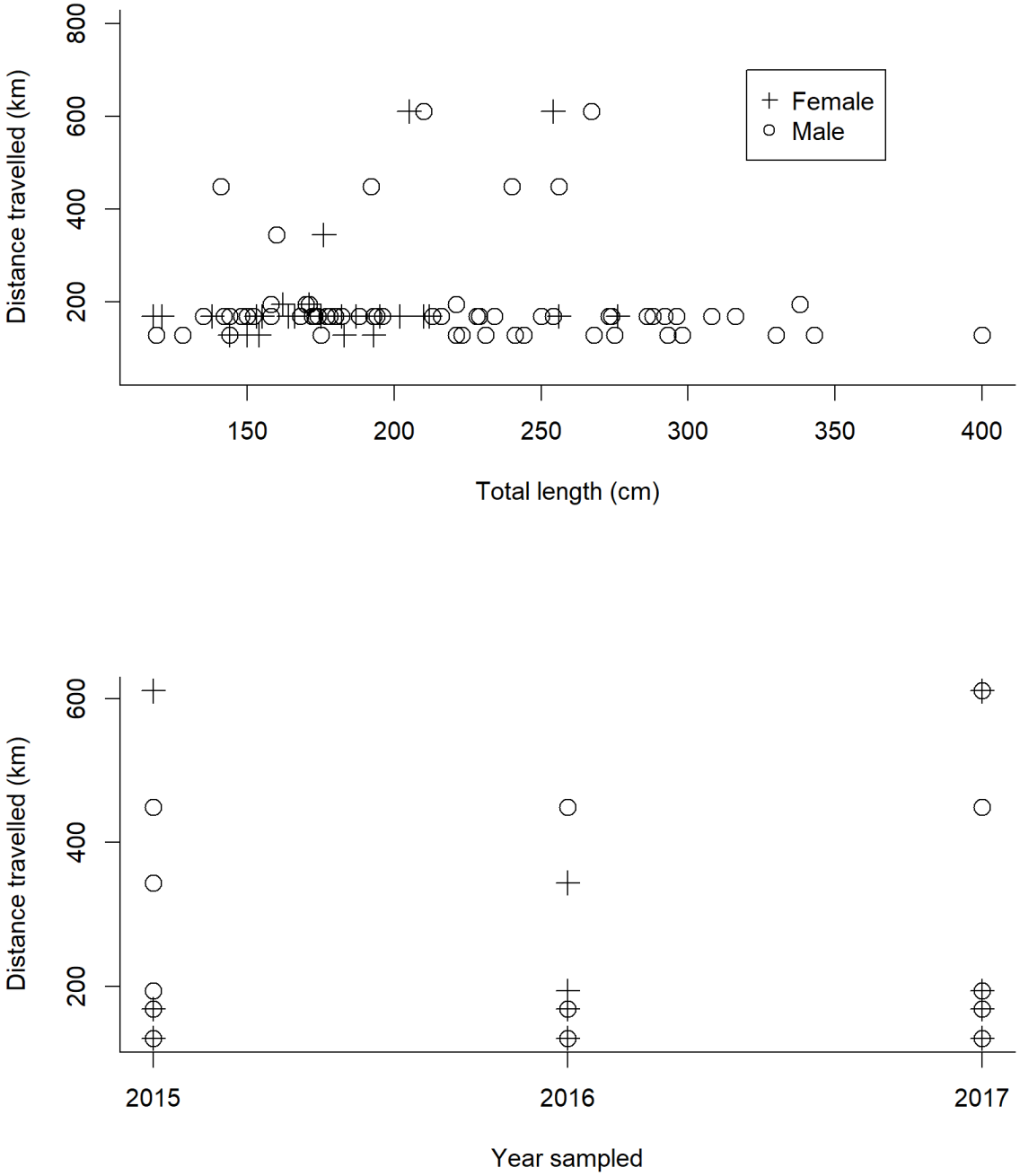

Figure 4.7. Relationships between A) the distance and Total Length (TL) of saltwater crocodiles caught as problem crocodiles (N = 95) in the Darwin Harbour, Northern Territory, Australia, 2015–2017 and B) the distance and year when the crocodiles were caught.

4.5. Discussion

Our results indicate that the region encompassing the Adelaide River, Mary River and Kakadu, to the east of Darwin, is the source of about 50% of the animals found in Darwin Harbour, with

the remainder originating from a mixture of the different sources across the NT. The combined Adelaide River, Mary River and Kakadu National park region has a very high density of the species and individuals in Kakadu National Park are not subject to any commercial egg collection, harvest or culling (Fukuda et al. 2011, Saalfeld et al. 2016). The second largest source region (about 20-25%) as estimated in both models for Darwin Harbour is the Daly, Finniss and Reynolds Rivers. These have extensive freshwater floodplains that are highly productive breeding habitats (Fukuda and Cuff 2013) subject to intensive but regulated egg harvesting for the ranching program (Saalfeld et al. 2015, 2016). The Tiwi Islands were the third largest source region for Darwin Harbour (about 15-20%), which is also subject to large egg harvesting (Saalfeld et al. 2015). The other regions were located further away (>200 km) from Darwin Harbour and inferred as possible sources of crocodiles in much lower numbers.

These results are consistent with a previous finding that environmental resistance, which is a combination of geographic distance and landcover types, impacts the species' dispersal patterns (Fukuda et al. 2022b, Chapter 3). For example, the Cobourg Peninsula functions as a geographic barrier to east-west and west-east movement (Fukuda et al. 2019), and the relatively small proportion of the Darwin Harbour crocodiles assigned to the sources beyond the Peninsula (<10% for Arnhem Land and Goyder River combined) reflects this. The Goyder River is associated with the Arafura Swamp, one of the largest breeding sites in the Northern Territory (Fukuda and Cuff 2013, Saalfeld et al. 2016), but contributes little to Darwin Harbour. One of the crocodiles assigned to the Goyder River by all 4 methods was confirmed to be an escapee from a commercial farm near Darwin. This crocodile was harvested as an egg from the Arafura Swamp and brought to Darwin, hatched in an incubator, and escaped from the facility to the Darwin Harbour, but was able to be identified when caught as a problem crocodile. It is possible that the others from the Goyder had a similar history.

Based on the population assignment results, the distance most commonly traveled by the crocodiles in the Darwin Harbour was 100-200 km for both females and males of all sizes. This is consistent with the previous study, where the most commonly traveled distance by crocodiles outside the Harbour was 150-200 km in the NT (Fukuda et al. 2022b, Chapter 3). Both studies identified a few individuals that traveled long distances (>400 km), and in our study, the long-distance travelers were medium-sized crocodiles (200-250 cm TL). This size range includes males, not sexually mature, and females at or near maturity. They may be dispersing from core habitats in search of new resources or territories (Campbell et al. 2013, Baker et al. 2019, 2022, Fukuda et al. 2022b). This contrasts situations in QLD where densities are much lower than the

NT (Read et al. 2004, Brien et al. 2017, Taplin et al. 2020) and crocodiles display greater site fidelity with their dispersal generally limited to <50 km from the site of origin (Lloyd-Jones et al. 2023). These differences in the carrying capacity (and gene flow) are largely influenced by the availability of breeding sites in freshwater floodplains that are much more limited in QLD (Fukuda et al. 2007, Taplin et al. 2020).

Two crocodiles captured in Darwin Harbour (268 and 293 cm long) had individual barnacles (*Chelonibia testudinaria*) on the tail and dorsal osteoderms. The population assignments suggested that both originated from the Daly, Finniss and Reynolds Rivers that are approximately 200 km west to Darwin Harbour. These marine barnacles are commonly found on sea turtles, but cannot survive in low salinity water (505 cyprid survival at 10 psu; Yasuyuki et al. 2011). Given the size of the largest barnacles (approximately 25 mm in diameter) on the larger crocodile and their growth rates (Sloan et al. 2014, Ewers-Saucedo et al. 2015, Doell et al. 2017), this crocodile is likely to have spent at least 90 days in seawater before it was caught in Darwin Harbour.

With regard to public safety, crocodiles over 3 m long arriving in Darwin Harbour represent an immediate threat to swimmers (Fukuda et al. 2014, 2015), and their interception is a management priority. However, smaller crocodiles, if not removed, may become established in the various tributaries feeding into Darwin Harbour, some of which are popular swimming sites (e.g. Berry Springs and Howard Springs). It is worth noting that all the larger crocodiles (>3 m) sampled in Darwin Harbour appeared to originate from the nearby sites (100-200 km), not from long distances away (>400 km).

We were not able to assign the Darwin Harbour samples to the individual catchments with enough confidence (61.8% of the samples had the probability <80%), thus we combined catchments into genetically distinct clusters. There is a clear trade-off between the geographic precision and the confidence of the genetic assignment. It should be noted that this study did not collect genetic samples from all the major river systems in the NT. The Wildman and West Alligator Rivers in Kakadu are major gaps, as are many of the creeks, rivers and swamps in the Gulf region of the NT. Given more samples, more detailed population assignment with greater confidence may be possible in the future. Full genome sequencing that can generate many more SNPs data may also help (Pérez-Reche et al. 2020). Tracking tools in telemetry such as acoustic, radio, and satellite transmitters may complement the genetic methods (Balaguera-

Reina et al. 2016, Combrink et al. 2017, Fukuda et al. 2019, Baker et al. 2022, Mascarenhas-Junior et al. 2023).

Management implications

Current government programs manage approximately 32 traps across Darwin Harbour to maintain crocodile density at minimal levels for public safety (Fukuda et al. 2014, Saalfeld et al. 2016, Department of Environment, Parks and Water Security (DEPWS) 2021). Our analyses suggested that crocodiles found in Darwin Harbour are migrants from multiple sources. Emigration from a known source may be able to be reduced by strategic harvesting (pre-migration), but individuals from other sources would still arrive. Thus, targeting a source population may not be effective in reducing management effort required to reduce the risk of crocodile attacks in the Harbour. Management should focus on continuing to remove crocodiles within the Harbour (post-migration). In addition, given that approximately 50% crocodiles caught in Darwin Harbour come from the rivers to the immediate east, intercepting them closer to the eastern mouth of the Harbour may be a strategy worth testing.

4.6. Ethics and acknowledgement statement

This study was conducted in accordance with the Code of Practice on the Humane Treatment of Wild and Farmed Australian Crocodiles (Environment 2013). The protocol was approved by the Animal Ethics Committee of the Australian National University (Animal Ethics Protocol Numbers A2017/11 and A2020/09), Parks and Wildlife Commission of the Northern Territory (Research Permit Number 57902 and 69126), Department of Parks and Wildlife of the Western Australian Government (Permit Numbers 08-002527-1 and 08-002527-2), and Parks Australia of the Australian Government (Permit Number AU-COM2017-361).

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4.7. Data Accessibility

The data and analysis codes that support the findings of this study are available from the corresponding author upon reasonable request.

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CHAPTER 5: Spatial population structure of saltwater crocodiles in Australia and Southeast Asia

5.1 Abstract

Understanding spatial population structure is important to the management of a species with high mobility and international distributions, such as the saltwater crocodile, *Crocodylus porosus*. This species is of substantial management interest for conservation, commercial exploitation, and human safety, and understanding patterns of their population connectivity within and among countries provides valuable information for such management programs. To quantify their genetic structure and identify geographic features that contribute to it, we sampled *C. porosus* across northern Australia and Southeast Asia and analysed the single nucleotide polymorphism data. There was a consistent pattern across analyses that the highest level of population structure involved a split into two groups comprising Oceania (Australia and New Guinea) and Southeast Asia (Borneo, Java, Peninsular Malaysia, Mindanao, and Sumatra), broadly aligned with Sunda and Sahul shelves of the Last Glacial Maximum as in other taxa previously observed. Resistance surface and effective migration surface modellings suggested that the genetic structure rreflected barriers such as deep straits and high mountains that effectively isolated the populations. High genetic connectivity was found between: 1) the NT and WA in Australia, 2) QLD (Australia) and New Guinea south, 3) Kalimantan (Indonesia) and Sarawak (Malaysia), and 4) the Philippines and Sulawesi. We found no genetic evidence of gene flow between Australia and Timor-Leste, which has been posited as a possible explanation for apparently increasing crocodile attacks in Timor-Leste. The occurrence of widespread connectivity between nations indicates that intergovernmental cooperation, such as strategic partnership agreements between different jurisdictions, may be beneficial for more effective management of crocodiles.

5.2 Introduction

The population structure of a species affects its adaptability and resilience to environmental variables such as climate change, habitat alteration, and human disturbance (Hughes and Stachowicz 2004; Hughes *et al.* 2008; Coates *et al.* 2015; Coates *et al.* 2018). Accounting for spatial population structure in management is important for a species with high mobility and extensive distributions (Sergeev *et al.* 2000; Cadrin and Secor 2009; Ying *et al.* 2011), and the saltwater crocodile, *Crocodylus porosus*, is one such species. They are adapted to freshwater,

brackish and saline water (Grigg *et al.* 1980; Taplin and Grigg 1981; Grigg *et al.* 1986) and widely distributed across the Indo-Pacific region (Webb *et al.* 2010; Webb *et al.* 2021). While the status varies significantly between populations [e.g. >120,000 non-hatchlings estimated in Australia and a few hundred in the Philippines (Webb *et al.* 2021; UNEP 2023)], the species is of substantial management interest for conservation (Saalfeld *et al.* 2016), commercial exploitation [e.g. ranching and tourism (Webb and Manolis 1993; Cohen 2019; Fukuda *et al.* 2021)], cultural use [e.g. traditional ceremonies (Brackhane *et al.* 2019; Ligtermoet *et al.* 2023)], and human safety (Fukuda *et al.* 2014; Amarasinghe *et al.* 2015; Brien *et al.* 2017).

Measuring the genetic connectivity between populations provides useful information for inferring migration rates (Hedgecock *et al.* 2007; Lowe and Allendorf 2010). Earlier studies in Australia showed that the immigration and emigration rate could vary drastically between populations, depending on habitat quality, especially the availability of habitat suitable for nesting (Fukuda *et al.* 2022; Lloyd-Jones *et al.* 2023), and these require different approaches to management. For example, populations with high recruitment (thus, higher emigration to other rivers), such as the Arafura Swamp and Tiwi Islands in the NT (Fukuda *et al.* 2022), can be a subject of egg harvest for farming under increased pressure, while those with limited connectivity, as in the QLD populations (Lloyd-Jones *et al.* 2023), may take more conservative consideration for interventions (e.g. removal of crocodiles).

Understanding possible migration rates is of a particular interest in Timor-Leste, where crocodile attacks have been increasing (Sideleau et al. 2017; Brackhane et al. 2018), and local residents widely believe that crocodiles, especially dangerous, large individuals, come from northern Australia (Abbott 2019; Brackhane *et al.* 2019). Given the species' high mobility, with odd individuals observed hundreds of kilometers away from a known population (Brackhane *et al.* 2018; Spennemann 2021), and their distinctive dispersal patterns shown in the NT (Fukuda *et al.* 2019; Fukuda *et al.* 2022; Fukuda *et al.* 2024, Chapters 2-4), some migration between these populations may be feasible. However, in this Indo-West Pacific region, ocean currents have been shown to limit the population connectivity of marine corals as geographic barriers by preventing their larval dispersal (Kool *et al.* 2011; Treml *et al.* 2015). If these populations are found genetically connected through recent migration, high-level consideration around the need for international cooperation may be a beneficial option for effective HCC management.

In this study, we aim to identify the spatial population structure of *C. porosus* on a international scale by measuring the genetic connectivity between populations in Australia and the surrounding countries and identifying the effective migration areas and geographic features that contribute to it. We also develop a population history tree that provides a historical context for the connectivity between the populations by identifying their likely ancestral clusters and divergence patterns.

5.3 Materials and methods

Study species

This study focused on *C. porosus* in Australia and the neighbouring countries. *C. porosus* is the largest and most aggressive extant crocodilian (Britton *et al.* 2012; Brien *et al.* 2013a; Brien *et al.* 2013b). While negative interactions with people and domestic animals are a serious issue in many countries (Fukuda *et al.* 2014; Brien *et al.* 2017; Sideleau *et al.* 2017; Brackhane *et al.* 2018), they are exploited for commercial uses such as skin production and tourism (Webb and Manolis 1993; Ryan 1998; Cohen 2019; Caldwell 2021; Rosenblatt *et al.* 2021). *C. porosus* is distributed in the Indo-Pacific region, ranging from India in the west across southeast Asia and Oceania to New Caledonia in the east, from the Philippines in the north to Australia in the south. This study focuses on the central range of the species, with Papua New Guinea and Singapore at the eastern and western boundaries, respectively (Figure 5.1).

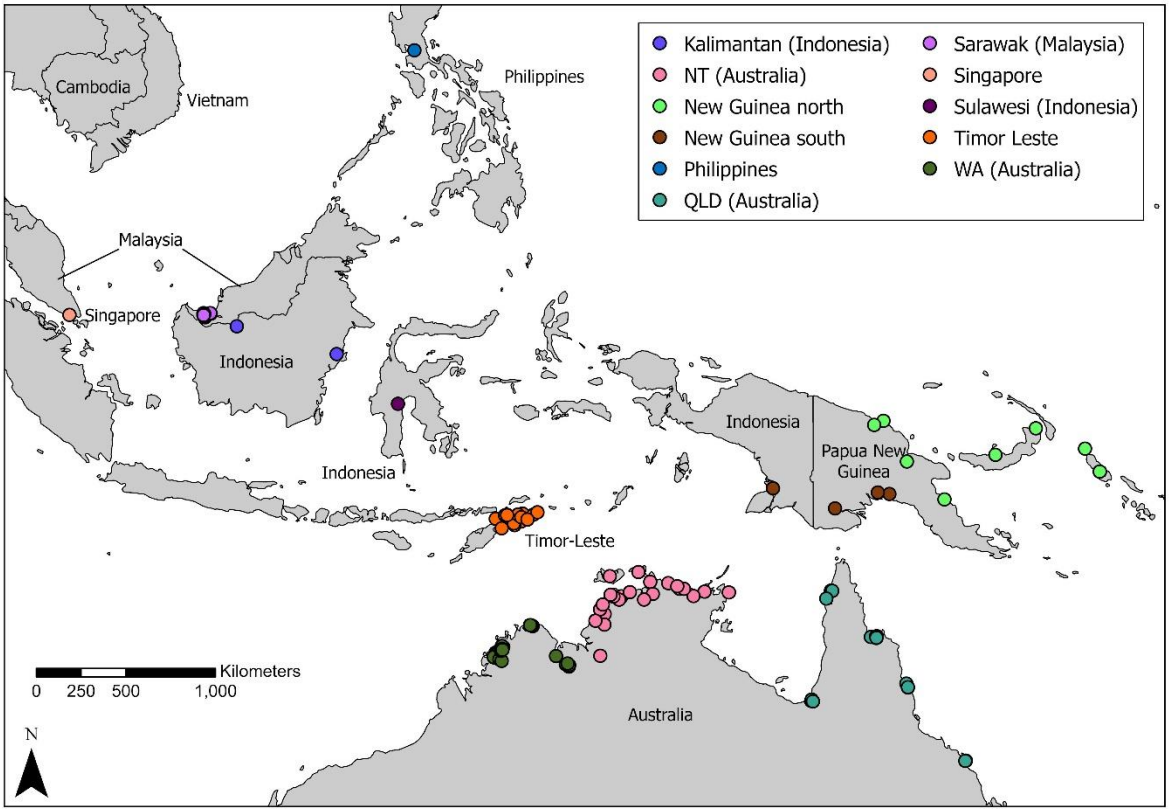

Figure 5.1. Filtered genetic samples of *C. porosus* collected from Australia and the neighbouring countries.

Data collection and filtering

We collected tissue samples of *C. porosus* from free-ranging animals using non-invasive biopsy (Barrow and Halford 2019), embryos that failed to hatch for ranching, animals processed for commercial use (Saalfeld *et al.* 2015) or removed for safety, or historical collection at museums (e.g. Queensland Museum) in Australia [Northern Territory (NT), Queensland (QLD), and Western Australia (WA)], Indonesia (Kalimantan, Sulawesi, and West Papua), Malaysia (Sarawak), Papua New Guinea, Philippines, Timor-Leste, and Singapore (Figure 5.1). We also collected samples from Cambodia, New Caledonia, the Solomon Islands, and Vietnam, but these were not included in the analysis because of their small sample sizes (N<10). To reduce potential bias from the significant variation in the sample size, we also limited the maximum size to 40 samples (see Supporting Information for the analyses with all the samples included).

DNA was extracted and genotyped at Diversity Arrays Technology (Canberra, Australia) using the DArTseq approach (Kilian *et al.* 2012). This method uses genome complexity reduction and next-generation sequencing conceptually similar to RADseq, in this case, based around a

SphI enzyme digestion of genomic DNA and Illumina HiSeq 2500 sequencing. We ran 30% of samples twice as technical replicates to assess the repeatability of single nucleotide polymorphism (SNP) calls. The reference genome assemblies were CroPor_comp1 (Mississippi State University 2016) and Cpor_3.0 (ICGWG 2020), and 21,183 SNPs were mapped to either of them.

We removed batch effects in the data by fitting a binomial GLM for each SNP with call rate (SNP genotype present or absent) as the response variable and batch ID from DArT as the grouping factor. We then plotted the histogram of pseudo-R-squared values, and based on visual inspection, a threshold of 0.5 identified a set of 2023 SNPs with strong associations between the batch ID and call rate. We retained 20,721 SNPs.

We plotted basic SNP metrics on sequence depth and other quality metrics as an initial data exploration step. We explored relationships between these metrics and basic population genetics statistics, including observed and expected heterozygosity. We used the graphical analysis to evaluate whether genotype properties (e.g. observed heterozygosity) were associated with SNP quality metrics such as mean sequencing depth. We filtered the SNPs using the dartR package in R 4.3.2 (Gruber *et al.* 2017; Mijangos *et al.* 2022), according to call rate (>0.75), repeatability (>0.95), mean total depth (7–200), and mean ratio of sequence depth (0.5–2). We set the allele depth ratio criterion to remove loci in which one allele was preferentially detected, indicating potential susceptibility to null alleles through polymerase chain reaction bias. We also dropped 1) SNPs (ranked by polymorphic information content) within sequences with >1 SNP present, 2) SNPs that did not align to the reference genomes (Mississippi State University 2016; ICGWG 2020) with an e-value threshold of -10 or lower, and 3) SNPs that mapped to >1 location on the reference genome. We dropped individuals for which >10% of SNPs were not genotyped. We also removed SNPs with particularly high factor loading (>0.05) for the first axis of a principal coordinates analysis (PCOA) conducted below. This was conducted following discussion with Diversity Arrays Technology staff because we noticed apparent effects of pre-2023 and post-2023 genotyping batches on genetic structure as indicated by PCOA.

We graphed the distribution of the genetic diversity statistics to visualise any unexpected patterns with regard to Hardy-Weinberg expectations within populations but did not filter any further based on this graphical analysis (see Supporting Information). We calculated the observed heterozygosity (H_O), expected Heterozygosity (H_E), and inbreeding coefficient (F_{IS})

for each population using GenAlEx (Peakall and Smouse 2006; Peakall and Smouse 2012). We also estimated the overall and pairwise genetic differentiation among populations (F_{ST}) using the dartR package.

Spatial and temporal analyses

Using software called GenAlEx 6.503 (Peakall and Smouse 2006; Peakall and Smouse 2012; Peakall and Smouse 2017), we estimated Euclidean genetic distances among the populations and performed a PCOA on the genetic distances to visualize patterns of the genetic similarity among the populations.

To visualize the genetic connectivity between the populations shown by the PCOA spatially, we generated a migration surface on a geographic map, using Fast Estimation of Effective Migration Surfaces (FEEMS) developed by Marcus *et al.* (2021). FEEMS is a related method of Estimating Effective Migration Surfaces (EEMS) initially developed by Petkova *et al.* (2016) for depicting spatial population structure. FEEMS uses a Gaussian Markov random field model in a penalized likelihood framework that allows for efficient optimization and output of effective migration surfaces, while EEMS is based on Bayesian inferences (Marcus *et al.* 2021). We converted the filtered genetic data in the genlight format to the PLINK format for FEEMS, using the dartR package (Gruber *et al.* 2017). FEEMS also required the geographic coordinates for each sample to calculate geographic distances to estimate the migration surface. When imputing missing SNPs in FEEMS, we used the default Mean strategy in the imputer. To avoid inferring gene flows inaccurately, where we could not collect genetic samples, we restricted the surface calculation to the sampled areas only by setting outer boundaries. In fitting the FEEMS model, we set 0.05 for the smoothness parameter, lambda (Marcus *et al.* 2021).

To find out what factors contributes to the migration and barrier areas identified by FEEMS, we plotted the bathymetric and elevation data (Natural Earth 2009; Danielson and Gesch 2011) and generated a resistance surface based on the SNP and altitude data using the resGF package (Vanhove and Launey 2023). Resistance Gradient Forest (resGF) estimates genetic resistance by first summarising allelic frequencies across the study area with principal component analysis and assessing the importance of environmental variables on the distribution of genetic diversity using the gradient forest approach (Ellis *et al.* 2012; Vanhove and Launey 2023). Based on their estimated importance, the environmental variable is transformed into a resistance surface that effectively represents the genetic resistance, highlighting areas where gene flow is likely impeded due to environmental factors (Vanhove and Launey 2023). We first

converted the genlight object to genind for resGF, using the dartR package (Gruber *et al.* 2017). We reclassed the raw values in the bathymetry and elevation raster into nine categories with 25 km cells as a univariate predictor. Based on how likely a crocodile is found at various altitudes (ranging from -9756 to 3991 m), we assigned arbitrary values of 1-9 in the order of -10 to 10 m, 10 to 50 m, 50 to 100 m, 100 to 500 m, over 500 m, -10 to -50 m, -50 to -100 m, -100 to -500 m, and over -500 m. We calculated Principal Coordinates of Neighbour Matrices (PCNM) using the vegan package (Dray *et al.* 2006) and retained the first half of the positive PCNMs for the gradient forest estimation as suggested previously (Manel *et al.* 2010; Vanhove and Launey 2023). We set 500 for the number of bootstrapped trees and 0.50 for the correlation. In addition, we compared the Mantel correlation of the genetic distance matrix [Euclidean genetic distance (Gruber *et al.* 2017)] between sampled populations with the resistance distances estimated from (1) the optimised resistance surface and (2) a constant resistance surface with a uniform value.. We used the commute distance method in gdistance (Etten 2017) to estimate resistance distances and the vegan (Oksanen *et al.* 2022) package for Mantel tests. We also conducted the Mantel test of pairwise F_{ST} and the geographic distance between the populations, with 999 permutations for the significance test, using dartR.

We used a spatial ancestry estimation program called TESS3 and its package, tess3r (Caye *et al.* 2016), to explore how the collected samples can be grouped into different numbers of genetic clusters and see their spatial distribution. Together with the migration and resistance surfaces, this provided a better picture of the species' population structure in terms of their spatial distribution and connectivity. Based on the genotypes and geographic coordinates of the samples, we estimated ancestry coefficients for each individual and determined the number of ancestral populations. The TESS3 function computes an Fst statistic for each locus, and returns test significant values for a null hypothesis of selective neutrality (Caye *et al.* 2016). For the calculation of the coefficients, we set 1-20 for K, which is the number of populations to explore, projected.ls (alternating projected least squares algorithm) for method, 2 (diploids) for ploidy, and 4 for openMP.core.num that is the number of central processing units to use. We determined the number of the genetic populations by inspecting the cross-validation plot that plotted the root mean-squared errors computed on a subset of the loci. We also plotted the proportions of the ancestral populations estimated by the function for each of the samples and mapped out the coordinates of the samples with their interpolated ancestry coefficients.

To further examine the temporal or historical relationships between the populations, we used TreeMix, which infers from the genetic data the population history in terms of migration events,

gene flow, and admixture (Pickrell and Pritchard 2012). Based on the allele frequency covariance, the model constructs a maximum likelihood tree representing the historical splits and mixture of the populations. We ran the model in blocks of 500 SNPs (k = 500) with different migration numbers (m = 0-10) with five iterations in each run. We selected the optimal number of migrations (m) using a package called OptM (Fitak 2021). The package provides a quantitative and reproducible measure of the optimal number of migration edges that best explain the tree graph by estimating the second-order rate of change in the likelihood weighted by the standard deviation (Δm).

5.4 Results

After a series of data filtering, we retained 3457 SNPs in 321 individuals with a minor allele frequency of >0.02, corresponding to ≥20 allele copies for analysis. The filtered dataset showed a high overall genetic differentiation (F_{ST} = 0.226, SE = 0.002) and low inbreeding coefficient (F_{IS} = 0.094, SE = 0.002). The genetic diversity and differentiation varied between the sampling sites (Tables 5.1 and 5.2). New Guinea north showed a particularly high inbreeding coefficient because this population combined the samples from the other islands. The PCOA showed the genetic relationships of the sampled populations (Figure 5.2) that roughly resemble their spatial distribution (Figure 5.1).

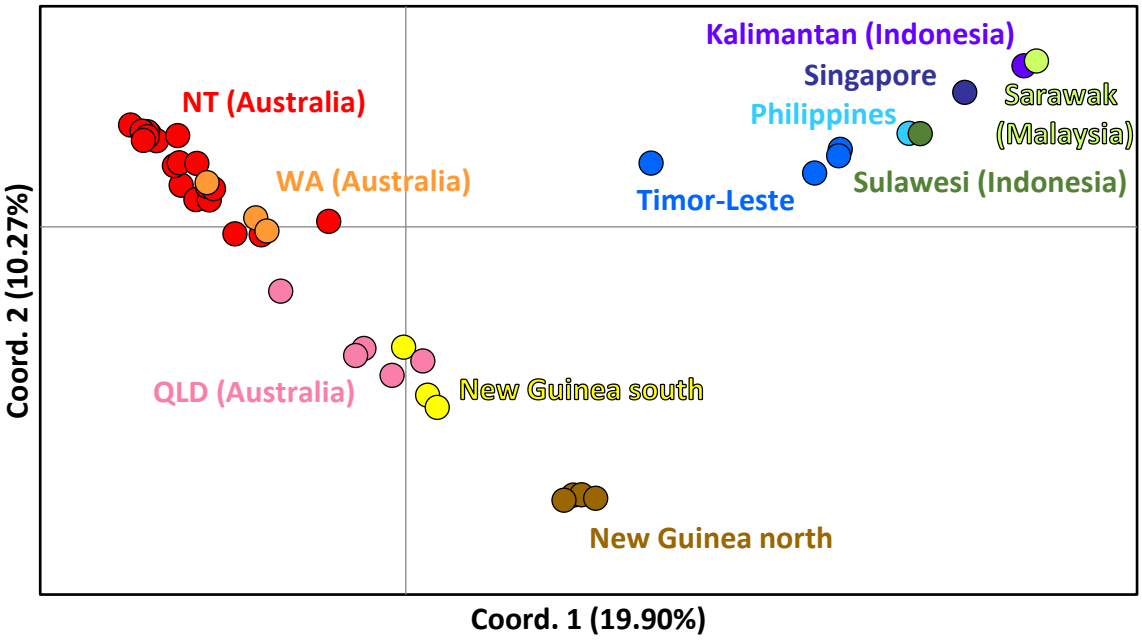

Figure 5.2. PCOA of the populations based on Euclidean genetic distance estimated at the population level.

Table 5.1. Genetic diversity statistics of each population, including sample size (n), mean observed heterozygosity (H_O), mean expected Heterozygosity (H_E), and inbreeding coefficient (F_{IS}). We note that many F_{IS} values are positive and that this is expected because the sampling regions typically included a number of sites.

Population	**N**	**H_E**	**H_O**	**F_{IS}**
Kalimantan (Indonesia)	14	0.200	0.167	0.16
New Guinea north	40	0.174	0.116	0.34
New Guinea south	22	0.207	0.159	0.23
NT (Australia)	40	0.208	0.189	0.09
Philippines	29	0.233	0.231	0.01
QLD (Australia)	40	0.213	0.195	0.08
Sarawak (Malaysia)	40	0.173	0.162	0.06
Singapore	17	0.234	0.231	0.01
Sulawesi (Indonesia)	11	0.204	0.202	0.01
Timor Leste	28	0.238	0.223	0.06
WA (Australia)	40	0.192	0.178	0.07

Table 5.2. Pairwise genetic differentiation (F_{ST}) between the populations (P <0.01 for all). The sample size is provided in Table 5.1.

Population	**QLD (Australia)**	**NT (Australia)**	**WA (Australia)**	**Sarawak (Malaysia)**	**Singapore**	**Timor Leste**	**Sulawesi (Indonesia)**	**Kalimantan (Indonesia)**	**New Guinea north**	**New Guinea south**
NT (Australia)	0.11									
WA (Australia)	0.14	0.08								
Sarawak (Malaysia)	0.44	0.44	0.47							
Singapore	0.30	0.30	0.34	0.15						
Timor Leste	0.23	0.23	0.27	0.27	0.14					
Sulawesi (Indonesia)	0.32	0.32	0.36	0.26	0.13	0.16				
Kalimantan (Indonesia)	0.37	0.37	0.41	0.17	0.07	0.18	0.16			
New Guinea north	0.21	0.25	0.28	0.48	0.35	0.28	0.36	0.42		
New Guinea south	0.07	0.13	0.16	0.45	0.29	0.22	0.31	0.37	0.17	
Philippines	0.28	0.29	0.32	0.21	0.10	0.15	0.15	0.13	0.33	0.27

The estimated migration surface with a few barriers to the inferred gene flow (Figure 5.3A) was consistent with the genetic distance between the populations indicated by the PCOA (Figure 5.2). The barriers implied by the migration surface coincided with areas with high elevation on land and shallow water in the sea (Figure 5.3B), as highlighted in the resistance surface estimated by resGF (Figure 5.3C). The Mantel scores for the correlation with the resistance distance from the optimised and constant resistance surfaces were 0.48 and 0.57 ($P < 0.01$ for both), respectively. The Mantel test also suggested a significant correlation between the pairwise F_{ST} and geographic distance between populations ($R^2 = 0.59$, $P < 0.01$).

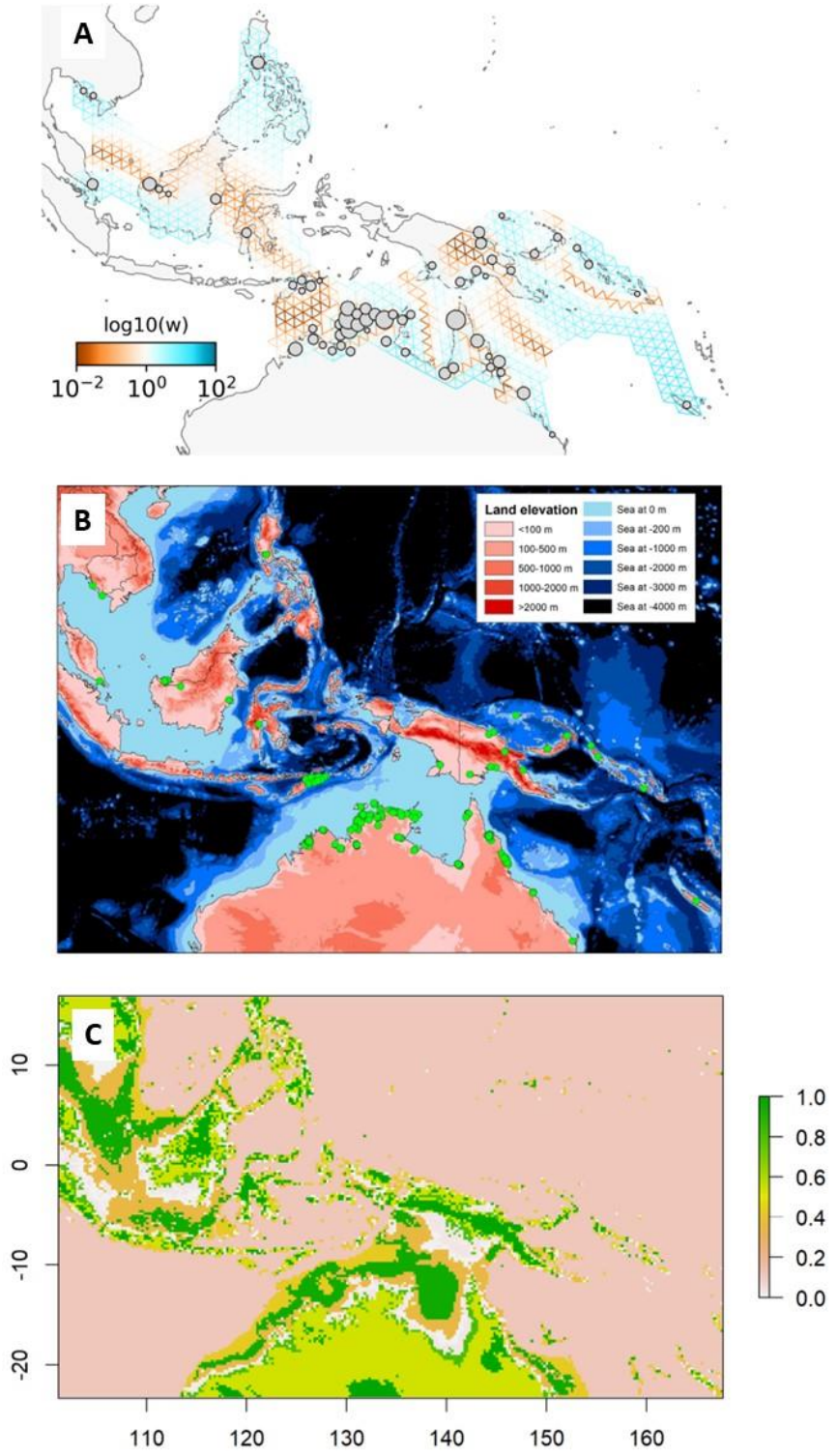

Figure 5.3. A) Migration surface estimated by FEEMS, indicating effective migration areas (blue) and barriers to migration (brown) with the location of the genetic samples whose symbol size is relative to the sample size, B) land elevation and bathymetry, and C) resistance surface estimated by resGF.

The ancestral clustering with the smallest number of ancestral populations (K = 2) was consistent with the PCOA that the entire population may be separated into two broad groups of Asia and Oceania (Figure 5.4A). Similarly, with the increased number of groups (K = 5 and 10), the ancestral clustering showed relatively distinctive separation, consistent with the barriers estimated in the migration surface. The cross-validation score consistently decreased with increasing K (Figure 5.4B).

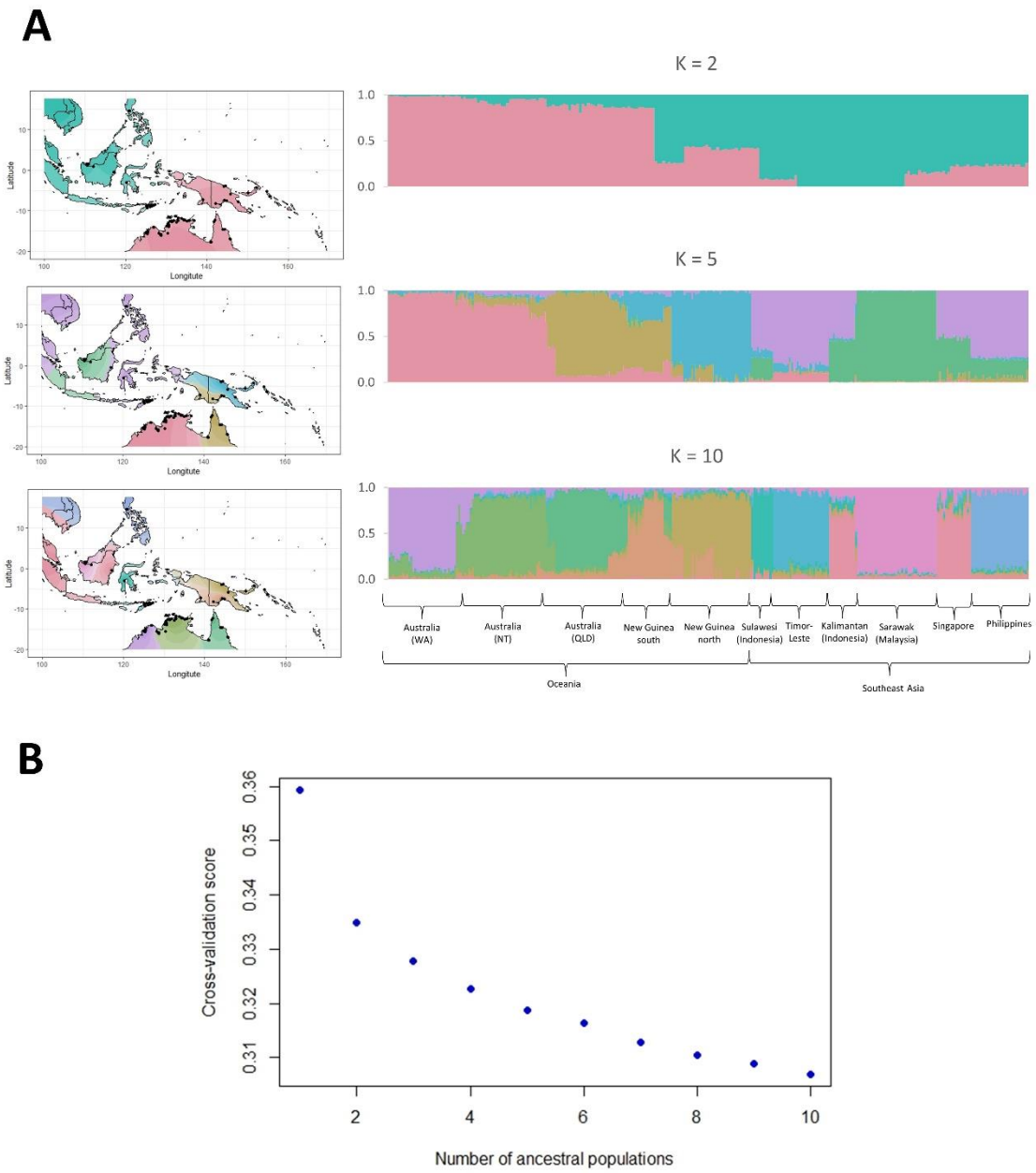

Figure 5.4. A) Interpolated ancestral clustering of *C. porosus* (K = 2, 5, and 10), inferred spatial distribution of the clusters, and the estimated proportions of the ancestral populations for each sample, and B) the cross-validation plot indicating root mean-squared errors computed on a subset of the loci used for cross-validation (Caye *et al*. 2016).

The estimation of the optimal number of migration events in the population trees generated by TreeMix showed a high peak in Δm at 1 (Figure 5.5A), suggesting that this was the most likely number of migration edges to be included in the model. Consequently, the migration event shown in the population history tree was from the common ancestor of the eastern group to the

Philippines (Figure 5.5B). The structure of the population tree resembled the ancestral clustering, especially with 10 populations, estimated by tess3R.

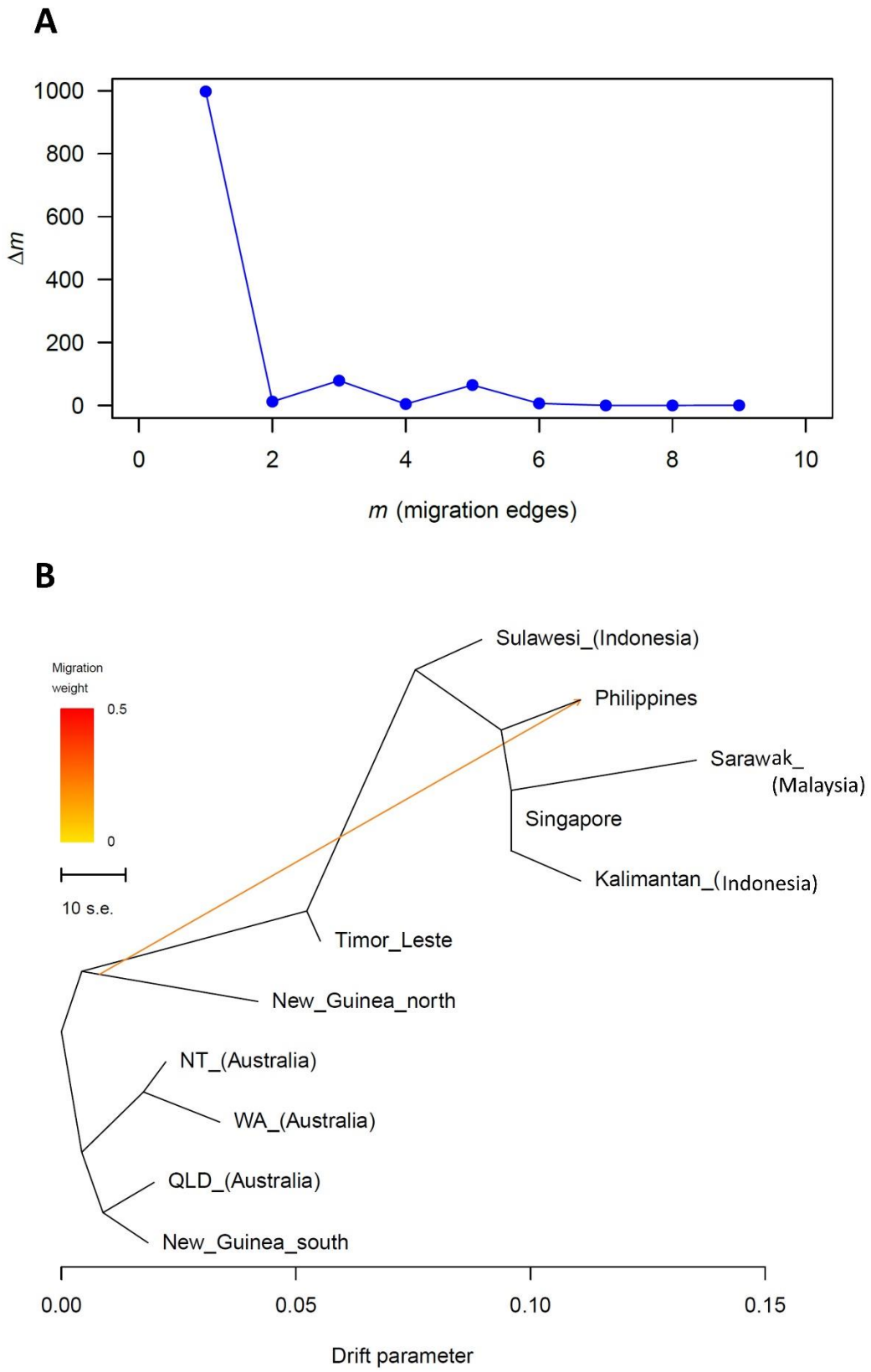

Figure 5.5. A) Second-order rate of change (Δm) across the different numbers of migration (m) estimated by OptM (Fitak 2021), and B) population history tree generated by TreeMix (Pickrell and Pritchard 2012) with one recent migration event suggested to the Philippines.

5.5 Discussion

Our results consistently showed that *C. porosus* populations have distinctive genetic structure and can be separated at the broadest level into two groups of Oceania (Australia and New Guinea) and Southeast Asia (Borneo, Java, Peninsular Malaysia, Mindanao, and Sumatra), roughly following the conventional biogeographic lines such as the Wallace, Webber, and Lydekker Lines (Mayr 1944; Simpson 1977; White *et al.* 2021; Ali and Heaney 2021; Ali and Heaney 2023). These broad groups likely reflect the Sunda and Sahul shelves during the Last Glacial Maximum 20,000-26,000 years ago when the sea level was considerably lower than today (Clark and Mix 2002; Carlson 2011; Du *et al.* 2023). The migration and resistance surfaces (Figure 5.3) highlighted the role of oceanographic and topographic features, such as deep-water straits and high mountain ranges, in driving the genetic structure by isolating the populations. This is consistent with the fact that the distribution of *C. porosus* is typically restricted to the coastal areas with shallow brackish and freshwater within a low altitude range (Webb and Manolis 1989; Fukuda *et al.* 2007; Fukuda and Cuff 2013). Fukuda *et al.* (2022) showed that the sea had a higher resistance to their dispersal than other habitat types, such as tidal rivers and freshwater floodplains. It is likely that these major geographic features (e.g. deep water and highlands) shaped the genetic structure of such ancient species, being around for more than 200,000,000 years (Grigg and Kirshner 2015) over many glacial-interglacial cycles. The spatial ancestry estimation (Figure 5.4) and the population history tree (Figure 5.5) indicated that the broad groups could be divided into finer substructures depending on their genetic lineages. It was not our expectation that the correlation of the genetic distance with the resistance distances estimated from the constant surface was higher than that with the optimised one. This may be because the mean resistance value that we estimated from the 25 km cells may have been too coarse to detect some variations rapidly changing in the short distance.

The apparent connectivity between some of the populations was no surprise, given their proximity to each other and the absence of major barriers. For example, within Australia, the NT populations showed closer relationships with the WA populations than those in QLD. Previous studies indicated that the genetic connectivity among populations in the NT was relatively high, with approximately 42% of individuals in each population being migrants from other rivers (Fukuda et al. 2022), while the gene flow between populations in QLD was extremely limited (Lloyd-Jones *et al.* 2023). However, some of the QLD populations were relatively close to those in New Guinea south as expected from a likely connection via the Torres Strait Islands as stepping stones between QLD and New Guinea. This pattern of genetic

connectivity was also reported in other fauna (Kool *et al.* 2011; Mirams *et al.* 2011; Wasef *et al.* 2021; Hernawan *et al.* 2021). While New Guinea north and south were separated by a geographic barrier of the New Guinea Highlands (>4000 m), the north populations were close to each other, and probably further east with the Solomon Islands and New Caledonia (see Supporting Information). In the Southeast Asia group, Kalimantan (Indonesia) and Sarawak (Malaysia) were genetically close to each other, suggesting that Borneo Island may be one group at a broad level. Likewise, the Philippines and Sulawesi were close to each other, although the ancestral population tree indicated an unexpected migration from near New Guinea north to the Philippines (Figure 5.5B). These Philippine samples were from captive crocodiles, and they potentially have recent ancestry in crocodiles brought from Papua New Guinea for ranching in the 1980s (pers.com. Manalo, R. Crocodylus Porosus Philippines, INC). Singapore also showed a high connectivity to all these Asian countries as expected from its close proximity to the other countries.

Contradicting the strong belief that crocodiles migrate from the NT to Timor-Leste (Brackhane *et al.* 2019), crocodiles from Timor-Leste were genetically similar to those in Southeast Asia, and not to crocodiles in Australia. The deep water in the Timor Sea and the complex monsoonal currents called the Indonesian Throughflow, connecting the tropical Pacific and Indian Oceans (Wijeratne *et al.* 2018; Peña-Molino *et al.* 2022; Wang *et al.* 2022), likely prevent the movement of crocodiles between the countries. Ocean currents between the Northern Territory and Timo-Leste have been suggested as potential barriers to the dispersal of multiple marine species (Treml *et al.* 2015). Nevertheless, given the limited sample size for Timor-Leste (N = 28) and some observations of a large individual wandering in the sea far from a coast (Brackhane *et al.* 2018), further examination with more genetic sampling across the country, especially from the coastal areas, should confirm the conclusion.

In this study, we could not access genetic samples in many populations, especially from Indonesia (e.g. Java, Maluku, Nusa Tenggara, and Sumatra), which forms a central part of the range. The western boundary of the range (e.g., India and Sri Lanka) also remained unstudied. The inclusion of these countries and those excluded from the analyses because of the small sample sizes (e.g. New Caledonia and Solomon Islands) would provide a more comprehensive understanding across the range. Pending further examination with more sampling, the patterns of the genetic structure shown here can be reflected in the high-level planning of how crocodiles should be managed within and between the countries. Intergovernmental cooperation, such as strategic partnership agreements, may be helpful for more effective

management to promote a variable balance between the objectives concerning conservation, sustainable commercial gain, and human safety. For example, the Northern Territory Government has been assisting the Timor-Leste Government in capacity building for removing problem crocodiles and conducting population monitoring (Northern Territory Government 2023, pers.com. Fukuda, Y. Northern Territory Government). Although crocodiles are unlikely to travel between these two countries as shown in this study, such technical cooperation and knowledge sharing should be maintained.

5.6. Ethics and acknowledgement statement

This study was conducted in accordance with the Code of Practice on the Humane Treatment of Wild and Farmed Australian Crocodiles (Environment 2013). The protocol was approved by the Animal Ethics Committee of the Australian National University (Animal Ethics Protocol Numbers A2017/11 and A2020/09), Parks and Wildlife Commission of the Northern Territory (Research Permit Number 57902 and 69126), Department of Parks and Wildlife of the Western Australian Government (Permit Numbers 08-002527-1 and 08-002527-2), and Parks Australia of the Australian Government (Permit Number AU-COM2017-361).

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5.7. Data Accessibility

The data and analysis codes that support the findings of this study are available from the corresponding author upon reasonable request.

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CHAPTER 6: Discussion overall

The thesis comprises four analytical chapters (Chapters 2-5), each focusing on different aspects of the movement by *C. porosus* in and around Australia. This chapter summarises the key findings from each chapter, offers recommendations for management and future studies, and presents the conclusion of the study.

6.1 Summary of key findings

Findings from Chapter 2: homing ability and geographic barriers

This chapter focused on the movement and dispersal patterns of translocated saltwater crocodiles in the NT of Australia, using satellite tracking and genetic analysis. The key findings include:

1. The Cobourg Peninsula is a significant geographic barrier that disrupts the movement and dispersal of crocodiles. Genetic analysis revealed that limited gene flow occurs across the peninsula.
2. Satellite tracking observed none of the crocodiles translocated over the Cobourg Peninsula crossed or swam around the peninsula despite their persistent attempts to return to the capture sites, indicating their navigational abilities.
3. In contrast, none of the non-translocated crocodiles travelled to the other side of the Cobourg Peninsula, and they all remained in their distinct activity centers within and between rivers.
4. These results have important implications for the management of human-crocodile conflicts. First, translocating problem crocodiles may not be an effective management strategy as they will likely return to their capture sites. Second, geographic barriers such as the Cobourg Peninsula may interrupt their homing, but their behaviour, when displaced and unable to return to their capture site because of a barrier, is unknown.

Findings from Chapter 3: Environmental resistance to dispersal

This chapter aimed to understand the factors influencing the dispersal patterns and population connectivity of saltwater crocodiles across the NT. The key findings from the study include:

1. Environmental resistance, which is related to the quality and permeability of the landscape, was a crucial factor in determining the dispersal of crocodiles. Crocodiles

were more likely to move through areas with lower environmental resistance and higher habitat quality.

2. The availability of breeding habitat influenced both the emigration and immigration processes. Crocodiles tended to leave areas with a high proportion of breeding habitat, likely due to competition from significant recruitment, and settle into areas with less breeding habitat, likely indicating a search for new territories with available resources. Productive populations had tthe highest proportion of emigration than immigration.
3. Approximately 42% of crocodiles in a given population were migrants from other populations. The distance most commonly travelled by these migrants was 150–200 km, although a few travelled much longer, up to 600–700 km. Given their extensive dispersal range, hydrographic regions that combine two or three adjacent catchments seem like an appropriate scale for population management.
4. Landscape features and habitat characteristics shape the dispersal patterns of crocodiles, which has implications for their conservation and management. Dispersal can be predicted by monitoring habitat quality and population dynamics. Understanding the dispersal patterns can inform management strategies, for example, by identifying likely sources of problem crocodiles.

Findings from Chapter 4: Natal origins of problem crocodiles

This chapter identified the natal origins and movement patterns of problem crocodiles in Darwin Harbour to inform management strategies that ensure public safety without adversely affecting populations in other parts of the NT. The key findings are:

1. Crocodiles caught in Darwin Harbor originated almost exclusively in the NT. The primary sources of the problem crocodiles were the Adelaide River, Mary River, and Kakadu National Park (30-50%), the Finniss River, Reynolds River, and Daly River (20-30%), and the Tiwi Islands (15-20%).
2. A few individuals originated from remote populations up to 700 km away. The presence of such long-distance travellers and marine barnacles found on some of them suggest that they spent prolonged time (up to 90 days) in the marine environment before they were captured in Darwin Harbour.
3. The most common crocodiles caught in the harbour were immature (10-180 cm) males. Most were estimated to have come from the sources 100-200 km away, while a few had to travel much further (<700 km) to reach the harbour. These distances from the inferred

origins were not correlated with the size and sex of the crocodiles or the year of capture because the migrants included both females and males in a wide range of sizes (118-400 cm).

4. The findings supported the current strategy of targeted removal of problem crocodiles from Darwin Harbor to reduce the risk to the public in the harbour while maintaining the integrity of the source populations that may be used commercially (e.g. egg collection for ranching).

Findings from Chapter 5: Population structure between countries

This chapter identified the spatial population structure of the species by measuring the genetic connectivity between the populations in Australia and the neighbouring countries throughout Oceania and Southeast Asia. The key findings are:

1. The populations are separated into two broad groups of Oceania (Australia and New Guinea) and Southeast Asia (Indonesia, Malaysia, Philippines, and Singapore), reflecting the Sahul and Sunda shelves of the LGM. These groups may be divided further (e.g. K = 10), depending on their ancestral lineages.
2. High genetic connectivity was observed between 1) the NT and WA, 2) QLD and New Guinea south, 3) Kalimantan (Indonesia) and Sarawak (Malaysia), and 4) the Philippines and Sulawesi.
3. In contrast, we did not find any support for the widely believed migration of crocodiles between Australia and Timor-Leste.
4. Effective migration areas between these populations, estimated from the genetic and geographic distances, mostly matched the variation in the bathymetry and elevation data. Deep straits, such as the Indonesian Throughflow between Australis and Timo-Leste, and mountain ranges, such as the New Guinea Highlands, appear to function as barriers to their movement.

6.2 Recommendations for management and future studies

More sampling is required

There is no doubt that the genetic approaches used in this study served as a valuable tool to better understand the movement of saltwater crocodiles at different scales, which would have been difficult otherwise to study. More extensive and intensive sampling to cover the entire species range and make the sample sizes consistent would enable more detailed and robust

analyses to identify their movement more precisely and confidently. The largest gaps at the international level are Indonesia, which embeds numerous island populations in the center of the range, and India and Sri Lanka, which form the western boundaries of the range. The political and bureaucratic challenges in accessing samples in these countries might remain problematic for future collaborative studies. Our largest bottleneck was that all collected samples had to be genotyped in Australia to use the DArTseq (Kilian *et al.* 2012) for consistent analysis. The complex and time-consuming process of organising necessary permits for exporting and importing the samples was a clear burden on the collaborators and us. Within our main study area, the NT, we were not able to collect samples from the West Alligator River and Wildman River in Kakadu National Park and many distant sites in the Gulf region (e.g. Blue Mud Bay, Groote Eylandt, and Limmen Bight River). The inclusion of these populations should provide a more comprehensive and detailed picture of their migration patterns within the NT, where the largest population of *C. porsus* occurs.

Translocation is not a good idea

Results from our studies and another (Lloyd-Jones *et al.* 2023) consistently showed that *C. porosus* populations are genetically structured at various levels between the rivers, regions, islands, and countries. From the perspective of conserving genetic diversity, crocodiles should not be translocated, especially over geographic barriers. Translocation is not desirable also as a means to manage problem crocodiles for safety because crocodiles have a strong homing ability (Walsh and Whitehead 1993; Woolard *et al.* 2004; Campbell *et al.* 2010; Brunell *et al.* 2023). However, a piece of magnet temporarily attached to the head of the multiple crocodilian species (*Caiman crocodilus*, *Crocodylus actutus*, and *C. moreletii*) reportedly deterred the return of the relocated animals to the capture sites (Dominguez-Laso 2008). Some individuals were not able to return even from a short distance (approximately 3 km) with no apparent barriers. This approach may warrant future trials for its use for HCC management once some ethical considerations, such as the impact of translocation on the behaviours, health, and survival of treated animals, as observed in other predatory animals (Bradley *et al.* 2005; Massei *et al.* 2010; Devan-Song *et al.* 2016; Bauder *et al.* 2021), are addressed. Detailed tracking by telemetry, as widely done on different crocodilians around the world (Mascarenhas-Junior *et al.* 2023), may help monitor the fate of treated individuals.

An intergovernmental approach may help

C. porosus is distributed over many countries. As shown by our analyses, some populations are connected through migration over different jurisdictions. For effective management, whether for conservation or human safety, intergovernmental cooperation, such as strategic partnership agreements, should be considered. For example, cooperation between the Torres Strait Islands and Queensland in Australia in managing problem crocodiles could be extended to other nearby countries like Papua New Guinea and the Solomon Islands, as has already been seen on occasion (Wasuka 2018; Messenger 2024). Even if populations are not genetically connected, the benefits from sharing the skills and knowledge required for managing crocodiles would be tremendous, as already seen between the NT and Timor-Leste (Northern Territory Government 2023). In 2014, the Northern Territory Government demonstrated the removal of problem crocodiles and monitoring of crocodile populations to the representatives of the Timor-Lest Government. Furthermore, the standardised methods for monitoring crocodiles documented by Messel *et al.* (1981) and Fukuda *et al.* (2013) have been passed on from the NT to other states [e.g. WA (Halford and Barrow 2017; Corey, *et al.* 2022)] and countries [e.g. Singapore, and Sri Lanka (Samarasinghe and Chandrasiri 2013; Tan, *et al*. 2023)]. Challenges often faced by such regional or international cooperations, including securing funding and engaging diverse stakeholders, must be addressed for effective and consistent implementation across different jurisdictions (Prager 2010; Kark et al. 2015).

Evolutionarily Significant Units (ESU) could exist

With more comprehensive sampling across the species range, we may be able to identify populations with distinctive genetic traits, such as different ancestral lineages. Such unique groups could be considered Evolutionarily Significant Units (ESU) or even subspecies (Moritz 1994; Moritz 2002; Coates *et al.* 2018). Especially, those threatened by previous hunting or ongoing habitat loss may need to be protected as a conservation priority before their genetic legacy is lost, as already was in China, Cambodia, Thailand, and Vietnam, where wild populations are considered extinct (Webb *et al.* 2010; Webb *et al.* 2021; UNEP 2023). Genetic analyses should not be solely relied on to identify ESU but integrated with other approaches such as morphology. Also, difficulty in recognising ESU with no clear criteria available requires careful interpretation of genetic differences in the context of total variation within the species (Moritz 1994, 2002). Insufficient sampling with too few individuals can lead to the false recognition of ESU, while sampling too few nuclear loci might result in failing to

recognise important genetic patterns. The latter can be overcome by whole genome or high-density sequencing that can generate numerous SNPs.

More environmental data are needed

Significant variations in bathymetry and elevation appear to explain the genetic differentiation between populations at a large scale. Additional environmental data, particularly on the complex sea currents that can vary seasonally in the region, may further explain the isolation of some populations. At a finer scale, in conjunction with more comprehensive genetic data, well-detailed habitat mapping, such as species-level vegetation covers and seasonal hydrography (Fukuda and Cuff 2013), should improve the prediction of the dispersal patterns between local populations. A better understanding of their local movement would directly contribute to management, such as identifying the source and route of problem crocodiles, adjusting harvest pressure on populations, or reflecting in development plans for public facilities (e.g. boat ramps and water recreational areas).

6.3 Conclusions

This study revealed the little-known movement patterns of *C. porosus* in and around Australia. Saltwater crocodiles in the NT, where most populations are saturated, were highly mobile, especially within a 200 km range, although the Cobourg Peninsula appeared to interrupt their movement. Their dispersal was influenced by environmental resistance, such as land covers, habitat types, and ecological distances. Problem crocodiles caught in Darwin Harbour came from multiple sources around the harbour, suggesting that the current strategy of targeted removal is efficient at lowering crocodile numbers in the harbour. On a larger scale, populations in different countries are separated by geographic barriers such as deep straits and tall mountains. Some isolated populations may have unique ancestral lineages that could be considered ESU, pending more sampling. An intergovernmental approach may be useful for managing the species, accounting for their population structure.

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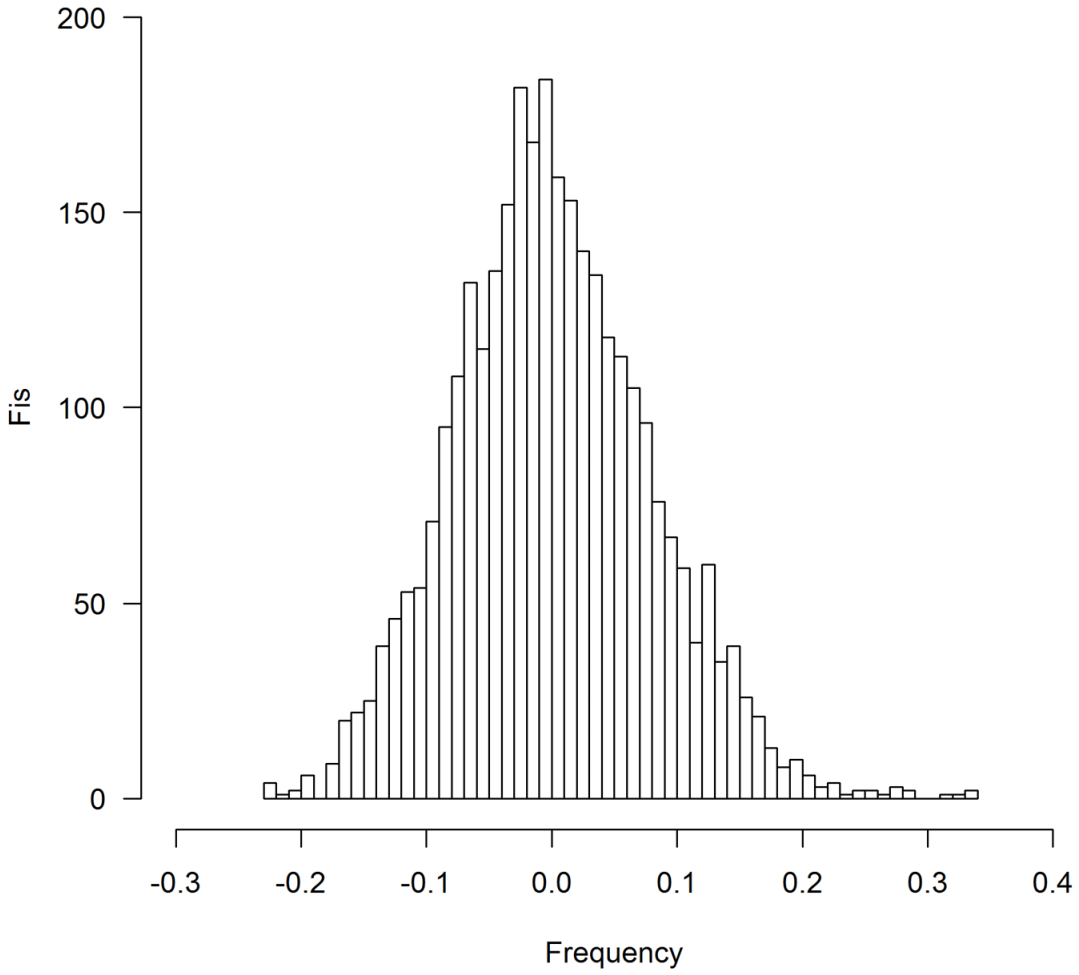
Supporting information

Chapter 2

Supporting information for this chapter is fully available at

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205862#sec011.



S2.1. Histogram of the Fis values at the filtered SNPs.

S2.2. Bootstrap F_{ST} estimates between saltwater crocodile sampling regions with 95% confidence intervals (CI).

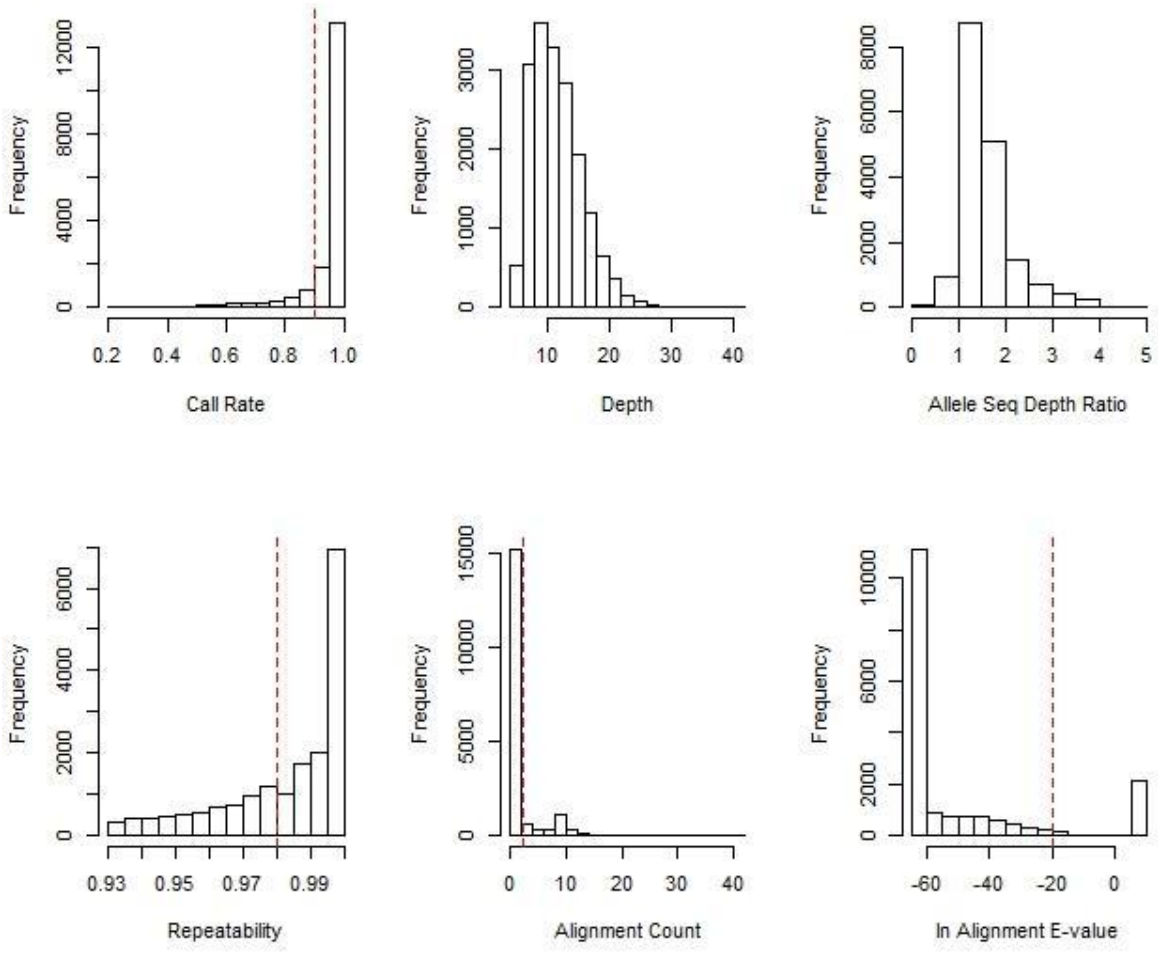
Region 1	**Region 2**	**Bootstrap F_{ST} (95% CI)**
Cobourg Murganella Creek	Adelaide River	0.019 (0.017-0.021)
Cobourg Murganella Creek	Finniss River	0.023 (0.021-0.025)
Cobourg Murganella Creek	Mary River	0.018 (0.016-0.02)
Cobourg Murganella Creek	Arafura Swamp*	0.061 (0.058-0.064)
Cobourg Murganella Creek	Maningrida King River*	0.027 (0.024-0.028)
Cobourg Murganella Creek	Tiwi Islands	0.012 (0.011-0.014)
Adelaide River	Finniss River	0.038 (0.035-0.04)
Adelaide River	Mary River	0.029 (0.026-0.031)
Adelaide River	Arafura Swamp *	0.076 (0.073-0.079)
Adelaide River	Maningrida King River*	0.041 (0.039-0.044)
Adelaide River	Tiwi Islands	0.023 (0.022-0.025)
Finniss River	Mary River	0.037 (0.034-0.04)
Finniss River	Arafura Swamp *	0.075 (0.072-0.077)
Finniss River	Maningrida King River*	0.044 (0.042-0.046)
Finniss River	Tiwi Islands	0.031 (0.03-0.033)
Mary River	Arafura Swamp *	0.069 (0.066-0.073)
Mary River	Maningrida King River*	0.034 (0.031-0.037)
Mary River	Tiwi Islands	0.023 (0.021-0.025)
Arafura Swamp *	Maningrida King River*	0.015 (0.014-0.016)
Arafura Swamp *	Tiwi Islands	0.061 (0.059-0.064)
Maningrida King River*	Tiwi Islands	0.031 (0.03-0.033)

*Regions on the eastern side of the Cobourg Peninsula.

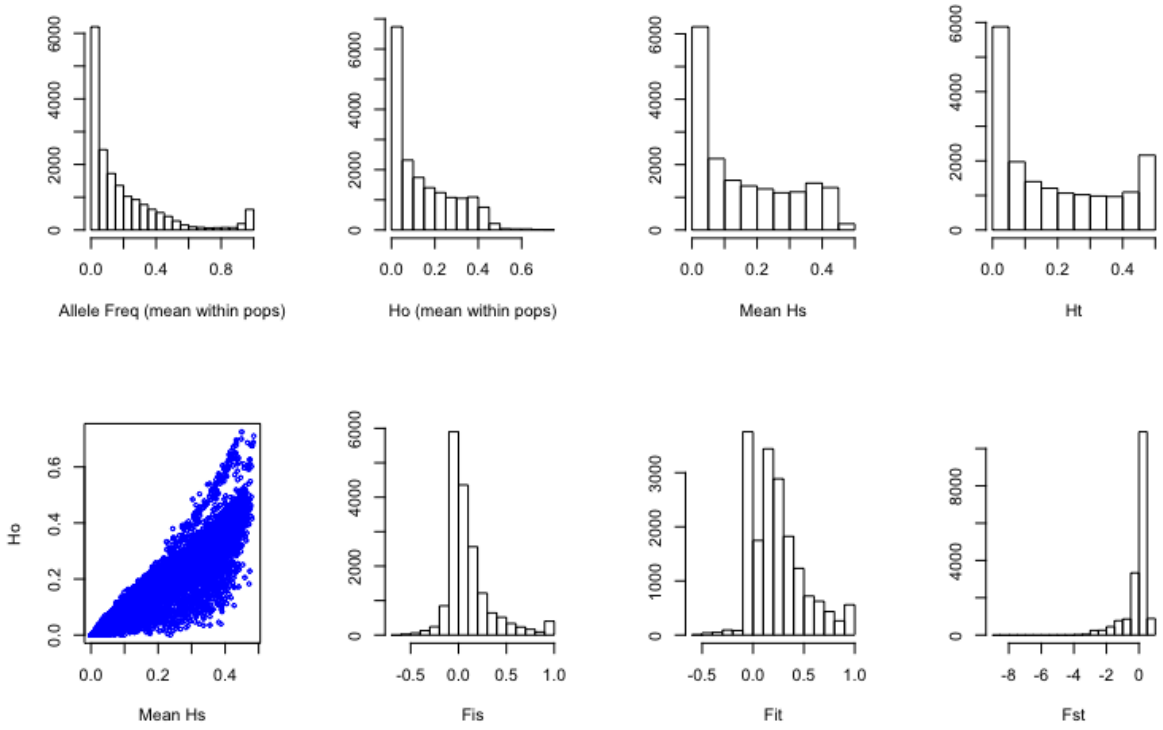
Chapter 3

Supporting information for this chapter is fully available at

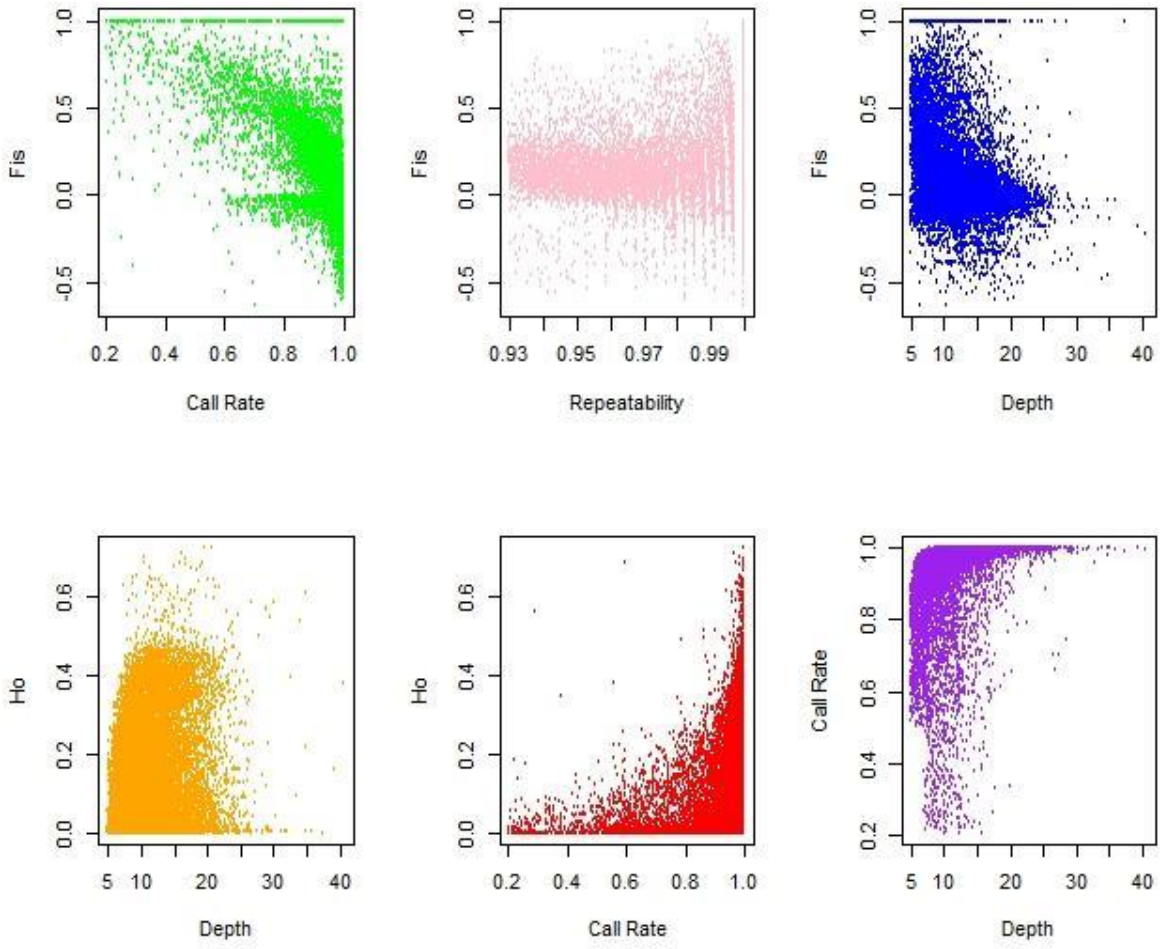
https://doi.org/10.5061/dryad.bcc2fqz94, https://doi.org/10.5061/dryad.q2bvq83gb, and https://doi.org/10.5061/dryad.2z34tmpj1.



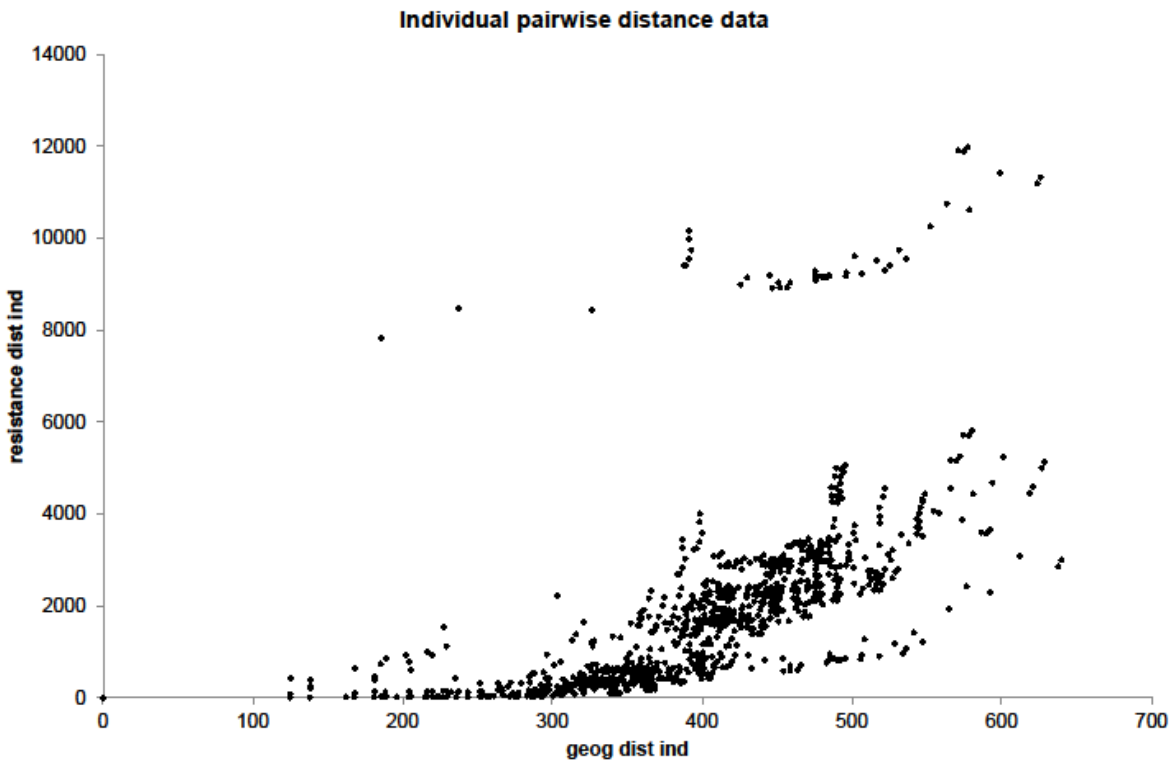
S3.1. Histograms of the SNP quality indexes before the filtering. Thresholds we used for the filtering are shown in broken lines.

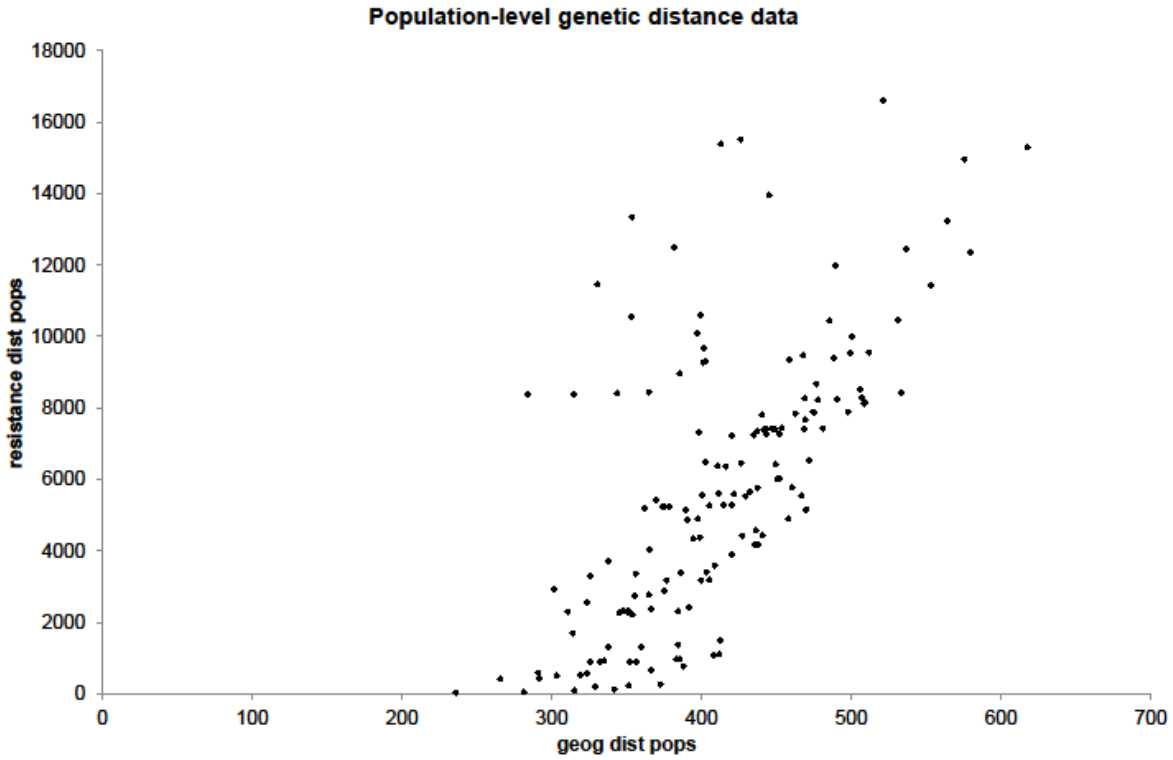


S3.2. Histograms of the population genetics statistics before the filtering. Y axis is the number of SNPs unless labeled differently.



S3.3. Comparison between the population genetics statistics and the SNP quality indexes before the filtering.



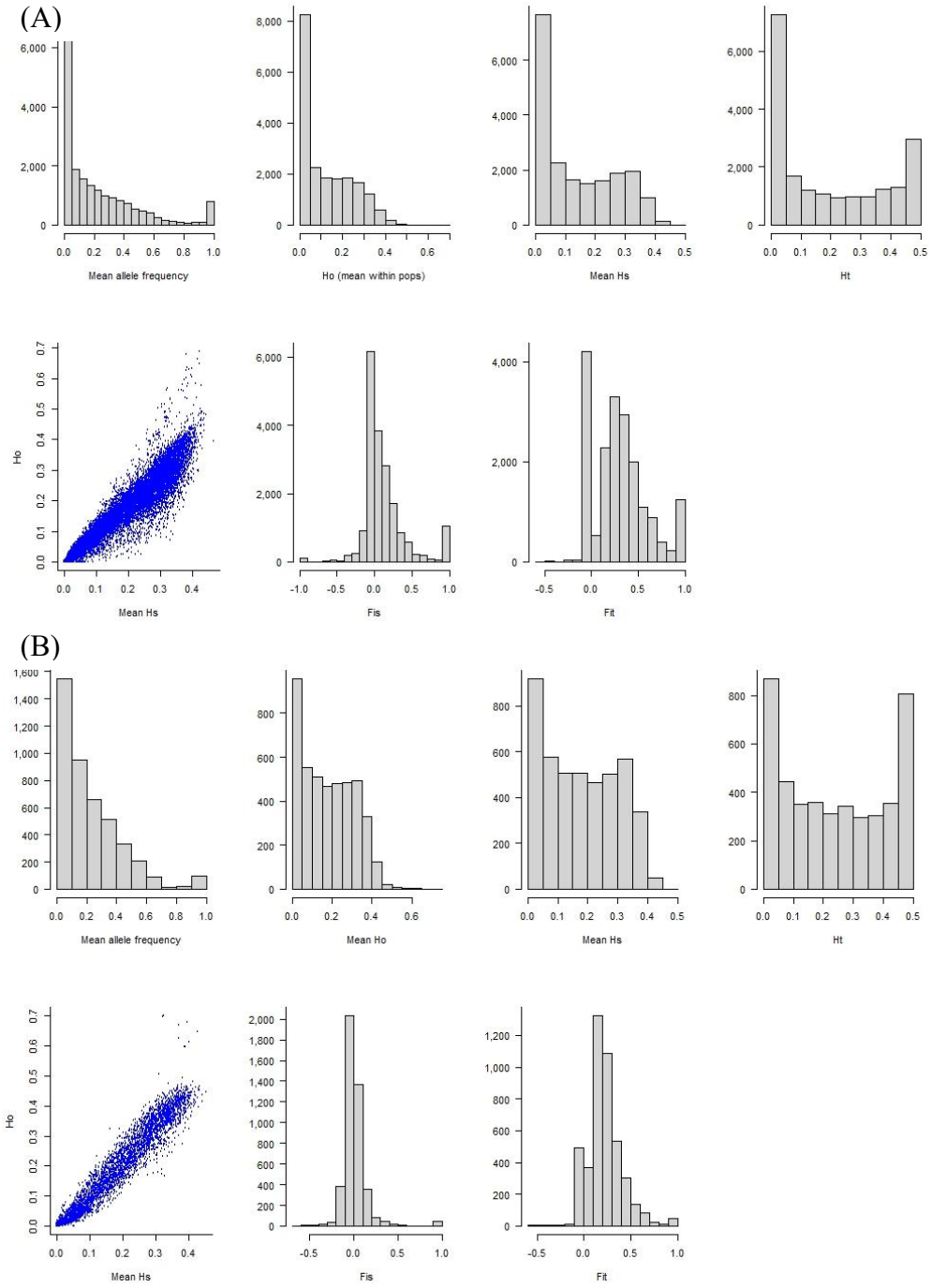


S3.4. Scatterplots of the matching geographic distances and resistance distances (random walk commute distances) for the pairwise population and individual-level datasets.

Chapter 4

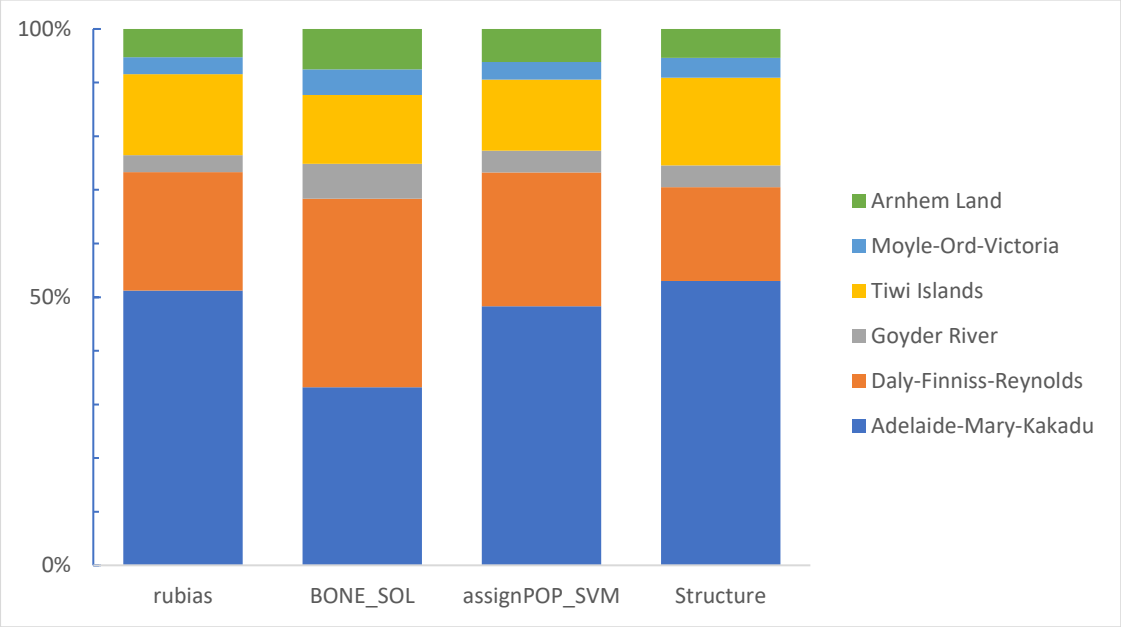
Supporting information for this chapter is fully available at

https://wildlife-onlinelibrary-wiley-com.virtual.anu.edu.au/doi/10.1002/jwmg.22525#.

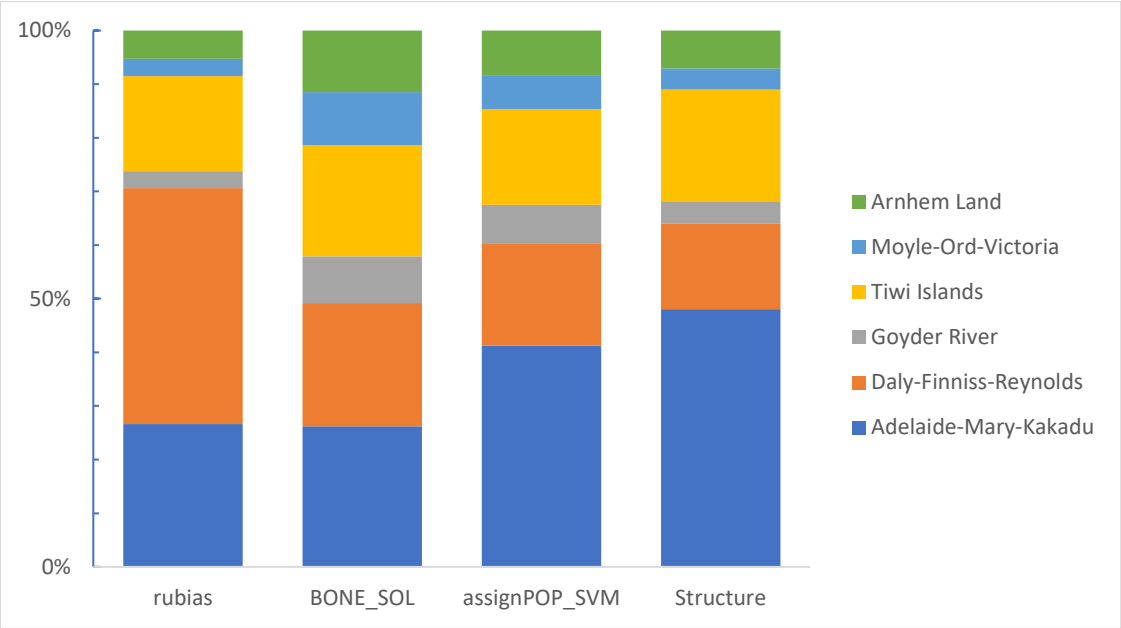


S4.1. Histograms of population genetics statistics A) before filtering (N = 939) and B) after filtering (N = 912) for *Crocodylus porosus* sampled in Australia and the neighbouring countries between 1997 and 2022. Y axis is the number of SNPs unless labeled differently.

(A)



(B)



S4.2. Comparison of the probabilities of saltwater crocodiles sampled in the Darwin Harbour (N = 95) being assigned by assignPOP, BONE, rubias and Structure between A) balanced (N = 33 for all populations) and B) unbalanced sample sizes [N = 33 (WA), 52 (Tiwi Islands), 60 (Goyder River), 62 (Arnhem Land), 106 (Adelaide/Mary/Kakadu), 137 (Daly/Finniss/Reynolds)] to the six clusters identified by Discriminant Analysis of Principal Components (Figure 3) that grouped rivers in the Northern Territory and Ord River, Western Australia, Australia between 1997 and 2022.

S4.3. Paired genetic differentiation among the catchments (F_{ST}; p values all <0.05) of saltwater crocodiles sampled in the Northern Territory and Western Australia, Australia between 1997 and 2022.

	Adelaide River	**Cobourg Murganella Creek**	**Goyder River**	**Tiwi Islands**	**Daly River**	**Finniss Reynolds Rivers**	**Blyth River**	**Mary River**	**King Goomadeer Rivers**	**East Arnhem**	**Victoria River**	**South Alligator River**	**East Alligator River**	**Ord River**
Cobourg Murganella Creek	0.011													
Goyder River	0.077	0.064												
Tiwi Islands	0.015	0.012	0.066											
Daly River	0.020	0.019	0.076	0.028										
Finniss Reynolds Rivers	0.020	0.017	0.074	0.028	0.001									
Blyth River	0.035	0.023	0.020	0.029	0.040	0.039								
Mary River	0.015	0.013	0.065	0.021	0.021	0.021	0.029							
King Goomadeer Rivers	0.026	0.017	0.034	0.024	0.027	0.027	0.005	0.021						
East Arnhem	0.045	0.033	0.032	0.036	0.050	0.047	0.008	0.035	0.007					
Victoria River	0.074	0.075	0.110	0.076	0.076	0.078	0.077	0.071	0.068	0.073				
South Alligator River	0.013	0.011	0.066	0.019	0.022	0.023	0.029	0.010	0.020	0.038	0.070			
East Alligator River	0.015	0.012	0.071	0.024	0.028	0.027	0.031	0.013	0.024	0.042	0.077	0.006		
Ord River	0.047	0.045	0.086	0.049	0.045	0.043	0.046	0.042	0.036	0.046	0.021	0.044	0.043	
Moyle River	0.058	0.062	0.086	0.068	0.066	0.068	0.072	0.061	0.064	0.077	0.059	0.054	0.059	0.058

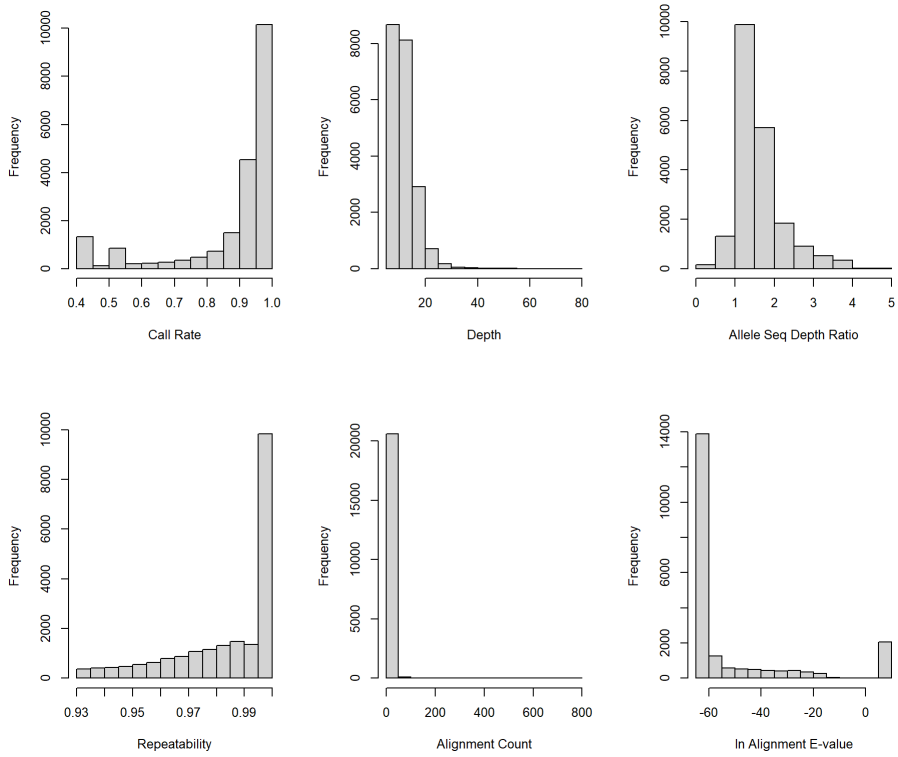
S4.4. Paired genetic differentiation among the clusters (F_{ST}) (p values all <0.05) of saltwater crocodiles sampled in the Northern Territory and Western Australia, Australia between 1997 and 2022.

	Adelaide, Mary, Kakadu	**Goyder River**	**Tiwi Islands**	**Daly, Finniss, Reylolds Rivers**	**West East Arnhem**
Goyder River	0.065				
Tiwi Islands	0.014	0.066			
Daly, Finniss, Reynolds Rivers	0.016	0.076	0.028		
West East Arnhem	0.024	0.026	0.028	0.036	
Moyle, Ord, Victoria Rivers	0.043	0.083	0.054	0.051	0.051

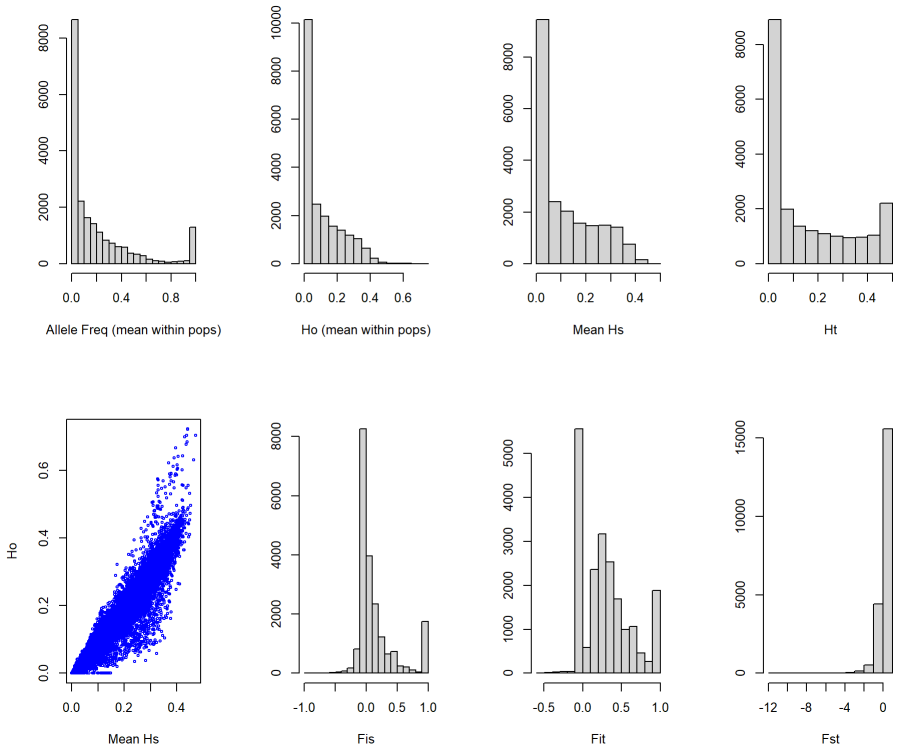
Chapter 5

Supporting Information

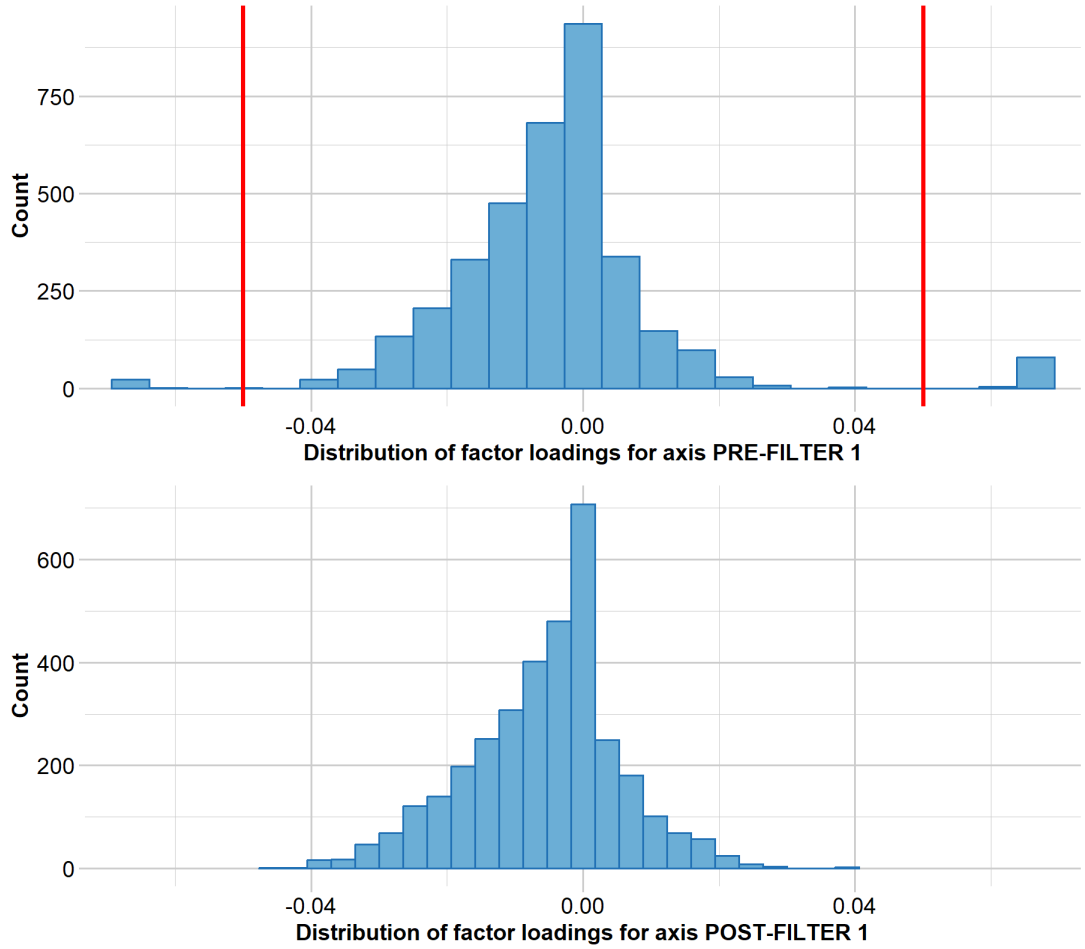
Data filtering



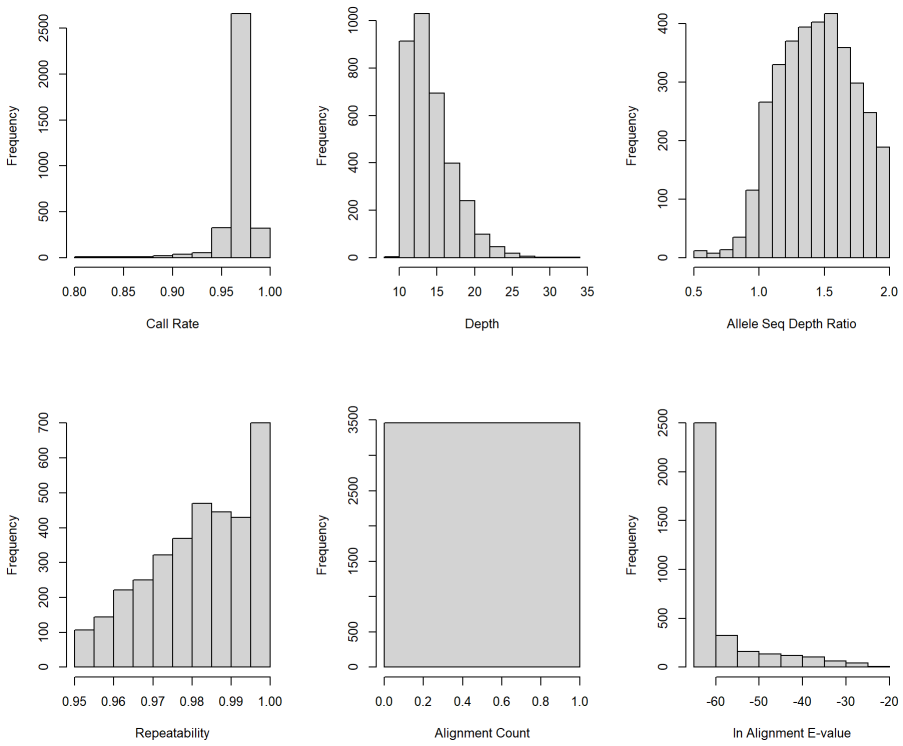
S5.1. Histograms of the SNP quality indexes before the filtering.



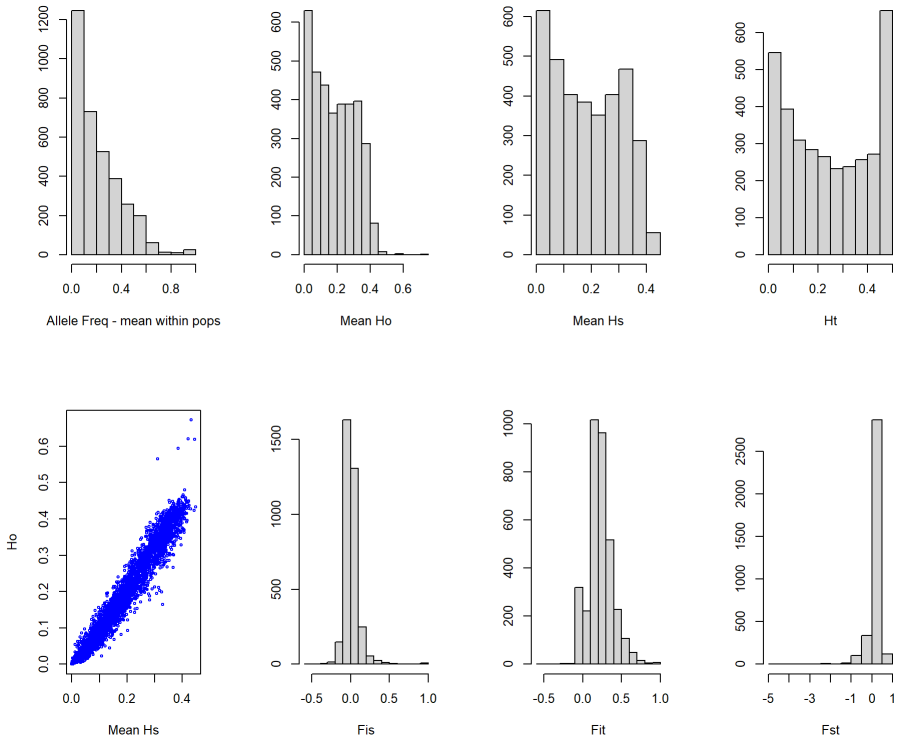
S5.2. Histograms of the population genetics statistics before the filtering. Y axis is the number of SNPs unless labeled differently.



S5.3. Filtering of SNPs with high factor loading (>0.05) for the first axis of the principal coordinates analysis.



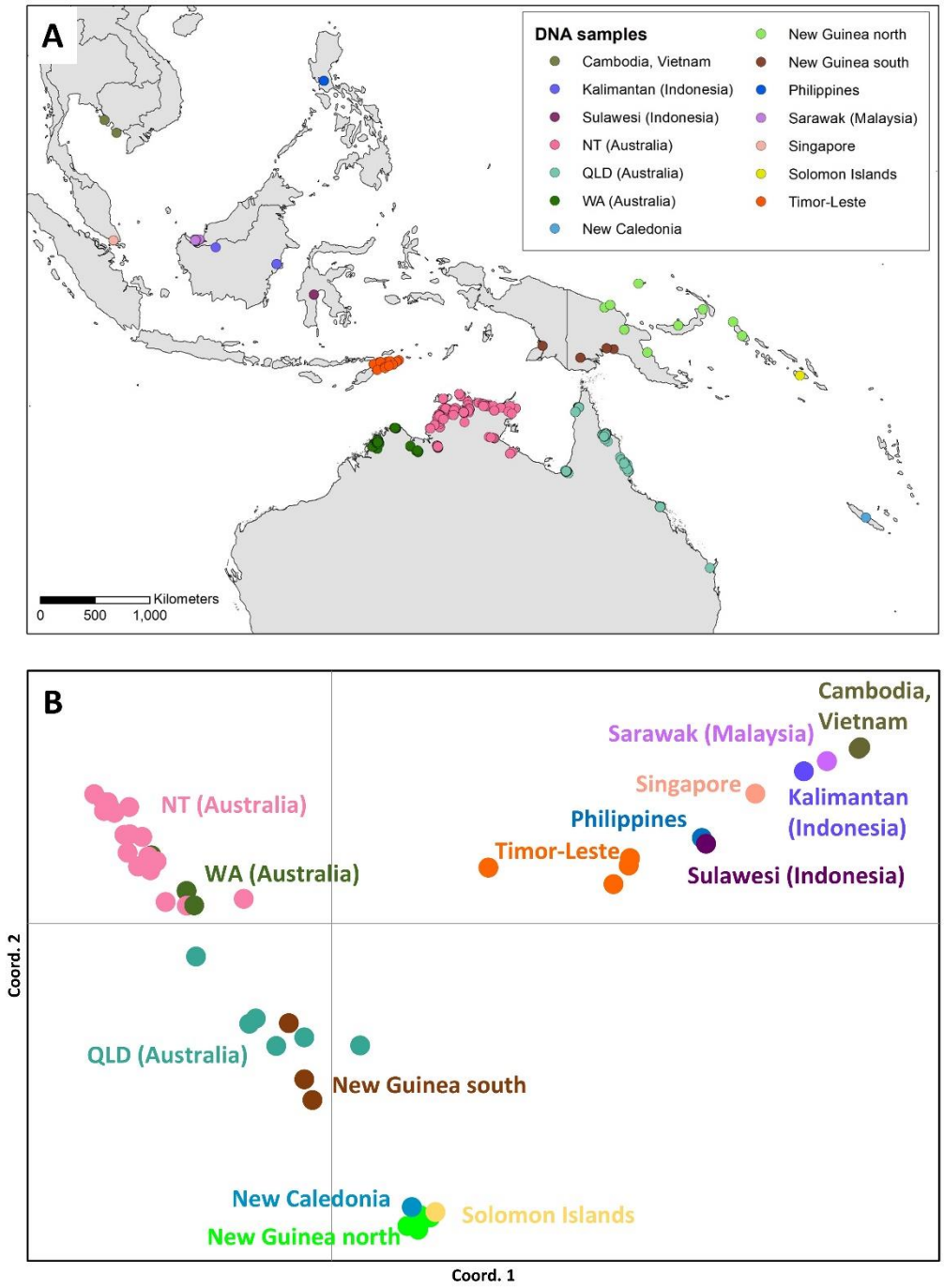
S5.4. Histograms of the SNP quality indexes after the filtering.



S5.5. Histograms of the population genetics statistics after the filtering. Y axis is the number of SNPs unless labeled differently.

Results with the full dataset

After a series of data filtering, we retained 3458 SNPs in 1280 of the 1329 genotyped individuals with a minor allele frequency of >0.02, corresponding to ≥20 allele copies for analysis. The filtered dataset showed a high overall genetic differentiation (F_{ST} = 0.179, SE = 0.005) and low inbreeding coefficient (F_{IS} = 0.011, SE = 0.001). The genetic diversity varies among the populations, partially depending on their different sample sizes (Table S5.1). The PCOA showed the genetic relationships of the sampled populations (S5.6.B) that roughly resemble their spatial distribution (S5.6.A).

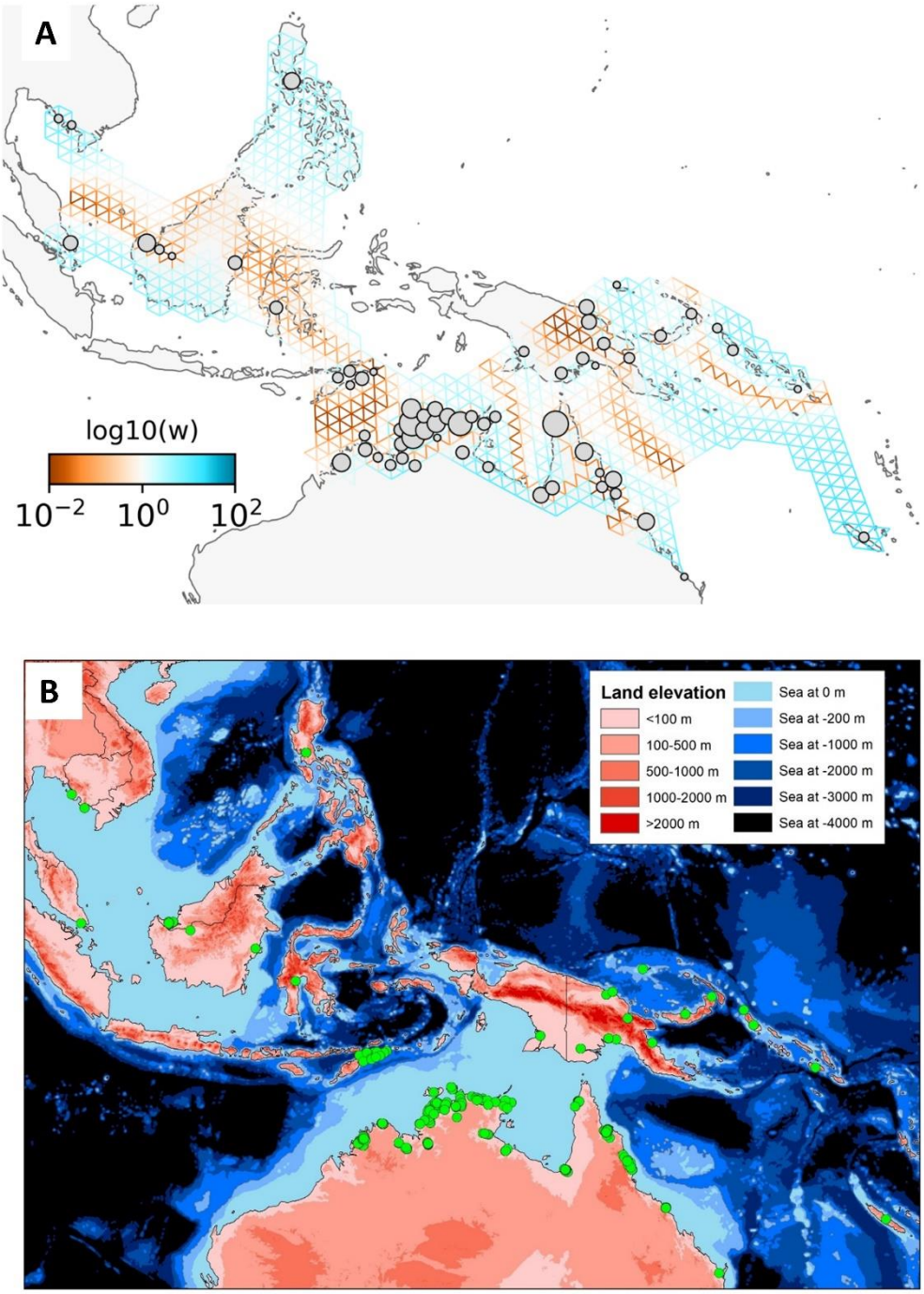


S5.6. A) Genetic samples of *C. porosus* collected from Australia and the neighbouring countries, and B) PCOA of the populations based on the genetic distance estimated by GenAlEx (Peakall and Smouse 2017).

Table S5.1. Genetic diversity statistics of each population estimated by GenAlEx (Peakall and Smouse 2006; Peakall and Smouse 2012), including sample size (n), mean observed heterozygosity (H_O), mean expected Heterozygosity (H_E), and inbreeding coefficient (F_{IS}) that was calculated as (mean H_E - mean H_O)/mean H_E.

Cluster	**n**	**H_E**	**H_O**	**F_{IS}**
Cambodia, Vietnam	4	0.11	0.13	-0.17
Kalimantan (Indonesia)	14	0.20	0.17	0.16
New Caledonia	4	0.14	0.16	-0.15
NT (Australia)	659	0.21	0.19	0.09
Papua (Indonesia)	4	0.18	0.18	-0.02
Philippines	29	0.23	0.23	0.01
PNG north	73	0.17	0.12	0.32
PNG south	18	0.20	0.15	0.23
QLD (Australia)	312	0.22	0.19	0.12
Sarawak (Malaysia)	40	0.17	0.16	0.06
Singapore	17	0.23	0.23	0.01
Solomon Islands	1	0.06	0.13	-1.00
Sulawesi (Indonesia)	11	0.20	0.20	0.01
Timor-Leste	28	0.24	0.22	0.06
WA (Australia)	66	0.19	0.18	0.08

The estimated migration surface with a few barriers to the inferred gene flow (S5.7.A) was consistent with the genetic distance between the populations indicated by the PCOA (S5.6.B). The barriers implied by the migration surface coincided with areas with high elevation on land shallow water in the sea (S5.7.B).



S5.7. A) Migration surface estimated by FEEMS (Marcus *et al.* 2021), indicating effective migration areas (blue) and barriers to migration (brown) with the location of the genetic samples whose symbol size is relative to the sample size and B) land elevation and bathymetry in the study area.

The ancestral clustering with the smallest number of ancestral populations (K = 2) was consistent with the PCOA that the entire population may be separated into two broad groups of Asia and Oceania (S5.8). Similarly, with the increasing groups (K = 5, 10, and 15), the ancestral clustering showed relatively distinctive separation, consistent with the barriers estimated in the migration surface.

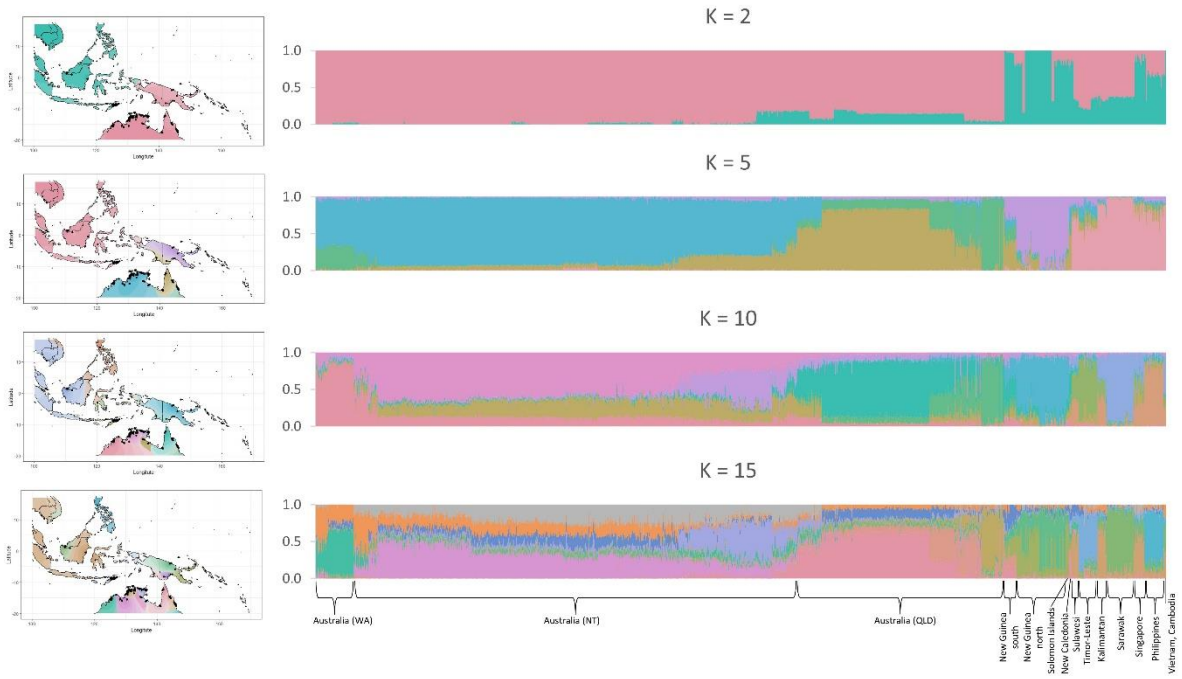
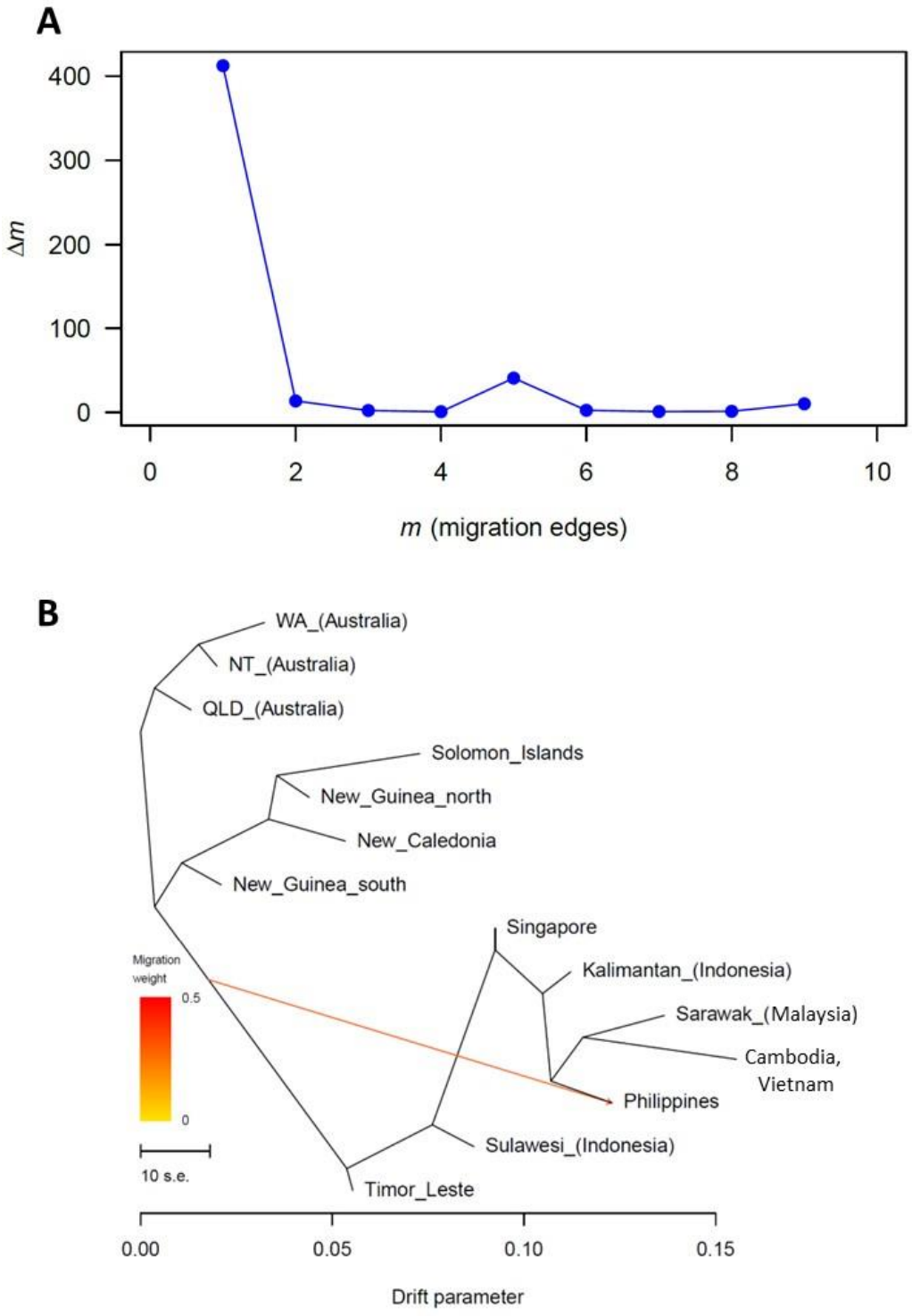


Figure S5.8. Interpolated ancestral clustering of *C. porosus* (K = 2, 5, 10, and 15) and the proportions of the ancestral populations for each sample estimated by tess3r (Caye *et al.* 2016).

The estimation of the optimal number of migration events in the population trees generated by TreeMix showed a high peak in Δm at 1 (S5.9.A), suggesting that this was the most likely number of migration edges to be included in the model. Consequently, the migration event shown in the population history tree was from near New Guinea south to the Philippines (S5.9.B). The structure of the population tree resembled the ancestral clustering, especially with 15 populations, estimated by tess3R.



S5.9. A) Second-order rate of change (Δm) across the different numbers of migration (m) estimated by OptM (Fitak 2021), and B) population history tree with one migration generated by TreeMix (Pickrell and Pritchard 2012).