Life history and genetic structure of three commercially targeted sharks in temperate eastern Australian waters

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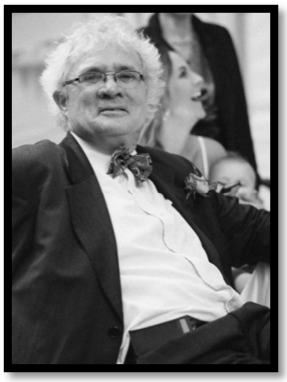
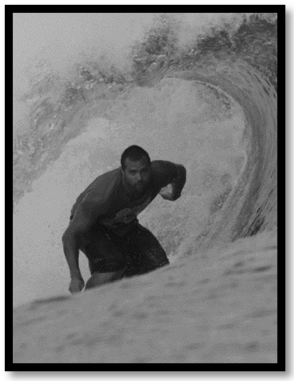
MACQUARIE UNIVERSITY

NSW GOVERNMENT Industry & Investment

Dedication

For your love, patience, support, encouragement, companionship and our surf trips, I dedicate this body of work to my mother and father, Adele and Chris, to my brother Patrice, and to my beautiful wife Emily.



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General Abstract

The removal of large predatory sharks from the world's oceans poses profound threats to marine community structure and species conservation over a range of spatial scales. In a context of increased harvest pressure worldwide, the effective management of exploited shark populations relies on a sound understanding of target species' life histories, genetic diversities and metapopulation structures.

Molecular genetic techniques and vertebral ageing analysis were employed here in conjunction with accurate fishery-observer catch data to investigate the genetic diversity and structure, growth dynamics and reproductive characteristics of dusky (*Carcharhinus obscurus*), spinner (*Carcharhinus brevipinna*) and sandbar (*Carcharhinus plumbeus*) sharks in temperate eastern Australian waters, where they support a demersal longline fishery. We also establish basic estimates of scientific observer accuracy in the identification of these species within the fishery. These data were used to qualitatively evaluate the susceptibility of these species, and the fishery as a whole, to stock decline and to recommend appropriate spatial scales of management.

Genetic analyses revealed varying levels of diversity among the three study species. *Carcharhinus obscurus* and *C. plumbeus* demonstrated a range of similarities in their genetic structures that were in contrast to that of *C. brevipinna*; the latter appearing to have been shaped by a very different evolutionary history in the sampling area. Genetic differentiation, albeit weak, was detected in *C. obscurus* between eastern and western Australian waters, suggesting the delineation of two independent populations. For *C. brevipinna*, the Indian Ocean was found to be a reasonably robust barrier to contemporary gene flow between Australia and South Africa, and we detected weak evidence for restricted gene flow on a fine-scale along a continuous continental margin within Australian waters. Limitations inherent in

our genetic analyses, however, highlighted the need for further sampling to achieve greater population structure resolution for these species.

Examination of the life histories of the three target species revealed a range of both contrasts and consistencies in their age, growth and reproductive characteristics off Australia's temperate east coast. Nevertheless, all three were characterised by low productivity (i.e. long-lived, relatively slow-growing, late-maturing species of low fecundity and lengthy gestation), highlighting their vulnerability to stock depletion. Interestingly, many aspects of their life histories in New South Wales waters appeared to challenge findings emanating from conspecific populations in other parts of the world. Comparison of biological parameters between studies, however, must be treated with some caution given potentially confounding factors.

We also demonstrated micro-computed tomography to be a valid and repeatable alternative means of shark ageing that offers several distinct advantages over more traditional methods. In spite of this, it is not sufficiently cost effective at present to be widely applied.

This thesis, via comprehensive assessments of demographic parameters and genetic population structure, raises important implications relating to the resilience of *C. obscurus*, *C. brevipinna* and *C. plumbeus* to fishing-induced population decline in the region and, in turn, the sustainability of the local fishery. It also provides valuable information pertaining to the allocation of management units for these species in Australian and surrounding waters.

Declaration

I certify that the contents of this thesis have not previously been submitted for a degree nor have they been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that this thesis is an original piece of research and has been written by me. Any help and assistance has been appropriately acknowledged along with all information sources and literature used.

The research presented herein was approved by the Primary Industries (Fisheries) Animal Care & Ethics Committee (ACEC REF 07/03 – CFC) on 6th June 2007.

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June 2013

Acknowledgements

As per my dedication, I owe this achievement entirely, and equally, to my family (Chris, Adele and Patrice Geraghty) and to my wife Emily, all of whom have seen this through from the very beginning. I'd like to thank them from the bottom of my heart for the patience, understanding and tolerance that was demanded of them throughout this (unintentionally) very selfish time. I've anxiously awaited my chance to give back, and so it's with great pleasure and relief that I can finally say that I now feel free and able to do so.

I owe a very special thankyou to Will Macbeth for taking such an interest in me, for involving me so closely in your work, and for ultimately presenting me with what has been a wonderful opportunity. Our like-mindedness and friendship made your supervision one of the highlights.

To Jane Williamson, thank you very much for trusting in me and welcoming me into your diverse Marine Ecology Research Group. Taking on a student you're not familiar with is a big gamble, but I hope this has been as good an experience for you as it has been for me.

For financial support, both direct and in-kind, I'd like to extend my deepest gratitude to the people of the NSW DPI Cronulla Fisheries Research Centre (R.I.P), Macquarie University, the Molecular Fisheries Laboratory QLD (R.I.P), and to those responsible for deeming me worthy of an Australian Postgraduate Award.

Finally, my humblest appreciation goes to Paul Moody, Graeme Coleman, Gary Thorncraft and Ken Hynes for welcoming me so warmly, for opening your homes to me and for allowing me a window into the fascinating world that is commercial fishing – undoubtedly one of the greatest experiences I've ever had. The public's generally negative perception of commercial fishers stems from a combination of ignorance and the actions of a select few, short-sighted operators. The respect, restraint, skills and resourcefulness shown by many should be given greater recognition.

Thesis Structure and Co-Author Contribution

Chapter 1. *General Introduction*

Literature review and written content were produced entirely by myself. Guidance and feedback were provided by my supervisors, Jane Williamson and William Macbeth.

Chapter 2. *Genetic structure and diversity of two highly vulnerable carcharhinids in Australian waters*

&

Chapter 3. *Population expansion and genetic structure in Carcharhinus brevipinna in the southern Indo-Pacific*

For both Chapters 2 and 3, tissue sample collection in New South Wales (NSW) waters was conducted by myself and other NSW Department of Primary Industry (NSW DPI) scientific observers on-board commercial shark-fishing vessels. For use in Chapter 2, additional samples were contributed from Northern Territory (NT) waters by Grant Johnson and from Western Australian and Indonesian waters by Jennifer Ovenden. For use in Chapter 3, samples were contributed from South African waters by Sabine Wintner, from Queensland waters by Alastair Harry, and again from NT waters by Grant Johnson.

Project design, aims and hypotheses for both chapters were developed by myself with assistance from Michael Gillings, Jane Williamson, Jennifer Ovenden and William Macbeth. Both chapters share common laboratory procedures, guidance for which was provided by Michael Gillings, Jess Morgan and Jennifer Ovenden. All three helped also with troubleshooting issues.

For both chapters, all aspects of laboratory work (i.e. DNA extraction, PCR assays, and sequence analysis), as well as all data and statistical analyses, interpretation and written content, were conducted by myself. Both chapters were strengthened by additional statistical analyses made possible by Dean Blower, who developed specific software for performing random sequence re-sampling simulations.

Sequencing was performed by the Macquarie University Sequencing Facility, and Macrogen.

All co-authors (Jane Williamson, William Macbeth, Dean Blower, Jess Morgan, Grant Johnson, Jennifer Ovenden and Michael Gillings) participated in review and provision of constructive comments for Chapter 2. Review of Chapter 3 was carried out by Jennifer Ovenden, Michael Gillings, Sabine Wintner and Alastair Harry.

Chapter 4. *Micro-computed tomography: an alternative method for shark ageing*

Original concept and funding was provided by John Stewart. Experimental design was subsequently developed by myself and John Stewart, with assistance from William Macbeth. Vertebrae samples utilised were collected as part of Chapter 5 (see below).

MicroCT equipment was operated by Allan Jones, with scanning methodology optimised by myself and Allan. All vertebral ageing data collection, statistical analyses, interpretation and writing for this chapter were conducted by myself. Review of drafts was conducted by William Macbeth and John Stewart.

Chapter 5. *Robust age and growth parameters for three heavily exploited shark species off temperate eastern Australia*

Vertebrae samples were collected on-board commercial shark fishing vessels by myself and other NSW DPI scientific observers. Laboratory procedures (i.e. vertebrae cleaning and sectioning) were carried out by myself, with assistance from Jacqui Bell.

Vertebral ageing data collection was conducted by myself (Reader1) and by Michelle Yerman (Reader 2). I was exclusively responsible for all data and statistical analyses, interpretation and written content, and used a range of *R* scripts provided by Alastair Harry including for non-linear growth model fitting and multi-model inference analysis.

Constructive comments on drafts were provided by William Macbeth, Jane Williamson, Jacqui Bell and Alastair Harry.

Chapter 6. *Reproductive parameters for dusky, spinner and sandbar sharks (Family Carcharhinidae) in the south-western Pacific Ocean and their implications for population status.*

Reproductive data was collected by myself and other NSW DPI scientific observers on-board commercial shark-fishing vessels in NSW waters.

With the aid of *R* scripts provided by Alastair Harry (see Chapter 5), I was responsible for all data and statistical analyses, interpretation and written content for this chapter.

William Macbeth and Jane Williamson participated in review of drafts.

Chapter 7. *General Discussion and Conclusions*

Writing for this chapter was performed entirely by myself. Constructive feedback was provided by Jane Williamson and William Macbeth.

Additional Published Works Relevant to this Thesis

Peer-Reviewed

Morgan JAT, Harry AV, Welch DJ, Street R, White J, **Geraghty PT**, Macbeth WG, Tobin A, Simpfendorfer CA, Ovenden JR (2012) Detection of interspecies hybridisation in Chondrichthyes: hybrids and hybrid offspring between Australian (*Carcharhinus tilstoni*) and common (*C. limbatus*) blacktip shark found in an Australian fishery. *Conserv Genet* 13: 455-463.

Plate 1. First, and only known, image of a genetically-confirmed hybrid shark (*C. limbatus*-*C. tilstoni*). Individual captured in northern NSW waters, Australia. Photo by P. Geraghty.

Technical Reports

Macbeth WG, **Geraghty PT**, Peddemors VM, Gray CA (2009) Observer-based study of targeted commercial fishing for large shark species in waters off northern New South Wales. *Fisheries Final Report Series 114*. Industry & Investment NSW.

Morgan J, Ovenden J, Street R, **Geraghty PT**, Welch DJ (2011) Genetic stock structure exists along the east coast of Australia for blacktip sharks, *Carcharhinus limbatus* and *C. tilstoni* based on mitochondrial DNA. In: Welch DJ, Ovenden J, Simpfendorfer C, Tobin A, Morgan JAT, Street R, White J, Harry A, Schroeder R, Macbeth WG (2011) Stock structure of exploited shark species in north eastern Australia. Report to the Fisheries Research & Development Corporation, Project 2007/035. *Fishing & Fisheries Research Centre Technical Report 12*. James Cook University.

Oral Presentations Delivered During Candidature

Conferences

Sharks International! Cairns, Australia (2010) – "A biological basis for fishery-management strategies for carcharhinid sharks commercially exploited in New South Wales waters".

2nd Annual Marine and Freshwater Student Symposium. Moreton Bay Research Station, North Stradbroke Island, Australia (2010) – "Developing biology-based fishery management strategies for carcharhinid sharks commercially exploited in eastern Australian waters".

Cronulla Fisheries Research Centre of Excellence Seminar Series. Industry & Investment, Sydney, Australia (2010) – "Developing biology-based management strategies for a multi-species carcharhinid shark fishery in eastern Australian waters".

Macquarie University Postgraduate Conference 2010. Macquarie University, Sydney, Australia (2010) – "The biology of four co-occurring, commercially-important carcharhinid shark species in eastern Australian waters".

Macquarie University Postgraduate Conference 2011. Macquarie University, Sydney, Australia (2011) –"Genetic population structure of three commercially-important shark species in eastern Australian waters".

American Elasmobranch Society Meeting at the 7th World Congress of Herpetology. University of British Columbia, Vancouver, Canada (2012) – "Contrasting population structures suggest different evolutionary histories for three large, coastal shark species off eastern Australia".

Macquarie University Postgraduate Conference 2012. Macquarie University, Sydney, Australia (2012) – "Genetic population structure and evolutionary history of three shark species in the southern Indo-Pacific".

Committees

NSW Ocean Trap and Line Fishery Management Advisory Committee. Industry & Investment NSW, Sydney, Australia (2010) – "A biological-basis for fishery-management strategies for carcharhinid sharks commercially exploited in eastern Australian waters".

Fishery Scientific Committee NSW. Industry & Investment NSW, Sydney, Australia (2010) – "Current research on dusky whalers – *Carcharhinus obscurus*".

NSW Fisheries Resource Assessment Workshop. Industry & Investment NSW, Sydney, Australia (2010) – "*Carcharhinus* spp. – Research in progress".

Fishery Scientific Committee NSW. Sydney Institute of Marine Science, Australia (2012) – "Genetic population structure of three shark species in the southern Indo-Pacific".

Public and University Lectures

Science Research Lecture Night – Public Lecture. Macquarie University, Sydney, Australia (2012) – "Getting to know our sharks".

Student Science Experience. Macquarie University, Sydney, Australia (2012) – "Getting to know our sharks".

Vertebrate Evolution, 3rd Year Biology Lecture. Macquarie University, Sydney, Australia (2012 & 2013) – "Radiation of Chondrichthyes".

CHAPTER 1. General Introduction

Plate 2. A shark-fishing vessel heading to sea off the eastern Australian coast.

1.1 Shark diversity, ecology & decline

Sharks are a diverse group of cartilaginous fishes (class Chondrichthyes) belonging to the subclass Elasmobranchii (shared with rays, skates and sawfishes). The result of over 400 million years of evolution, sharks are represented by *c.* 500 extant species worldwide, which together exhibit astonishing morphologic variation (Compagno 1984, Last & Stevens 2009, Last & White 2011). Remarkably, sharks have successfully adapted to almost all aquatic habitats and niches – from riverine freshwater, to surface waters of the open ocean, to abyssal plains of the deep ocean floor – and occur in equatorial to polar waters (Compagno 1984, Nelson 2006, Last & Stevens 2009). The Indo-Australasian region – Australia, Indonesia, Papua New Guinea, New Caledonia and New Zealand – is recognised as a focal point of global shark (and chondrichthyan) biodiversity and endemism. Australian seas, in particular, boast the greatest species richness of this mega-diverse zone and where *c.* 36 % of all described shark species occur (Last & Stevens 2009, Last & White 2011). Moreover, of the 182 species comprising Australia's shark fauna, *c.* 40 % are endemic (Last & Stevens 2009).

Many sharks are apex predators in the ecosystems they inhabit, thereby playing vital roles in the maintenance of community structure and biodiversity through regulation of mesopredator and prey abundance (Ritchie & Johnson 2009). The removal of such sharks can initiate profound cascading effects on lower trophic levels (Stevens et al. 2000, Shepherd & Myers 2005, Myers et al. 2007, Heithaus et al. 2008, Baum & Worm 2009, Ferretti et al. 2010).

Despite their evolutionary and adaptive success, shark populations have coped poorly with the dramatic rise of anthropogenic influences in recent decades; in particular, the advent and subsequent expansion of industrialised fishing (Bonfil 1994). Throughout human history sharks have been exploited for their liver oil, vertebrae, skin, teeth, flesh and, more recently,

their fins for medicinal, medical, consumptive, practical, cultural and industrial purposes (Walker 1998, Musick 2005a, Clarke et al. 2006). Intense historical harvest pressure, coupled with non-existent or ineffective management strategies, has driven precipitous population declines (Baum et al. 2003, Baum & Myers 2004, Ferretti et al. 2008, Baum & Blanchard 2010), and even fishery collapse (Musick 2005b), in a range of shark species around the world. Consequently, growing global concern surrounds the sustainability of directed shark fisheries and the conservation status of various target and by-catch species.

While magnitudes of stock decline are debatable (Burgess et al. 2005), the inherent vulnerability of many sharks to overexploitation is attributed to a combination of life-history traits and a susceptibility to multiple fishing gears. Sharks are typically characterised by long life-spans, slow rates of growth, late onset of maturity, along with low reproductive output and natural abundance (Cortés 2000). This low productivity renders most shark species able to withstand only modest levels of fishing mortality, in turn translating to a low capacity for population recovery in the event of stock collapse (Smith et al. 1998, Musick 1999, Cortés 2000, 2002, García et al. 2008, Field et al. 2009).

The abovementioned issues have highlighted the urgent need for further research into, and improved management of, current shark fisheries and their target and by-catch species. Such research is needed to arrest stock depletion and ensure the maintenance of biodiversity and the ongoing provision of ecosystem services (Heithaus et al. 2008).

1.2 Targeted shark fisheries in Australia

Sharks are actively targeted in Australian coastal waters by domestic, commercial fisheries employing a range of specialised harvest methods. Substantial increases in effort and catch have occurred in these fisheries in recent decades, coinciding with those observed in other regions of the world (Bonfil 1994, Barker & Schluessel 2005).

In tropical north-eastern Australia, neonate and small juvenile sharks are targeted off the east coast of Queensland (QLD) in the East Coast Inshore Finfish Fishery (ECIFF) (Harry et al. 2011a). Following a 200 % increase in shark landings between 1993 and 2004, this small-scale gillnet fishery accounted for ~1,084 tonnes (t) of shark in 2008; over 50 % (by number) of which was comprised of the carcharhiniform species *Carcharhinus tilstoni* (Australian blacktip shark), *Carcharhinus limbatus* (common blacktip shark), *Carcharhinus sorrah* (spot-tail shark), *Carcharhinus brevipinna* (spinner shark), *Sphyrna lewini* (scalloped hammerhead) and *Rhizoprionodon acutus* (milk shark) (Simpfendorfer et al. 2007, Anon. 2010, Bensley et al. 2010, Harry et al. 2011a). In 2009 an annual Total Allowable Commercial Catch (TACC) of 600 t was introduced for shark in the ECIFF as a means of limiting shark catch while uncertainty existed regarding the status of populations in the region (Anon. 2010).

Along Australia's northern coastline, sharks are landed via pelagic net and demersal longline in the recently formed Northern Territory Offshore Net and Line Fishery (NT ONLF) (Field et al. 2012, Tillett et al. 2012a). In addition to teleost species, this small-scale fishery targets neonate and small juvenile *C. tilstoni*, *C. limbatus* and *C. sorrah* in inshore waters, which together account for ~75 % (by number) of the fishery's total shark catch (Field et al. 2012). Fishery landings of these species have increased gradually from the time of the fishery's inception in 1983 to 2010 (Field et al. 2012), with fishery-dependent reporting indicating landings of 371 t of *C. tilstoni* and *C. limbatus* combined, and 86 t of *C. sorrah* in 2009 (Handley 2010).

In Western Australian waters, two geographically distinct demersal shark fisheries operate concurrently. Off the south-western coast, neonate and small juvenile *Carcharhinus obscurus* (dusky shark) are the primary target of a temperate gillnet fishery, with secondary-target species including *Furgaleus macki* (whiskery shark), *Mustelus antarcticus* (gummy shark), *Carcharhinus plumbeus* (sandbar or thickskin shark), *Galeorhinus galeus* (school

shark) and several species of squalid (deepwater spurdog) (Simpfendorfer & Donohue 1998). This fishery saw a 500 % increase in catch of *C. obscurus* (from ~100 to 600 t) over a ten-year period to the late 1980s prior to management input reducing and stabilising catch at ~300 $t \cdot year^{-1}$ (Simpfendorfer & Donohue 1998, McAuley 2006a). Off the tropical north-western coast, demersal longlines are used to target predominantly adult *C. plumbeus* and, to a lesser degree, *C. obscurus* (Simpfendorfer & Donohue 1998, McAuley 2006b). Combined catches from both fisheries revealed a > 300 % increase in *C. plumbeus* landings to 415 t between 1995 and 2004 (McAuley 2006a, 2006b).

Southern Australian waters are characterised by a demersal gillnet and longline fishery targeting *G. galeus* and *M. antarcticus* off the coasts of South Australia, Victoria and Tasmania; collectively termed the Southern Shark Fishery (SSF) (Walker 1999). Combined landings of both species in this fishery varied between 2,234 and 4,226 t during the period from 1970 to 2000, with *M. antarcticus* and *G. galeus* constituting 69 and 11 %, respectively, of the catch in the latter year (Pribac et al. 2005). Having experienced severe stock depletion following intense historic fishing pressure, the *G. galeus* resource has been locally assessed as overexploited (Punt et al. 2000, Walker et al. 2002). In contrast, stable catches and stock assessment modeling indicate that *M. antarcticus* is harvested sustainably at a level close to the maximum sustainable yield, and is widely referred to as one of the few examples of successful shark-fishery management (Walker 1998, Pribac et al. 2005). A fishery targeting *Carcharhinus brachyurus* (bronze whaler or copper shark) also operates in South Australian waters, with incidental catches of juvenile *C. obscurus* also recorded (Rogers et al. 2013).

Finally, off Australia's temperate east coast, recent years have seen the sudden expansion of a multi-species fishery targeting large, coastal and pelagic sharks in New South Wales (NSW) waters as part of the wider NSW Ocean Trap and Line Fishery (NSW OTLF) (Macbeth et al. 2009).

1.3 The NSW OTLF shark fishery

Commercial log-book records revealed a substantial increase in fishing effort for, and catches of, sharks in the mid-2000s by line fishers in the NSW OTLF. More specifically, the annual catch of sharks increased by 200 % (from 152 to 457 t) over a two-year period between 2004/05 and 2006/07 (Figure 1.1). These fishery-dependent data, however, were deficient in spatial and temporal resolution, as well as in species identification accuracy; the vast majority of the abovementioned catch increase having been reported by the fishers as 'Shark, Unspecified' (Macbeth et al. 2009).

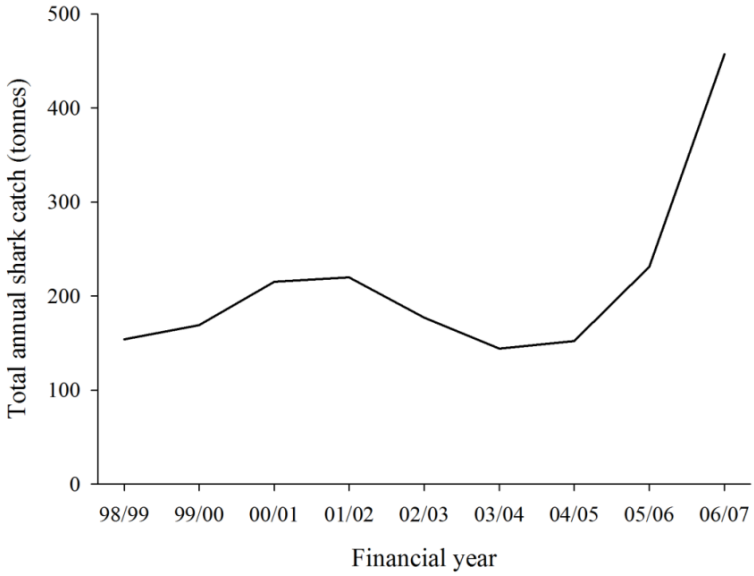

Figure 1.1 Historic trend in commercial shark catch in the New South Wales Ocean Trap and Line Fishery. Adapted from Macbeth et al. (2009).

To redress this lack of scientifically-robust operational and catch data, and also to address management concerns regarding shark by-catch composition and the sustainability of these increased fishing activities, an observer study was conducted onboard NSW OTLF shark-fishing vessels during 2008/09; continuing, albeit less-intensely, until 2011 (Macbeth et al. 2009). It was demonstrated that the elevated catch and effort indices emanating from the

fishery were the direct result of an increased and active targeting of large sharks – particularly carcharhiniform species – using demersal longlines in the waters off northern NSW (Figure 1.2). Sharks were being targeted primarily for the high value of their fins, although the dressed trunks (i.e. headed, gutted and finned carcass) were also being sold at low financial benefit (Macbeth et al. 2009).

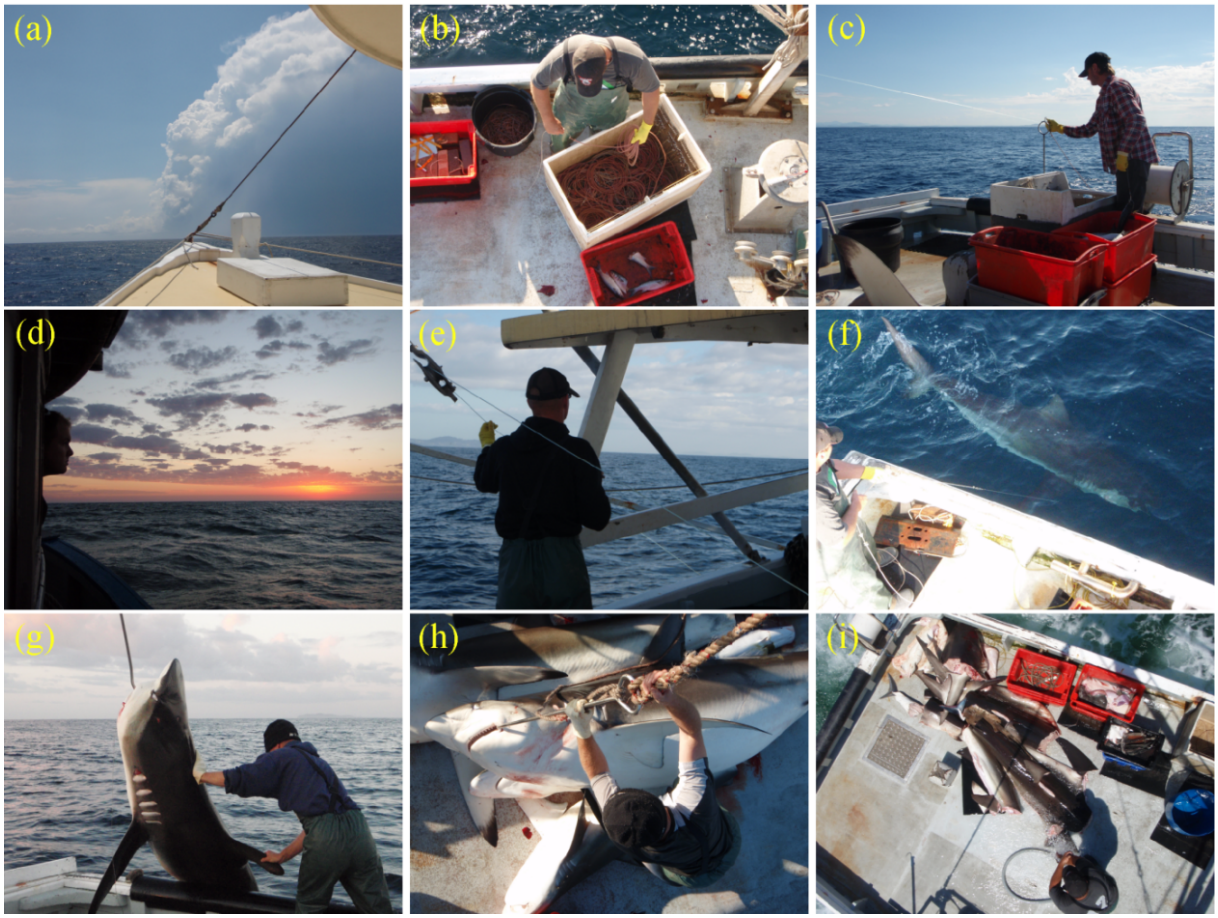

Figure 1.2 Demersal longline shark fishing in the New South Wales Ocean Trap and Line Fishery as observed during 2008/09: (a) afternoon trip to fishing grounds for overnight fishing gear set; (b) baiting branch lines; (c) deploying demersal longline; (d) searching for longline floats the following morning; (e) retrieval of longline; (f) captured dusky shark being manoeuvred alongside the vessel; (g) hoisting catch on-board; (h) arranging of catch for examination by scientific observer; and (i) dressed trunks (i.e. headed, gutted and trimmed carcasses with fins still attached) ready for landing. All photos by P. Geraghty.

The observer program demonstrated that the catch composition (by number) of the shark fishery was dominated by three species – *C. plumbeus, C. obscurus* and *C. brevipinna* – which together accounted for > 60 % of the total observed catch (Table 1.1, Figure 1.3). Furthermore, it was found that individuals spanning the entire size range of these species were being captured, but with a major focus on adult size classes for *C. obscurus* and *C. plumbeus* in particular (Figure 1.3). These catch distributions were most likely due to choice of fishing grounds with some, albeit lesser, influence from gear-selectivity.

The targeting of these species in NSW waters was cause for considerable management and conservation concern given the poor record of management for *C. plumbeus, C. obscurus* and *C. brevipinna* (but particularly the former two) on a global scale. Highly sought-after for their fins (Clarke et al. 2006), all three species represent important target and by-catch components of commercial and artisanal multi-species shark fisheries across the globe (e.g. Bonfil 1997, Amorim et al. 1998, Castillo-Géniz et al. 1998, McVean et al. 2006, Henderson et al. 2007, White 2007, Morgan et al. 2009, Manojkumar et al. 2012, Moore et al. 2012). In areas such as the north-west Atlantic, intense fishing mortality led to the collapse of the large, coastal shark fishery off the east coast of the U.S. (Musick et al. 1993, Anon. 1997), for which various datasets suggest population declines of up to 64–99 % in the two primary target species – *C. obscurus* and *C. plumbeus* (Anon. 2006a, Cortés et al. 2006, Myers et al. 2007, Baum & Blanchard 2010). Consequently, a complete prohibition on the landing of *C. obscurus* in US Atlantic waters was implemented in 2000 (Cortés et al. 2006, Anon. 2011a, Hale et al. 2011), as well as a prohibition on the commercial landing of *C. plumbeus* in 2007, unless participating in a special research fishery (Morgan & Carlson 2010, Anon. 2011b). While these same management controls remain in effect today, *C. obscurus* remains IUCN listed as 'endangered' in the north-west Atlantic and 'vulnerable', along with *C. plumbeus*, globally (Musick et al. 2009a, 2009b).

Table 1.1 Catch composition of observed shark-fishing trips in New South Wales waters as recorded from the 2008/09 NSW OTLF commercial shark-fishing observer project. Only species representing ≥ 1 % (by number) of the overall observed catch are shown. Adapted from Macbeth et al. (2009).

Common name	Scientific name	Proportion of overall observed catch (%)
Sandbar shark	*Carcharhinus plumbeus*	34.8
Dusky shark	*Carcharhinus obscurus*	15.2
Spinner shark	*Carcharhinus brevipinna*	10.6
Blacktip shark complex [a]		6.4
Tiger shark	*Galeocerdo cuvier*	5.9
Smooth hammerhead	*Sphyrna zygaena*	4.3
Smooth stingray	*Dasyatis brevicaudata*	4.3
Scalloped hammerhead	*Sphyrna lewini*	3.2
Spotted wobbegong	*Orectolobus maculatus*	1.5
Bronze whaler	*Carcharhinus brachyurus*	1.4
Cobia	*Rachycentron canadum*	1.4
Black stingray	*Dasyatis thetidis*	1.2
Shortfin mako	*Isurus oxyrinchus*	1.0
Port Jackson shark	*Heterodontus portusjacksoni*	1.0

[a] Includes *Carcharhinus limbatus*, *Carcharhinus tilstoni* & hybrids thereof (Morgan et al. 2012)

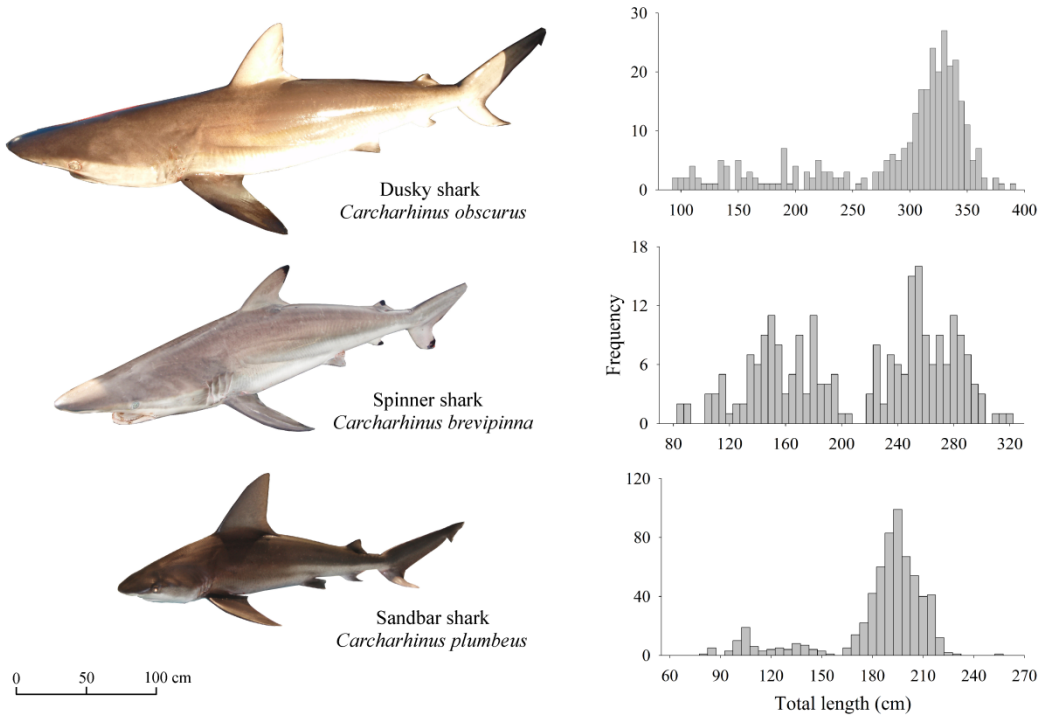

Figure 1.3 (Left) The three primary target shark species of the NSW OTLF and their relative attainable sizes in New South Wales waters as recorded during the observer program; (right) species-specific length-frequencies derived from fishery-observer catch data. Original photos by P. Geraghty.

Within this context, and amid lingering concerns regarding sustainable rates of harvest for *C. plumbeus* and *C. obscurus* in the waters of western Australia (McAuley et al. 2007a), a precautionary management approach was implemented in NSW waters. Specific conditions and restrictions were imposed upon shark fishing in the NSW OTLF (targeted and otherwise) in 2009. These included a TACC of 160 t (processed weight) for large shark species (Carcharhinidae, Sphyrnidae and Lamnidae – but excluding the protected *Carcharodon carcharias*), as well as daily catch and by-catch limits (Macbeth et al. 2009). Of the overall TACC, 100 t were designated to the catch of *C. plumbeus* via a restricted permit system; the remaining 60 t were assigned to non-permit holders for the catch of all other TACC shark species combined (i.e. excluding sandbar sharks) (Macbeth et al. 2009). Calibrated from unsustainable yields of *C. plumbeus* defined elsewhere, these management controls were designed to be conservative in the absence of locally-derived biological parameters necessary for accurate stock assessment.

1.4 Thesis rationale, objectives & structure

Knowledge of the local stock structure, spatial dynamics and biology of targeted species provides an essential framework for effective natural resource assessment and management (Welch et al. 2011). Moreover, the sustainable and rational use of a resource relies on rates of harvest being commensurate with the biological productivities of the target species (Walker 2005a). As such, commercial importance and cosmopolitan distributions have led to considerable research on the abundance, age and growth, behaviour, reproduction, habitat, diet, mortality, population status, movement, demography and genetic stock structure of *C. plumbeus*, *C. obscurus* and *C. brevipinna* in many parts of the world, including regions of Australia (e.g. for *C. plumbeus* – Casey et al. 1985, Casey & Natanson 1992, Heist et al. 1995, Joung & Chen 1995, Sminkey & Musick 1995, 1996, Carlson 1999, Heist & Gold

1999, Brewster-Geisz & Miller 2000, Merson & Pratt 2001, Joung et al. 2004, Thorpe et al. 2004, McAuley et al. 2005, 2006, 2007a, 2007b, 2007c, Saïdi et al. 2005, Torres et al. 2005, McElroy et al. 2006, Romine et al. 2006, Conrath & Musick 2007, 2008, Daly-Engel et al. 2007, Grubbs et al. 2007, Hazin et al. 2007, Portnoy et al. 2007, 2009, 2010, White 2007, Diatta et al. 2008, Hale & Baremore 2010, Andrews et al 2011, Anon. 2011b, Baremore & Hale 2012, Driggers et al. 2012). These studies revealed strong K-selected life-history traits typical of large predatory sharks in all three species (Musick 1999, Cortés 2000) as well as an affinity for shallow inshore waters for early development. Such characteristics confer a high vulnerability and low resilience to fishing mortality and, in turn, a propensity for rapid population decline and slow rates of recovery (Smith et al. 1998, Musick 1999, Cortés 2000, 2002, Stevens et al. 2000, Field et al. 2009). Given the practical issues associated with the robust sampling of highly-vagile marine taxa, however, many such studies were either limited by small sample sizes and/or gear-selectivity generated sample biases that compromised the accuracy of the reported biological parameters.

In spite of their documented vulnerability, poor global track-records of management (Musick et al. 1993, McAuley et al 2007a) and commercial targeting in the region (Macbeth et al. 2009), robust biological parameters for *C. plumbeus*, *C. obscurus* and *C. brevipinna* in temperate eastern Australian waters are conspicuously lacking. Such information is critical if assessments of their status off Australia's southeast coast are to be made and appropriate controls to underpin their sustainable management are to be developed (Cortés et al. 2006, McAuley et al. 2005, 2007a).

The over-arching objective of this thesis, therefore, was to provide robust demographic parameters for *C. plumbeus*, *C. obscurus* and *C. brevipinna* specific to the waters off Australia's NSW coast (Figure 1.4) and to elucidate their genetic population structures over a range of spatial scales; the view being for this information to be used in qualitative

evaluations of the susceptibilities of these commercially-important species to overexploitation in south-eastern Australia, and the identification of appropriate spatial scales of management.

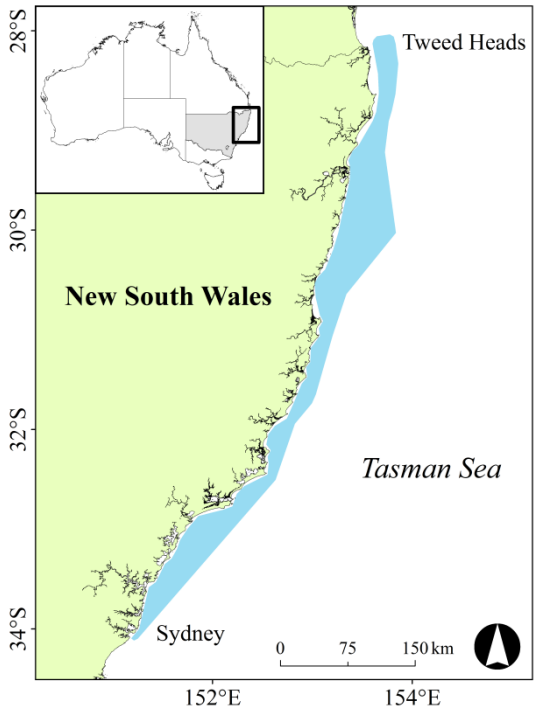

Figure 1.4 Primary study area and fishing/sampling zone (shaded blue).

In attempting to achieve this objective I have compiled the following data chapters, with the inclusion of an additional chapter describing a novel method of shark vertebral ageing developed during this study:

Chapter 2 presents a comparative assessment of genetic diversity in *C. obscurus* and *C. plumbeus* in south-eastern Australian waters using unprecedentedly high sample numbers, and examines the geographic extent of genetic homogeneity in *C. obscurus* in the Indo-Australian region. In addition to providing information relevant to the allocation of potential management units, this chapter informs on the comparative resilience of these two species to a loss of genetic diversity.

Chapter 3 quantifies genetic diversity as well as broad and fine-scale population structuring in *C. brevipinna* in Australian and Indian Ocean waters. This chapter hypothesises

on the evolutionary history of this species and the mechanisms responsible for the observed patterns of genetic diversity. As in the preceding chapter, the strengths and limitations of our findings are thoroughly assessed via novel rarefaction and random sub-sampling simulations analyses, and basic estimates of observer accuracy in the identification of the three target species within the NSW OTLF are established.

Chapter 4 describes the use of micro-computed tomography as a valid, non-destructive method of vertebral growth band visualisation for shark ageing purposes. We evaluate the advantages and disadvantages offered by this technique and provide a direct comparison with the most widely employed method.

Chapter 5 investigates the age and growth characteristics of *C. obscurus*, *C. brevipinna* and *C. plumbeus* in NSW waters. We compare longevity and modelled growth parameters among these species within the study area, and discuss our findings in relation to those reported by previous studies for conspecific populations.

Chapter 6 examines a range of aspects of the reproductive biology of the study species off the south-east coast of Australia. As in the preceding chapter, our results are presented in the context of previous works, thereby highlighting the importance of locally-derived demographic parameters for accurate population modelling.

Chapter 7 synthesises the main findings emanating from this thesis focusing on implications for the abilities of these species to withstand stock decline in the study region and the delineation of potential management units. This study therefore provides information directly relevant to the management and conservation of these species in NSW waters and beyond.

CHAPTER 2. Genetic Structure and Diversity of Two Highly Vulnerable Carcharhinids in Australian Waters

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Plate 3. A sandbar shark (*Carcharhinus plumbeus*) caught commercially via demersal longline off the northern New South Wales coast.

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2.1 Abstract

Molecular techniques were employed to investigate genetic structure and diversity in dusky (*Carcharhinus obscurus*) and sandbar (*Carcharhinus plumbeus*) sharks in the Indo-Australian region. Tissue samples of 423 *C. obscurus* and 442 *C. plumbeus* defined 18 and 11 mtDNA ND4 haplotypes, respectively. For *C. obscurus*, weak genetic differentiation was detected between east and west Australian waters (pairwise $\Phi_{ST} = 0.04437$, $p < 0.008$; pairwise $F_{ST} = 0.02403$, $p < 0.035$), suggesting the delineation of two independent populations, while patterns of gene flow between Australia and Indonesia were inconclusive. Rarefaction analysis indicated that robust population comparisons in these species were reliant on sample numbers > 100 at any particular location. Off Australia's east coast, *C. plumbeus* and *C. obscurus* exhibited strong similarities in genetic structure – suggestive of similar evolutionary histories in the region. In addition, genetic validation revealed observers to be highly accurate in the identification of both target species in an eastern Australian shark fishery. Our findings contribute valuable information for the management and conservation of both species.

2.2 Introduction

Topographic, biological and oceanographic limitations to dispersal result in reproductive isolation between groups of individuals. Over evolutionary time, this cessation of (or restriction to) gene exchange leads to quantifiable genetic differentiation as a result of intrinsic natural selection, genetic drift and mutation (e.g. Riginos & Nachman 2001, Hazlitt et al. 2006). In a context of increasing anthropogenic pressures, the identification of barriers to gene flow can assist with the conservation and management of a species' genetic diversity, which is an essential store of variety to meet future environmental challenges. This is

especially pertinent for taxa demonstrably vulnerable to human-induced population decline e.g. elasmobranchs (Stevens et al. 2000, Field et al. 2009).

Sharks have a demonstrated susceptibility to overexploitation on the basis of their life-history traits and a vulnerability to multiple fishing gears (Cortés 2000, Stevens et al. 2000). Recent global increases in commercial-fishing effort for sharks have resulted in grave population declines (Baum et al. 2003, Ferretti et al. 2008). While magnitudes of stock depletion are disputed (Burgess et al. 2005), there is international agreement regarding the urgent need for the effective management of shark fisheries to address issues of conservation and cascading ecological impacts catalysed by apex predator removal (Barker & Schluessel 2005, Myers et al. 2007, Ferretti et al. 2010).

The dusky shark (*Carcharhinus obscurus*) and the sandbar shark (*Carcharhinus plumbeus*) are two large-medium carcharhinid species widely regarded as among the most vulnerable of sharks to overfishing. As long-lived, late-maturing species of decidedly low productivity (e.g. Simpfendorfer et al. 2002, Dudley et al. 2005, McAuley et al. 2006, Baremore & Hale 2012), demographic analyses have reported abilities to withstand only very modest levels of fishing mortality in conjunction with slow rates of population increase (Sminkey & Musick 1996, Smith et al. 1998, McAuley et al. 2007a, Romine et al. 2009).

Nevertheless, being highly sought after for their fins (Clarke et al. 2006), both species are captured in commercial and artisanal fisheries across much of their respective cosmopolitan ranges (e.g. Amorim et al. 1998, Castillo-Géniz et al. 1998, McVean et al. 2006, White 2007, Morgan et al. 2009), with poor records of management in some regions. In particular, *C. plumbeus* and *C. obscurus* were subject to intense targeted harvest pressure in the now collapsed large, coastal shark fishery off the east coast of the U.S, where various datasets suggest population declines of up to 64–99 % in both species (Cortés et al. 2006, Myers et al. 2007, Baum & Blanchard 2010). As a result of these directed fishing activities, both sharks

are globally IUCN listed as 'vulnerable' and *C. obscurus* as 'endangered' in the north-west Atlantic (Musick et al. 2009a, 2009b).

Carcharhinus obscurus and *C. plumbeus* are also important components of commercial shark landings in Australian waters (Simpfendorfer & Donohue 1998, Macbeth et al. 2009). Dramatic increases in catches off both east and west coasts led to considerable concern regarding their sustainability under harvest pressure in the region (McAuley et al. 2007a, Macbeth et al. 2009), and emphasised the need for effective management input to arrest further stock decline.

Genetic techniques are useful tools for addressing shark fishery management issues. Population genetic analyses can help identify appropriate scales of management by investigating contemporary patterns of gene flow, genetic diversity and the spatial structure of stocks (Dudgeon et al. 2012). Carcharhiniformes are the most represented of the elasmobranchs in the population genetic literature, but few have been examined in any detail (Dudgeon et al. 2012). These studies have typically focused on elucidating genetic structure over broad spatial scales, consistently demonstrating large oceanic expanses to be robust barriers to gene flow (Duncan et al. 2006, Keeney & Heist 2006, Benavides et al. 2011a), including in *C. obscurus* (Benavides et al. 2011b) and *C. plumbeus* (Portnoy et al. 2010). Genetic subdivision on finer scales has also been reported for some shark species, raising important implications for regional fisheries management (Keeney et al. 2003, Karl et al. 2011, Tillett et al. 2012b, 2012c, Whitney et al. 2012).

Previous investigations of genetic structure in *C. obscurus* and *C. plumbeus* in Australian and neighbouring waters have yielded a variety of results. Portnoy et al. (2010) observed genetic subdivision between east and west Australia in *C. plumbeus* based on mitochondrial DNA (mtDNA), while Ovenden et al. (2009) and Benavides et al. (2011b) reported evidence for genetic homogeneity between the same two regions in *C. obscurus*. Ovenden et al. (2009)

also raised the possibility of limited dispersal across the Timor Trench in the latter species through a finding of genetic differentiation between western Australia and central Indonesia. However, the strength of the abovementioned findings was generally limited due to small sample sizes. Given their vulnerability to population decline, therefore, we believed that a more detailed assessment of genetic structure was warranted for these two species.

Using mtDNA NADH dehydrogenase subunit 4 (ND4) sequence data we re-assessed the genetic structure of *C. obscurus* on a regional scale, testing a null hypothesis of genetic homogeneity in Indo-Australian waters, and investigated the genetic structure of *C. plumbeus* for the first time off the east coast of Australia. We also applied these genetic data in establishing basic estimates of observer accuracy in an east Australian shark fishery, and explored the implications of our findings for the management and conservation of both species.

2.3 Materials & methods

2.3.1 Sample collection

Shark tissues were collected from a range of locations in Indo-Australian waters (Figure 2.1), focusing on a harvested population off Australia's east coast. Tissues were sampled from New South Wales (NSW) waters during 2007–2010 from landed-catch by observers on-board commercial shark-fishing vessels within the NSW Ocean Trap and Line Fishery (NSW OTLF). A small quantity (< 2 g) of white muscle tissue was excised from each specimen, immediately preserved in 95 % reagent grade ethanol, and stored at room temperature. Additional samples, collected during 2000–2012, were obtained from more distant locations, including *C. obscurus* and *C. plumbeus* samples from waters of the Northern Territory (NT) in Australia, as well as *C. obscurus* samples from Western Australia (WA) and Indonesia. Samples from NT and WA were collected from landed-catch by observers within their

respective commercial shark fisheries, and preserved in 20 % dimethylsulphoxide (DMSO) solution and 70 % ethanol respectively. Samples from Indonesia were collected from landed-catch by a fisheries biologist at the Tanjung Luar local market in eastern Lombok, and preserved in DMSO; exact capture locations were not confirmed. Additional *C. obscurus* tissues were obtained from NSW waters by sampling sharks caught in the NSW Shark Meshing (Bather Protection) Program (Reid et al. 2011). Tissues from NSW and NT were sampled from predominantly adult and sub-adult individuals, while those from WA were sampled from mostly small juveniles. Tissues from Lombok were sampled from processed trunks for which associated length measurements were unavailable.

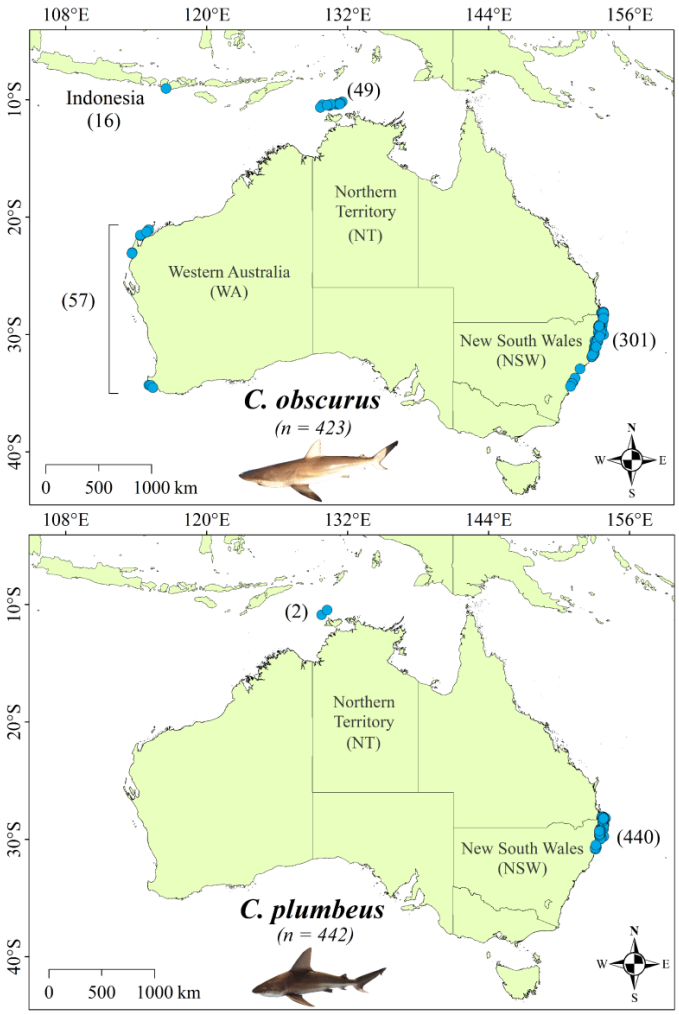

Figure 2.1 Collection locations and sample sizes (in brackets) for *Carcharhinus obscurus* and *Carcharhinus plumbeus* tissues included in genetic diversity and structure analyses.

2.3.2 DNA extraction, amplification and sequencing

To obtain mtDNA sequence data, total genomic DNA was first extracted from 5 mg of each tissue using a modified salting-out protocol (Sunnucks & Hales 1996). Samples were digested with 10 µl of Proteinase-K (10 mg·ml^{-1}) in 580 µl of TNES [50 mM Tris.HCl (pH 7.5), 400 mM NaCl, 20 mM EDTA and 0.5 % SDS] by incubation overnight at 55 °C. Proteins were precipitated by adding 170 µl of 5 M NaCl followed by microcentrifugation at 14,000 rpm for 5 min. Supernatant (600 µl) was recovered into a fresh tube and the DNA precipitated by adding 600 µl of ice-cold 100 % absolute ethanol. Tubes were stored at −20 °C for approximately 1 h. DNA was then recovered by microcentrifugation at 14,000 rpm for 15 min, and the ethanol decanted. The resulting DNA pellet was washed with 200 µl of 70 % ethanol, 100 mM sodium acetate solution, and microcentrifuged at 14,000 rpm for 3 min. Following decanting, all remaining ethanol was removed using a micropipette. DNA was air-dried, resuspended in 100 µl of TE buffer [10 mM Tris.HCl (pH 7.6) and 1 mM EDTA] and stored at −20 ^{o}C. DNA yield was checked on a 1.0 % agarose TBE (1×) gel, run at 110 V, and stained with GelRed (Biotium Inc.).

Polymerase Chain Reaction (PCR) was then used to amplify the mitochondrial ND4 gene from all tissue samples. This gene was selected for analysis following Dudgeon et al. (2009) and Ovenden et al. (2010) who demonstrated the ND4 gene to be the most polymorphic among a range of mtDNA markers (including the control region) in species related to those under study here. PCR reactions were carried out in 50 µl volumes containing 1 µl of DNA template, 1× GoTaq Colourless reaction buffer [consisting 1.5 mM $MgCl_2$ and 200 µM deoxynucleoside triphosphates (dNTPs)] (Promega), 0.5 µl of RNase (1 mg·ml^{-1}), and 0.5 µM of each of the primers ND4 (5’ CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC) (Arèvalo et al. 1994) and H12293-LEU (5’ TTG CAC CAA GAG TTT TTG GTT CCT AAG ACC) (Inoue et al. 2001). Amplifications were performed in an Eppendorf ep gradient S

Mastercycler (Eppendorf), using thermal cycling conditions consisting of an initial denaturation (94 °C for 3 min) followed by 35 cycles of 94 °C for 15 s, 60 °C for 30 s and 72 °C for 1 min, with a final extension step of 72 °C for 10 min, and held at 4 °C. PCR products were visualised on a 2.0 % agarose TBE (1×) gel, run at 110 V, and stained as above. PCR products were purified prior to sequencing using Exosap-IT (USB Corporation). Sequencing was performed with an Applied Biosystems 3130xl Genetic Analyzer 16-array capillary sequencer (Life Technologies), with sequencing reactions and analyses being carried out by the Macquarie University (MQ) DNA Sequencing Facility using Big Dye Terminator reactions and the forward PCR primer only.

2.3.3 Sequence alignment & ID validation

Sequences were trimmed and edited by eye. Edited sequences were entered into Biomanager (https://biomanager.info) and aligned using the ClustalW (accurate) algorithm (Thompson et al. 1994). No GenBank ND4 reference sequences were available for *C. obscurus* or *C. plumbeus* prior to this study. To validate that the two study species had been correctly identified, and to determine the species identity of any misidentified individuals, randomly-selected representatives from each separate haplotype determined from the alignment output were amplified for the mitochondrial cytochrome oxidase I (CO1) gene using the primers Fish F1 (5' TCA ACC AAC CAC AAA GAC ATT GGC AC) and Fish R1 (5' TAG ACT TCT GGG TGG CCA AAG AAT CA) (Ward et al. 2005). PCRs were carried out as above, with thermal cycling conditions consisting of an initial denaturation (95 °C for 5 min), followed by 30 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 1 min, with a final extension step of 72 °C for 7 min, and held at 4 °C. PCR products were purified and sequenced following the same protocol outlined above for the ND4 locus. Resultant CO1 sequences were compared to reference sequences in GenBank for species recognition.

2.3.4 ND4 sequence analysis

To identify and characterise mitochondrial haplotypes, aligned ND4 *C. obscurus* and *C. plumbeus* sequences were imported to Arlequin 3.5.1.2 (Excoffier & Lischer 2010). A sequence representing each haplotype was lodged in GenBank (Accession codes KJ004523 – KJ004551). The frequency of, and mutational steps between, haplotypes was assessed by generating statistical parsimony haplotype networks in TCS 1.21 using the default settings (Clement et al. 2000). Phylogenetic relationships among haplotypes were inferred using a maximum likelihood phylogram (or phylogenetic tree) based on the Tamura-Nei model (Tamura & Nei 1993), and generated in MEGA 5 (Tamura et al. 2011) with 1,000 bootstrap replicates. The best-fitting model of nucleotide substitution, as offered by MEGA 5, was determined by likelihood ratio tests and calculations of Akaike and Bayesian Information Criteria performed in jModelTest 2.1.1 (Darriba et al. 2012). To assess the ability of the ND4 region to differentiate between carcharhinids, the phylogram was rooted with a range of morphologically-similar species, as well as with two sphyrnid species as outgroups. Genetic diversity indices were also obtained with Arlequin using the Tamura-Nei substitution model (Tamura & Nei 1993), and included polymorphism statistics, number of haplotypes, haplotype diversity (h) and nucleotide diversity (π).

2.3.5 Rarefaction analysis

To determine whether sample sizes adequately represented population genetic variation, rarefaction exact curves were generated to qualitatively assess the proportion of haplotypic diversity sampled at each location for both *C. obscurus* and *C. plumbeus*. The expected number of haplotypes found for a given sample number was calculated using the rarefaction formula of Hurlbert (1971), and executed in the statistical package R (R Development Core Team 2010). A trend towards an asymptotic relationship infers haplotype saturation,

suggesting that the majority of the available genetic diversity was likely sampled at that location and that more intensive sampling is likely to yield few additional haplotypes. In contrast, a steep slope suggests that a large fraction of the available haplotype diversity remains unsampled.

2.3.6 **Carcharhinus obscurus** ***genetic structure***

Appropriate samples were available for one species (*C. obscurus*) to test a null hypothesis of panmixia (genetically homogeneity) in Indo-Australian waters. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) was implemented in Arlequin to evaluate the overall extent of genetic subdivision between sampling locations. We employed two *F*-statistic metrics of genetic divergence: Φ_{ST} (Excoffier et al. 1992) and F_{ST} (Wright 1965). While Φ_{ST} has been regarded as the superior metric on the basis of its incorporation of a measure of genetic distance between haplotypes, frequency based F_{ST} has been proposed as potentially a more appropriate measure of genetic differentiation among locations where migration is theoretically occurring at a faster rate than mutation (Bird et al. 2011). Φ_{ST} was calculated via the computing of a distance matrix using the Tamura-Nei model (Tamura & Nei 1993) for estimation of genetic distance between sequences, while F_{ST} used haplotype frequencies only. AMOVA partitioned genetic variance among, and within, populations and calculated Φ_{ST} and F_{ST} fixation indices. Genetic differentiation between sample locations was also measured by calculating pairwise Φ_{ST} and F_{ST} estimates. Statistical significance was determined following 20,000 permutations of the sequence data and, in the case of pairwise Φ_{ST} and F_{ST}, assessed at an initial critical significance level of $\alpha = 0.0083$ (adjusted from $\alpha = 0.05$) following sequential Bonferroni correction for six, simultaneous comparisons (Holm 1979). The AMOVA structure consisted of one group made up of the following four putative

populations: NSW ($n = 301$), NT ($n = 49$), WA ($n = 57$) and Indonesia ($n = 16$) (Figure 2.1). The analysis outlined above is henceforth referred to as the 'original analysis'.

Carcharhinus obscurus sample sizes were strongly biased towards NSW, where sampling intensity was an order of magnitude greater than at the remaining three locations (Figure 2.1). We evaluated the influence of this sampling bias on the *F*-statistics of pairwise population comparisons involving NSW via random re-sampling simulations. Ten thousand replicate random sample sets of $n = 100$, $n = 50$ and $n = 16$ (for comparison with Indonesia only) were selected without replacement from the NSW population, while NT, WA and Indonesian sample sizes were kept unchanged. Population pairwise Φ_{ST} and associated *p* values were generated for each replicate random sample set in Arlequin using the batch processing function and permutation settings as outlined above. Resultant Φ_{ST} and *p* value distributions were plotted, and the likelihood of producing a contradictory result to that of the original analysis was calculated as either the percentage of *p* values ≤ 0.05 or > 0.05, depending on the outcome of the original analysis.

2.4 Results

2.4.1 Fishery-observer accuracy in NSW waters

The ND4 gene region proved to be an excellent marker for carcharhinid species recognition (Figure 2.2), as also shown by Tillett et al. (2012a), hence confirming its suitability for use in the present study.

Genetic validation was possible for a total of 296 sharks visually identified by scientific observers as *C. obscurus* in the NSW OTLF from 2007–2010. Of these, 286 were genetically confirmed to be *C. obscurus*, translating to an observer-accuracy estimate of 96.6 % for the identification of this species in the fishery (Table 2.1). Misidentified individuals ($n = 10$) were all of adult size and represented six different carcharhinid species (Table 2.1).

Genetic validation was possible for a total of 487 sharks visually identified by scientific observers as *C. plumbeus* in this same fishery over the same temporal period. Of these, 484 were genetically confirmed to be *C. plumbeus*, translating to an observer-accuracy estimate of 99.4 % for the identification of this species in the NSW OTLF (Table 2.1). Misidentifications (n = 3) once again were all of adult size and comprised three different carcharhinid species (Table 2.1). Overall observer accuracy was estimated at 98.3 % for the identification of these two target species combined.

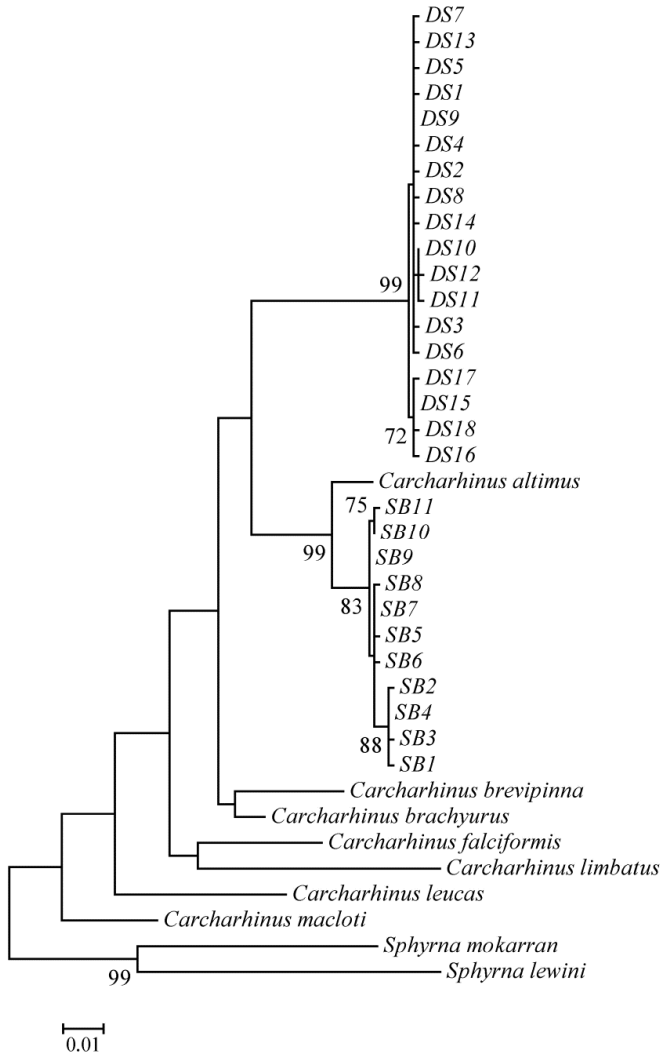

Figure 2.2 Inferred phylogenetic maximum likelihood tree for *Carcharhinus obscurus* (DS1–18) and *Carcharhinus plumbeus* (SB1–11) mtDNA ND4 haplotypes from Australian and Indonesian waters. Nodal bootstrap support is displayed where ≥ 70 %. Scale represents the proportion of polymorphic sites between haplotypes.

Table 2.1 Percentage (individual counts in parentheses) of each genetically-identified shark species from observer-identified *Carcharhinus obscurus* and *Carcharhinus plumbeus* in the New South Wales Ocean Trap and Line Fishery. Total lengths (cm) for misidentified individuals are displayed.

Genetic identification	Observer identification and measurement			
	C. obscurus (*n* = 296)	Mis-ID L_T[1]	*C. plumbeus* (*n* = 487)	Mis-ID L_T[1]
C. obscurus	96.6 (286)		0.2 (1)	302
C. plumbeus	0.3 (1)	210	99.4 (484)	
C. falciformis	1.0 (3)	235, 242, 256	0.2 (1)	214
C. leucas	0.7 (2)	220, 293		
C. limbatus	0.7 (2)	252, 254	0.2 (1)	208
C. brevipinna	0.3 (1)	276		
C. altimus	0.3 (1)	269		

[1] Total length (L_T, cm)

2.4.2 Genetic diversity & summary statistics

2.4.2.1 *Carcharhinus obscurus*

An 857 base pair mtDNA ND4 sequence was obtained for 423 *C. obscurus* individuals collected from Australian and Indonesian waters (Figure 2.1). A total of 18 haplotypes were defined, characterised by 18 polymorphic sites composed of 15 transitions and 3 transversions (Supplementary material). Phylogenetic analysis placed these haplotypes into two shallow clades (Figure 2.2). Two haplotypes (DS9 and, to a lesser degree, DS15) dominated the sample set, and were common at all four locations (Table 2.2a). Overall haplotype (h) and nucleotide (π) diversities were moderate and low, respectively (h = 0.5150, π = 0.0012) (Table 2.3). Notwithstanding sample-size differences, the greatest number of haplotypes (n = 12) was found in NSW waters, of which 5 were unique to the area (Table 2.3). Ten haplotypes were found in WA waters, 3 of which were unique, and 5 haplotypes were found in both NT and Indonesia, each exhibiting 1 unique haplotype. Haplotype and nucleotide diversities ranged across the putative populations; Indonesia displayed the highest diversity values (h = 0.7500,

π = 0.0016) and NT the lowest (h = 0.3520, π = 0.0008). Standard deviation estimates, however, rendered differences in diversity between the locations impossible to discern (Table 2.3).

Table 2.2 Mitochondrial DNA ND4 haplotype relative frequencies observed from putative populations in Indo-Australian waters for (a) *Carcharhinus obscurus* and (b) *Carcharhinus plumbeus*.

(a)

Haplotype	Relative frequency				GenBank Accession Codes
	NSW (n = 301)	NT (n = 49)	WA (n = 57)	Indonesia (n = 16)	
DS1	–	–	0.018	–	KJ004534
DS2	–	–	0.018	–	KJ004535
DS3	–	–	0.053	–	KJ004536
DS4	–	–	–	0.063	KJ004537
DS5	–	0.020	–	0.063	KJ004538
DS6	–	0.020	–	–	KJ004539
DS7	0.003	–	0.018	–	KJ004540
DS8	0.010	–	0.018	–	KJ004541
DS9	0.648	0.796	0.702	0.438	KJ004542
DS10	0.040	–	0.053	0.188	KJ004543
DS11	0.007	–	0.018	–	KJ004544
DS12	0.003	–	–	–	KJ004545
DS13	0.003	–	–	–	KJ004546
DS14	0.020	–	–	–	KJ004547
DS15	0.239	0.143	0.070	0.250	KJ004548
DS16	0.003	–	–	–	KJ004549
DS17	0.013	0.020	0.035	–	KJ004550
DS18	0.010	–	–	–	KJ004551

(b)

Haplotype	Relative frequency		GenBank Accession Codes
	NSW (n = 440)	NT (n = 2)	
SB1	0.011	–	KJ004523
SB2	0.014	–	KJ004524
SB3	0.005	–	KJ004525
SB4	0.841	1.000	KJ004526
SB5	0.005	–	KJ004527
SB6	0.002	–	KJ004528
SB7	0.102	–	KJ004529
SB8	0.009	–	KJ004530
SB9	0.005	–	KJ004531
SB10	0.005	–	KJ004532
SB11	0.002	–	KJ004533

2.4.2.2 *Carcharhinus plumbeus*

An 857 base pair mtDNA ND4 sequence was obtained for 442 *C. plumbeus* individuals collected from eastern and northern Australian waters (Figure 2.1). A total of 11 haplotypes were defined, characterised by 12 polymorphic sites composed exclusively of transitions (Supplementary material). Phylogenetic analysis placed these haplotypes into two shallow clades (Figure 2.2). Two haplotypes (SB4 and, to a far lesser degree, SB7) dominated the sample set (Table 2.2b). Overall haplotype and nucleotide diversities were low for *C. plumbeus*, at 0.2814 and 0.0009 respectively (Table 2.3). No unique haplotypes were found amongst the two NT samples, with both being the most common haplotype SB4 (Table 2.2b). Given the low sample size from NT, this location was henceforth excluded from further analyses, with detailed investigations focusing exclusively on eastern Australian (NSW) waters.

Table 2.3 Genetic diversity indices observed in the mitochondrial DNA ND4 region for *Carcharhinus obscurus* and *Carcharhinus plumbeus* sample locations from Australian and Indonesian waters.

Location	n [a]	n_H [b]	n_{Hq} [c]	h [d]	π [e]
			C. obscurus		
NSW	301	12	5	0.5224 (± 0.027)	0.0012 (± 0.0009)
NT	49	5	1	0.3520 (± 0.080)	0.0008 (± 0.0007)
WA	57	10	3	0.5031 (± 0.080)	0.0010 (± 0.0008)
Indonesia	16	5	1	0.7500 (± 0.078)	0.0016 (± 0.0012)
Pooled	423	18	•	0.5150 (± 0.025)	0.0012 (± 0.0009)
			C. plumbeus		
NSW	440	11	•	0.2826 (± 0.027)	0.0009 (± 0.0008)
NT [1]	2	1	•	•	•
Pooled	442	11	•	0.2814 (± 0.027)	0.0009 (± 0.0008)

[a] Sample size (n), [b] number of haplotypes (n_H), [c] number of unique haplotypes (n_{Hq}), [d] haplotype diversity (h), [e] nucleotide diversity (π). [1] Diversity indices not available for *C. plumbeus* from NT (n = 2); both samples were the same haplotype. Values in parentheses represent standard deviations (s.d.). (•), value not applicable.

2.4.3 Rarefaction & optimum sample size

Rarefaction exact curves indicated trends towards asymptotic relationships for the NSW populations in both *C. obscurus* and *C. plumbeus* (Figure 2.3), suggesting the majority of the available haplotypic diversities were likely sampled at this location in both species. Steep slopes, however, were observed for the remaining three *C. obscurus* populations (Figure 2.3), indicating that a proportion of the available genetic diversities were unsampled. These analyses suggest that sample sizes in excess of 100 are required to adequately represent levels of genetic variation in any given *C. obscurus* or *C. plumbeus* population in Indo-Australian waters.

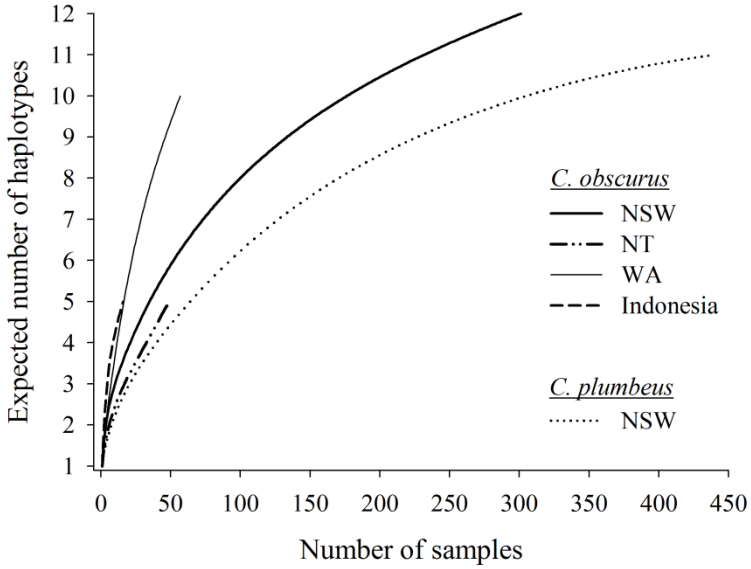

Figure 2.3 Rarefaction exact curves for *Carcharhinus obscurus* and *Carcharhinus plumbeus* collection locations in Australian and Indonesian waters.

2.4.4 **Carcharhinus obscurus** *regional genetic structure*

A haplotype network incorporating the four putative populations of *C. obscurus* demonstrated the presence of two shallow clades centred on the two most common haplotypes DS9 and DS15, both of which were shared between all four sample locations (Figure 2.4). Low-frequency variants shared between, and unique to, locations were also present. AMOVA

fixation indices detected significant levels of differentiation between putative populations for both *F*-statistic metrics (Φ_{ST} = 0.02462, $p < 0.03$; F_{ST} = 0.02723, $p < 0.01$) (Table 2.4). We therefore rejected the null hypothesis that *C. obscurus* are panmictic in Indo-Australian waters. Pairwise comparisons revealed weak genetic subdivision between eastern and western Australia – significant after sequential Bonferroni adjustment for Φ_{ST} only (NSW v WA; Φ_{ST} = 0.04437, $p < 0.008$; F_{ST} = 0.02403, $p < 0.05$) (Table 2.5). Evidence for weak differentiation between NT and Indonesia (F_{ST} = 0.13925, $p < 0.05$) and between WA and Indonesia (F_{ST} = 0.07440, $p < 0.05$) was also detected based on haplotype frequencies, with neither comparison significant after Bonferroni correction (Table 2.5).

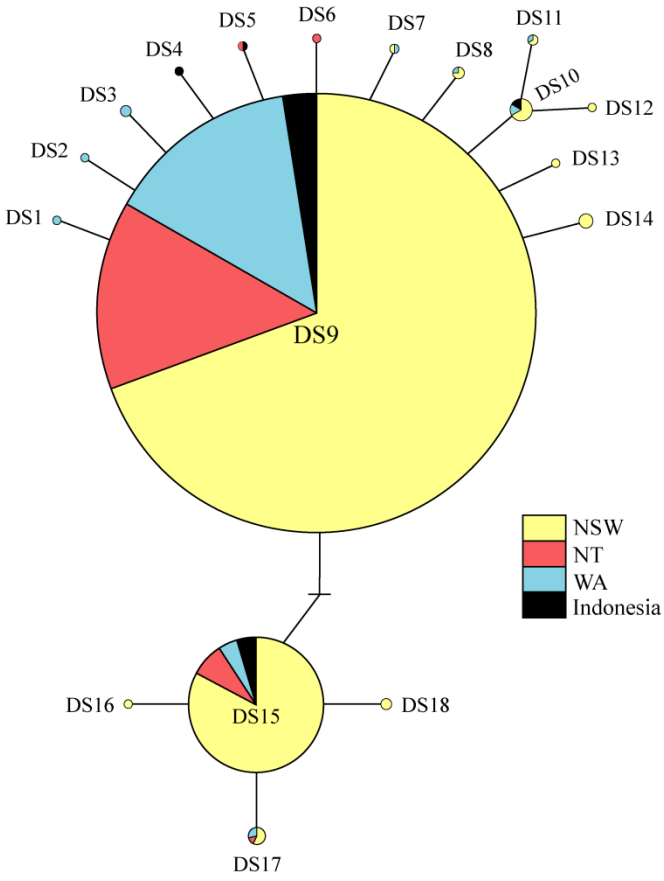

Figure 2.4 Mitochondrial DNA ND4 haplotype network for *Carcharhinus obscurus* (*n* = 423) from Australian and Indonesian waters. Sizes of circles correspond to the number of individuals displaying each haplotype. Shading indicates the proportion observed from each of the four putative populations. (–), mutational step/missing haplotype.

Table 2.4 AMOVA analyses of spatial genetic variation of mitochondrial DNA ND4 sequences for *Carcharhinus obscurus* from Australian and Indonesian waters.

Source of variation	d.f.	Test statistic	Sum of squares	Variance components	Percentage of variation (%)
Among populations	3	Φ_{ST}	3.875	0.01235	2.46
		F_{ST}	2.149	0.00712	2.72
Within populations	419	Φ_{ST}	205.056	0.48939	97.54
		F_{ST}	106.517	0.25422	97.28
Fixation indices		$\Phi_{ST} = 0.02462$; $p = 0.02143$ (± 0.00099)			
		$F_{ST} = 0.02723$; $p = 0.00999$ (± 0.00069)			

Table 2.5 Mitochondrial DNA ND4 population pairwise Φ_{ST} (below diagonal) and F_{ST} (above diagonal) estimates for *Carcharhinus obscurus* collected from Indo-Australian waters. Bold italics indicate the pairwise value significant after sequential Bonferroni correction (initial $\alpha = 0.0083$); * denotes values significant at the $p \leq 0.05$ level.

	NSW (n = 301)	NT (n = 49)	WA (n = 57)	Indonesia (n = 16)
NSW		0.02208	0.02403*	0.03592
NT	0.01362		0.00668	0.13925*
WA	***0.04437***	0.00285		0.07440*
Indonesia	−0.00597	0.02476	0.03010	

Random re-sampling simulations demonstrated an increasing likelihood of finding a non-significant pairwise result between NSW and WA with decreasing NSW sample size (Figure 2.5). More specifically, 14.18 % of replicate comparisons where sample size was set to 100 for NSW (and left at 57 for WA) did not provide statistical support for the original analysis, where sample size was 57 for WA and 301 for NSW. This increased to 36.8 % when the NSW sample size was reduced to 50.

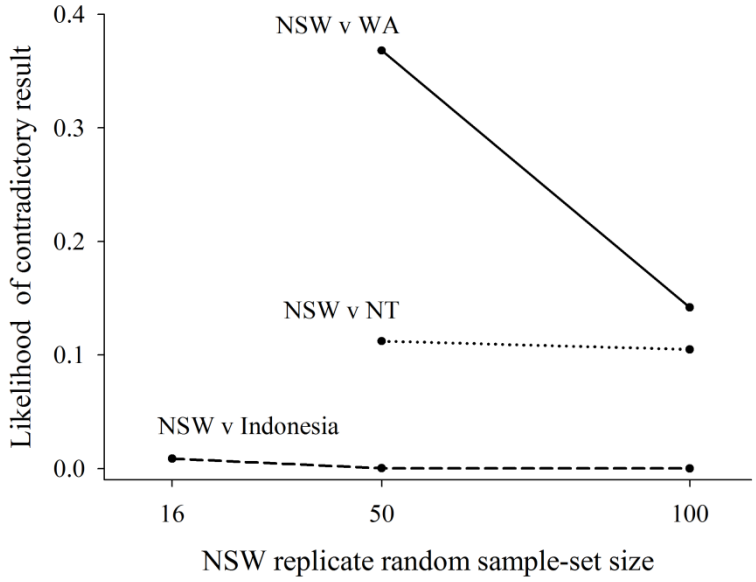

Figure 2.5 Likelihood of generating a contradictory pairwise result to that of the original analysis given 10,000 replicate random re-samples of the NSW *Carcharhinus obscurus* population at varying sample sizes.

In addition, pairwise Φ_{ST} distributions displayed stable mean Φ_{ST}'s (despite increased variation) but increasing mean *p* values relative to the output of the original analysis as random NSW sample-set size decreased (Figure 2.6). Simulations involving random NSW sample sets of $n = 100$ returned pairwise Φ_{ST}'s normally distributed around a mode (and mean) very near the Φ_{ST} produced by the original analysis, and a mean *p* value < 0.05 (Figure 2.6a). Simulations involving random NSW sample sets of $n = 50$, despite a more variable and skewed distribution, once again returned a mean Φ_{ST} very near that produced by the original analysis, but in contrast returned a non-significant mean *p* value (> 0.05) (Figure 2.6b). Replicate pairwise comparisons between NSW and NT and Indonesia, on the other hand, displayed little change in the likelihood of returning a contradictory result to the original analysis as random NSW sample size was altered (Figure 2.5).

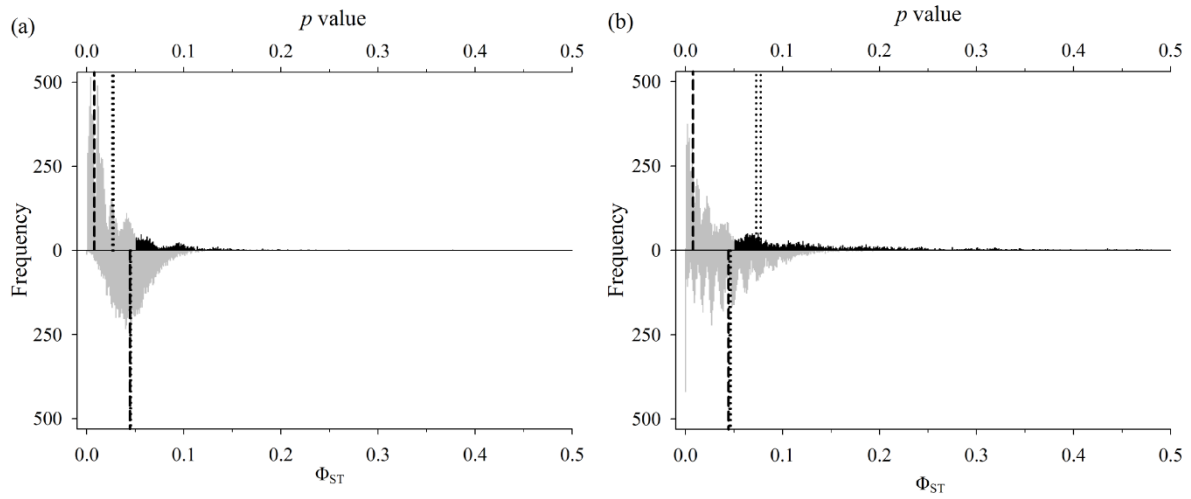

Figure 2.6 New South Wales versus Western Australia pairwise Φ_{ST} and *p* value distributions following 10,000 replicate random re-samples of the NSW *Carcharhinus obscurus* population at (a) $n = 100$ and (b) $n = 50$. Grey and black zones on simulated *p* value distributions represent $p \leq 0.05$ and $p > 0.05$, respectively. Dotted lines denote upper and lower 95 % confidence intervals around simulated means. Dashed lines indicate the pairwise Φ_{ST} and *p* value generated by the original analysis.

2.4.5 Species comparison off the NSW coast

There was a marked similarity in mtDNA features between *C. obscurus* and *C. plumbeus* samples collected from eastern Australian waters. Large sample sets revealed similar numbers of haplotypes for *C. obscurus* ($n_H = 12$, $n = 301$) and *C. plumbeus* ($n_H = 11$, $n = 440$) (Table 2.3). Comparative haplotype networks revealed strikingly similar topologies for the two species, with both networks being shallow and suggestive of the presence of two distinct, yet closely related, clades separated by 1–2 mutation steps (Figure 2.7). A difference between the two species, however, was observed in their diversity indices, where *C. obscurus* exhibited moderate genetic diversity ($h = 0.5224$) and *C. plumbeus* low genetic diversity ($h = 0.2826$) (Table 2.3).

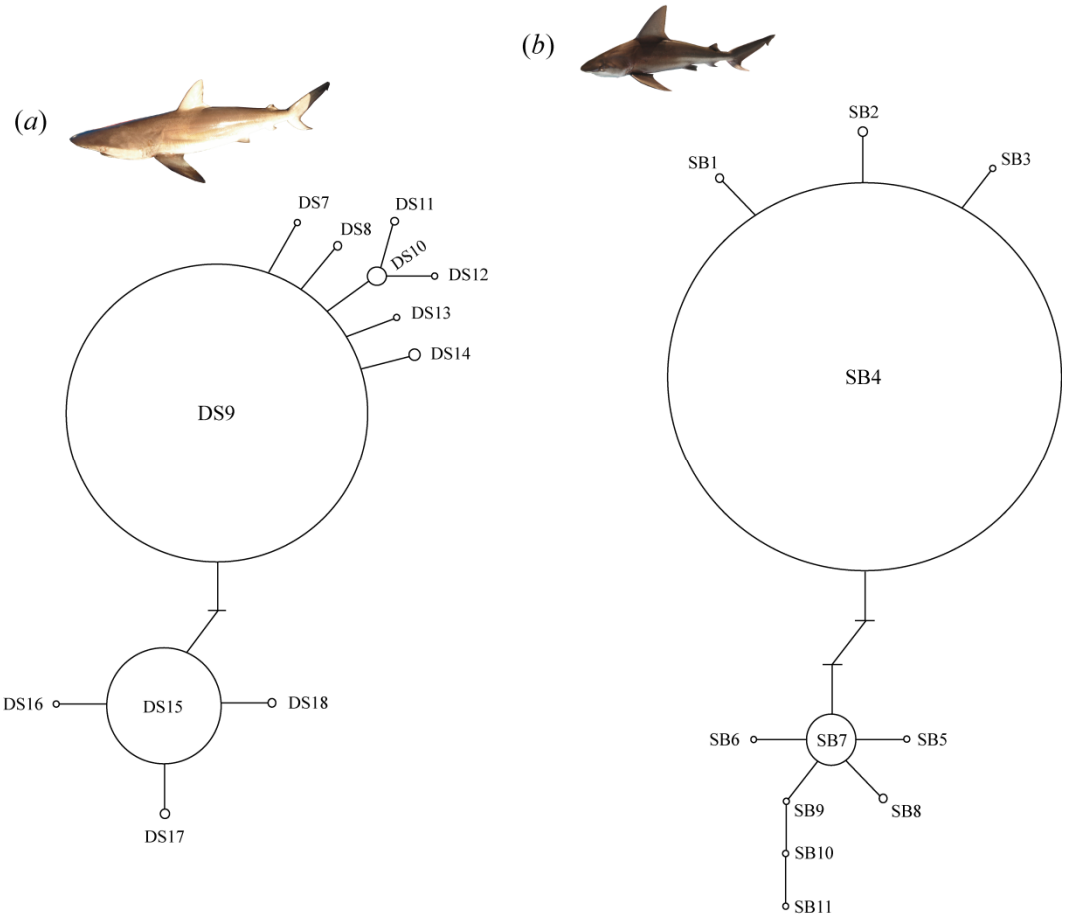

Figure 2.7 Comparative ND4 haplotype networks for (*a*) *Carcharhinus obscurus* (*n* = 301) and (*b*) *Carcharhinus plumbeus* (*n* = 440) in NSW waters. Sizes of circles correspond to the relative frequency of each haplotype. (–), mutational steps/missing haplotypes.

2.5 Discussion

2.5.1 Regional gene flow in Carcharhinus obscurus

This study represents a re-assessment of genetic structure in *C. obscurus* from Indo-Australian waters, following on from Ovenden et al. (2009). Using a different mtDNA marker, higher sample numbers and the addition of northern Australian samples, we detected weak genetic subdivision between east and west Australia. We observed genetic homogeneity, however, between northern Australia and both eastern and western Australia. In considering the Indonesian population, the application of two *F*-statistic metrics (Φ_{ST} and F_{ST})

produced contrasting results, with some evidence for differentiation between Indonesia and Australia based on haplotype frequencies. Discrepancies between these two metrics can arise due to their differing methods of calculation, and typically occurs when genetic subdivision is at the margins of statistical significance (Broderick et al. 2011).

Our finding of unencumbered gene flow between northern Australia and more southern regions (NSW and WA) was not surprising from a point of view of dispersal potential. *Carcharhinus obscurus* attains a large size (Last & Stevens 2009) and is suspected of undergoing long-range temperature-driven migrations on a seasonal basis, with tagging studies revealing an ability to travel considerable distances (Hussey et al. 2009, Rogers et al. 2013). Our finding of genetic subdivision between eastern and western Australia, however, challenge those of Ovenden et al. (2009) and also Benavides et al. (2011b), who failed to detect genetic differentiation between these same two locations using control region sequence data. We also provide evidence for and against the findings of Ovenden et al. (2009) relating to genetic subdivision between Australia and Indonesia. The conclusions drawn by the abovementioned authors, however, were suitably circumspect given the generally low sample numbers upon which their comparisons were based.

Despite the comparatively robust sample numbers used in the present study, we too have reason to be circumspect in our findings. Random-resampling simulations offered some evidence that our detection of significant genetic differentiation between NSW and WA was driven, in part, by the strong bias in sample sizes between the two locations. Replicate pairwise comparisons indicated an increasing likelihood of finding a non-significant result between the two regions as the NSW sample size was decreased towards a more balanced analysis. These simulations either highlight the weak nature of genetic subdivision between Australia's east and west coast or draw its actual existence into question. Conversely, replicate pairwise comparisons between NSW and NT and Indonesia appeared to be

unaffected by a balancing of the NSW sample size - suggestive that the outcomes of the original analysis were robust to biased sample sizes in these instances.

Rarefaction analysis emphasised an additional limitation of our study, and in doing so cast considerable doubt over the reliability of population comparisons presented here. New South Wales was demonstrated as the only location at which an adequate proportion of the available genetic variability was likely sampled, with much of the available diversities appearing to have remained unsampled from NT and Indonesia, and possibly also WA. The rarefaction exact curves suggested that sample sizes in excess of 100 (and even up to 150) may be required to accurately represent levels of genetic diversity, and hence to confidently discern haplotype relative frequencies, at any given location. It is important to consider, however, that these results pertain specifically to the ND4 region and should not be applied to other mitochondrial genes. We would anticipate rarefaction curve trajectory, and therefore optimum sample size estimates, to be heavily reliant on the degree of polymorphism of the mtDNA region employed. These findings are particularly pertinent for studies investigating genetic structure over fine and regional spatial scales, where signals of genetic differentiation are unlikely to be strong, and reiterate that conclusions based on small sample sizes should be treated with considerable caution. For this reason, and given the contradicting metric results as well as our inability to confirm that the samples were actually collected from Indonesian waters, we have henceforth placed little emphasis on results involving the Indonesian location.

Notwithstanding the abovementioned limitations, evidence for regionally-restricted gene flow between eastern and western regions of Australia, as presented in this study for *C. obscurus*, is consistent with mtDNA research on a range of other shark species representing a broad spectrum of different ecologies and life-histories – scalloped hammerhead *Sphyrna lewini* (Duncan et al. 2006), grey nurse *Carcharias taurus* (Ahonen et al. 2009), *C. plumbeus*

(Portnoy et al. 2010), pigeye *Carcharhinus amboinensis* (Tillett et al. 2012b) and great white *Carcharodon carcharias* (Blower et al. 2012). On comparable geographic scales, genetic subdivision was detected in bull *Carcharhinus leucas* and common blacktip *Carcharhinus limbatus* sharks between Gulf of Mexico and north-western Atlantic waters (Keeney et al. 2005, Karl et al. 2011).

Regional and fine-scale genetic subdivision in sharks, based on mtDNA, is often attributed to reproductive philopatry – a sex-biased behavioural trait widely documented in this taxon (Hueter et al. 2005, Portnoy & Heist 2012). Discerning reproductive philopatry in a justifiable manner, however, requires a stringent experimental design (Keeney et al. 2005, Dudgeon et al. 2012), which the present study lacked; tissue collection was both spatially and temporally opportunistic, with the exception of WA where small individuals were sampled over consecutive days. While it is possible that our finding of regional subdivision reflects signs of philopatry, this study is unable to provide an informative test of this hypothesis.

Alternatively, the shallow divergence observed between eastern and western Australian regions may have resulted from repeated periods of isolation associated with the rise and fall of the Torres Strait land-bridge during the Pleistocene epoch, as is hypothesised for *C. amboinensis* by Tillett et al. (2012b). Unlike *C. obscurus* however, *C. amboinensis* exhibits a distribution restricted to northern areas in Australian waters (Last & Stevens 2009). Given the former species' Australia-wide distribution, genetic divergence between eastern and western regions based on this historic, northern physical boundary is difficult to reconcile for *C. obscurus*, and assumes restricted gene flow across southern Australia which we can neither refute nor support. Furthermore, under this hypothesis one would expect similar levels of divergence between NSW and NT, which we did not observe.

2.5.2 **Carcharhinus obscurus *a suitable proxy for* Carcharhinus plumbeus*?***

Carcharhinus obscurus and *C. plumbeus* exhibited strong similarities in their patterns of genetic diversity in eastern Australian waters. Rarefaction curves from this region suggested that our sample sets had likely captured the majority of the respective genetic diversities available in both species, and hence were accurate representations of each species' genetic structure in the area. The haplotype-network topologies for both species, resolved thus through highly robust sample numbers, were very similar – suggestive that *C. obscurus* and *C. plumbeus* populations have experienced related evolutionary histories off Australia's east coast. In light of this, given our finding of weak genetic differentiation between the east and west coast in *C. obscurus*, the concordant finding by Portnoy et al. (2010) for *C. plumbeus* is perhaps not unexpected. These similarities suggest that *C. obscurus* may, to some degree, be a suitable proxy for patterns of gene flow in *C. plumbeus* around Australia; excluding southern waters where the latter species is not found.

However, while comparable levels of diversity were found off the east coast based on haplotype numbers, diversity indices indicated low haplotypic diversity in *C. plumbeus* compared with moderate haplotypic diversity in *C. obscurus*. This low apparent diversity in *C. plumbeus* in NSW waters may be accounted for by the exclusive sampling of the species' southern-most distribution limit (Last & Stevens 2009). Extreme and/or unstable environmental conditions are associated with distribution boundaries, and have been hypothesised to result in low population density and increased genetic drift and inbreeding in peripheral populations (e.g. Arnaud-Haond et al. 2006, Lind et al. 2007). If this is indeed the case, one would anticipate the sampling of core Australian populations to reveal increased genetic diversity in *C. plumbeus*.

2.5.3 Observer-identification accuracy in an eastern Australian shark fishery

Genetic validation revealed high observer accuracy in the identification of *C. obscurus* and *C. plumbeus* in the NSW OTLF. This was not unexpected given morphologic distinctions coupled with a large modal size-at-capture within the fishery; the vast majority of the shark catch in the NSW OTLF is landed as mature, adult individuals (Macbeth et al. 2009). While morphologically similar to one another, and to a range of other species, at smaller sizes, *C. obscurus* and *C. plumbeus* are characterised by diagnostic traits that become increasingly discernible as the individual grows larger (Last & Stevens 2009).

Our estimates of observer accuracy were markedly higher than those reported by Tillett et al. (2012a) from the Northern Territory Offshore Net and Line Fishery (NT ONLF), who estimated overall observer accuracy at ~80 % compared with 98.3 % in the present study. Also, species-specific identification accuracy ranged from 70–92.7 % in northern Australia (Tillett et al. 2012a), compared to 96.6–99.4 % off the east coast as presented here. Lower observer accuracy in the NT ONLF can be attributed to the targeting of morphologically-similar species (e.g. Australian blacktip *Carcharhinus tilstoni* and *C. limbatus*; *C. leucas* and *C. amboinensis*) at predominantly neonate and small juvenile life-stages. In this way, the NSW OTLF is less vulnerable to observer-based catch-data inaccuracies than the northern Australian shark fishery.

2.5.4 Management implications & further work

Notwithstanding the limitations as discussed earlier, our results tentatively support restricted gene flow in *C. obscurus* between east and west Australia. This suggests the allocation of two management units for *C. obscurus* in Australian waters – eastern vs western regions. Under this scenario, stock recovery from a population collapse in the east would rely on reproduction by surviving local individuals and replenishment by immigrants from

northern Australia. Although the most suitable boundary between these two management units is uncertain, given the apparent genetic homogeneity involving northern Australia, our results nevertheless support a more integrated approach to management between adjacent Australian states in this species.

The closely-related genetic structures observed here in *C. obscurus* and *C. plumbeus* in NSW waters, resulting presumably from similar evolutionary histories, raise important implications for their management and conservation. Given that both species appear to have responded similarly to evolutionary influences over time and also exhibit related biological traits in the study area (Simpfendorfer et al. 2002, McAuley et al. 2006, 2007b, Geraghty et al. 2013a – *Chapter 5*, *Chapter 6*), it is likely that contemporary environmental and/or anthropogenic pressures will impact the two species' populations in a similar manner. Of concern, therefore, is that the majority of both species' genetic diversities in NSW waters is present as low-frequency haplotypes – suggestive of a vulnerability to rapid loss of genetic diversity under intense fishing pressure in the region.

High observer accuracy in the NSW OTLF, however, augers well for the management of these species and the fishery. Scientifically-sound catch-composition information is a valuable means of recognising fishing-induced ecosystem consequences such as species-specific shifts in abundance, size-at-capture and/or catch per unit effort (Burgess et al. 2005, Field et al. 2009). The maintenance of such high observer accuracy, however, is somewhat dependent on the fishery maintaining its focus on the more easily identified adults; identification success rate would presumably drop should effort shift to juveniles.

The use of only one mitochondrial marker limited the resolution of the present study, as did the exclusive use of mitochondrial sequence data. We were unable, therefore, to test a null hypothesis that gene flow between the putative populations is equal between males and females. Conflicting genetic structures between mitochondrial and bi-parentally inherited

nuclear data (or mito-nuclear discordance) is a widely identified phenomenon in sharks (Portnoy & Heist 2012). Researchers have typically hypothesised male-biased dispersal (e.g. Pardini et al. 2001, Daly-Engel et al. 2012), including in *C. plumbeus* between eastern and western Australia (Portnoy et al. 2010), which implies persistent male dispersal despite constrained female gene flow. Patterns of male-mediated gene flow, therefore, can have significant implications with respect to interpretations of genetic subdivision and, in turn, the allocation of appropriate management units (Toews & Brelsford 2012).

Southern Australian waters were unsampled in this study, highlighting a lack of knowledge regarding gene flow in this region. A recent satellite-tagging study by Rogers et al. (2013) demonstrated the mixing of *C. obscurus* between southern and south-western Australian waters, but not between southern and eastern waters. Their findings, however, were based on data from only three individuals tagged in the same location. Given, therefore, that definitive information pertaining to movement (or lack of) between east and west Australia is not currently available, genetic sampling of southern waters would greatly improve interpretations of the current data.

With the shortcomings of this study in mind, we strongly encourage further work aimed at achieving greater genetic structure resolution for *C. obscurus* and *C. plumbeus* in Australian and neighbouring waters via more extensive sampling and the use of more and varied genetic markers. We also urge evaluations of connectivity in these species around Australia, particularly between the east and west coasts. For *C. obscurus*, we suggest a focus on southern Australian waters. More robust assessments of contemporary gene flow, as well as physical tagging and tracking, would greatly assist the effective management of these species in Indo-Australian waters through the appropriate allocation of management units.

2.6 Acknowledgements

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2.7 Supplementary material – Polymorphic sites for mitochondrial DNA ND4 haplotypes defined from Australian and Indonesian waters.

(*a*) Dusky shark – *Carcharhinus obscurus*

Haplotype	Nucleotide polymorphism position (1–857)																	
	21	34	90	109	124	189	192	199	289	360	400	421	423	453	594	648	649	822
DS1	A	G	T	G	G	T	T	G	G	C	G	T	G	T	T	A	C	G
DS2	.	.	.	.	.	.	.	.	.	T	.	.	A	.	.	.	.	.
DS3	.	.	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.	.
DS4	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
DS5	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	.
DS6	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	T	.
DS7	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.
DS8	.	.	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
DS9	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
DS10	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	.	.	.
DS11	.	.	.	.	.	A	.	.	.	.	.	C	A	.	.	.	.	.
DS12	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	T	.	.
DS13	.	.	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.
DS14	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A
DS15	.	.	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.
DS16	.	.	.	.	.	.	C	A	.	.	.	.	A	C	.	.	.	.
DS17	.	.	.	.	.	.	C	.	.	.	.	.	A	C	C	.	.	.
DS18	.	A	.	.	.	.	C	.	.	.	.	.	A	C	.	.	.	.

(*b*) Sandbar shark – *Carcharhinus plumbeus*

Haplotype	Nucleotide polymorphism position (1–857)											
	9	72	120	160	186	199	209	327	531	600	650	655
SB1	G	T	T	T	A	G	T	C	T	C	T	T
SB2	.	.	C	.	.	.	C	.	.	.	.	.
SB3	.	.	.	.	G	.	C	.	.	.	.	.
SB4	.	.	.	.	.	.	C	.	.	.	.	.
SB5	A	.	.	.	.	.	C	T	.	T	.	C
SB6	A	.	.	C	.	.	C	T	.	.	.	C
SB7	A	.	.	.	.	.	C	T	.	.	.	C
SB8	A	C	.	.	.	.	C	T	.	.	.	C
SB9	A	.	.	.	.	.	C	T	C	.	.	C
SB10	A	.	.	.	.	.	C	T	C	.	C	C
SB11	A	.	.	.	.	A	C	T	C	.	C	C

(.) indicates the same nucleotide as in haplotype DS1 and SB1 in (*a*) and (*b*) respectively.

CHAPTER 3. Population Expansion and Genetic Structure in *Carcharhinus brevipinna* in the Southern Indo-Pacific

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Plate 4. An adult spinner shark (*Carcharhinus brevipinna*) landed in the New South Wales Ocean Trap and Line large shark fishery. Photos by P. Geraghty.

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3.1 Abstract

3.1.1 Background

Quantifying genetic diversity and metapopulation structure provides insights into the evolutionary history of a species and helps develop appropriate management strategies. We provide the first assessment of genetic structure in spinner sharks (*Carcharhinus brevipinna*), a large cosmopolitan carcharhinid, sampled from eastern and northern Australia and South Africa.

3.1.2 Methods & findings

Sequencing of the mitochondrial DNA NADH dehydrogenase subunit 4 gene for 430 individuals revealed 37 haplotypes and moderately high haplotype diversity ($h = 0.6770 \pm 0.025$). While two metrics of genetic divergence (Φ_{ST} and F_{ST}) revealed somewhat different results, subdivision was detected between South Africa and all Australian locations (pairwise Φ_{ST}, range 0.02717–0.03508, p values ≤ 0.0013; pairwise F_{ST} South Africa vs New South Wales = 0.04056, $p = 0.0008$). Evidence for fine-scale genetic structuring was also detected along Australia's east coast (pairwise $\Phi_{ST} = 0.01328$, $p < 0.015$), and between south-eastern and northern locations (pairwise $\Phi_{ST} = 0.00669$, $p < 0.04$).

3.1.3 Conclusions

The Indian Ocean represents a robust barrier to contemporary gene flow in *C. brevipinna* between Australia and South Africa. Gene flow also appears restricted along a continuous continental margin in this species, with data tentatively suggesting the delineation of two management units within Australian waters. Further sampling, however, is required for a more robust evaluation of the latter finding. Evidence indicates that all sampled populations were shaped by a substantial demographic expansion event, with the resultant high genetic diversity being cause for optimism when considering conservation of this commercially-targeted species in the southern Indo-Pacific.

3.2 Introduction

Patterns of genetic variability in extant taxa have been generated by events and processes occurring over evolutionary time scales. Genetic bottlenecks and demographic expansions, coupled with associated fluctuations in effective population size, are examples of such events, respectively manifesting as low and, eventually, high levels of genetic diversity (e.g. Nei et al. 1975, Excoffier 1990, Lyrholm et al. 1996, Zhang et al. 2002, Peakall et al. 2003, Díaz-Jaimes et al. 2006, Hoelzel et al. 2002, 2006). Evolutionary processes that influence genetic variability, however, need not be characterised by pronounced reduction or elevation in diversity. In a range of taxa, barriers to dispersal and gene flow caused by geographic separation or long-term behavioural traits have led to spatial partitioning of genetic diversity. Cessation of gene flow results in spatial genetic differentiation (e.g. Pope et al. 1996, Sivasundar et al. 2001, Beheregaray et al. 2004, Keeney et al. 2005, Steeves et al. 2005), and ultimately, speciation due to natural selection, genetic drift and mutation (Mayr 1963, Palumbi 1992, 1994). Quantifying genetic diversity and metapopulation structure, therefore, can provide insight into the evolutionary history and behaviour of a species and, in turn, the most appropriate strategy for its management.

In the marine environment, generating accurate, representative estimates of genetic diversity and population structure can be challenging. Cryptic barriers to dispersal and inherent uncertainties pertaining to the spatial extent of gene flow within a species make the most informative experimental designs difficult to determine, notwithstanding the practical issues associated with the collection of highly-vagile marine taxa. For example, various members of the Carcharhinidae represent large, cosmopolitan shark species occupying predominantly continental-shelf waters (Last & Stevens 2009). Species such as the dusky (*Carcharhinus obscurus*), sandbar (*Carcharhinus plumbeus*), bull (*Carcharhinus leucas*) and common blacktip (*Carcharhinus limbatus*) shark are capable of travelling considerable

distances, and are suspected to undertake long-range migrations (Kohler & Turner 2001, Merson & Pratt 2001, Grubbs et al. 2007, Hueter 2007, Hussey et al. 2009, Rogers et al. 2013). These species are also dependent on shallow coastal habitats for birthing and offspring development (e.g. Thorpe et al. 2004, Hussey et al. 2009, Conrath & Musick 2007, Heupel et al. 2007, Taylor & Bennett 2013), with mounting evidence demonstrating philopatric behaviour in juveniles and, more notably, in gravid females (Keeney et al. 2003, 2005, Hueter et al. 2005, Karl et al. 2011, Tillett et al. 2012c). This trait suggests that, for some carcharhinid sharks, spatial genetic connectivity may be lower than otherwise predicted based on vagility and demonstrated patterns of movement. The contrast between long-range dispersal ability and the potential for sex-specific disruption of gene flow between geographically proximate locations provides a complex context within which to decipher genetic structure. Given the implications for management and conservation, however, this same dichotomy highlights the importance of an understanding of spatial genetic subdivision in shark species.

Genetic structure has been investigated in several carcharhinids at a range of geographic scales (Dudgeon et al. 2012). Studies on global phylogeography have consistently shown that large oceanic expanses are robust barriers to gene flow (Keeney & Heist 2006, Schultz et al. 2008, Benavides et al. 2011a, 2011b, Portnoy et al. 2012, Whitney et al. 2012). Genetic subdivision has also been documented over finer spatial scales and attributed to either philopatric behaviour or historic events causing geographic isolation (Keeney et al. 2003, 2005, Ovenden et al. 2009, 2011, Portnoy et al. 2010, Karl et al. 2011, Portnoy & Heist 2012, Tillett et al. 2012b, 2012c).

The spinner shark (*Carcharhinus brevipinna*) has thus far been neglected in the population genetic literature. No research on genetic diversity or stock structure has been conducted in any part of its cosmopolitan range, which includes much of the world's tropical and warm-

temperate continental shelf waters (Last & Stevens 2009). *Carcharhinus brevipinna* is predominantly a by-catch or secondary target species, but is nevertheless an important component of commercial catches in multi-species shark fisheries around the world (Castillo-Géniz et al. 1998, Joung et al. 2005, Dudley & Simpfendorfer 2006, McVean et al. 2006, Henderson et al. 2007, White 2007, Hale et al. 2011, Carlson et al. 2012). Furthermore, owing to confusion with the 'blacktip' shark, commercial catch records of *C. brevipinna* are most likely gross underestimates in some regions. Recreational catch rates are also suspected to be substantial, however, as for most shark species, they remain unquantified. In Australian waters, considerable numbers of *C. brevipinna* are landed along the eastern, northern and western coastlines where they are harvested using demersal longlines, demersal and pelagic gillnets, and handlines (Simpfendorfer & Donohue 1998, Rose et al. 2003, Macbeth et al. 2009, Harry et al. 2011a, Tillett et al. 2012a). In eastern Australia, a fishery-observer study revealed this species to be the third most abundant large shark caught in the New South Wales Ocean Trap and Line Fishery (NSW OTLF) (Macbeth et al. 2009).

Carcharhinus brevipinna is a schooling species known to frequent nearshore waters as adults and utilise inshore nursery habitats as juveniles (Castro 1993, Carlson & Brusher 1999, Thorpe et al. 2004, White & Potter 2004, Reid et al. 2011). As such, *C. brevipinna* is considered highly vulnerable to fishing pressure and human-induced habitat alteration, and is hence globally IUCN listed as 'near threatened' (Burgess 2009). Despite this, long-term catch-data sets have provided evidence for stock stability in *C. brevipinna*. Carlson et al. (2012) proposed that growth overfishing had not occurred on this species in the heavily fished western North Atlantic, with the average landed size remaining stable from 1994–2009. Furthermore, the abundance of *C. brevipinna* in this fishery appears to have remained largely unchanged, with some evidence for increase over the same period (Carlson et al. 2012). Similar findings were reported by Dudley and Simpfendorfer (2006) from the western Indian

Ocean, who revealed stable catch per unit effort (CPUE) and stable/increasing size-at-capture from 1978–2003. Having experienced comparatively lower targeted-fishing pressure on a global scale, *C. brevipinna* has not been subject to the same concern or scrutiny regarding the status of its populations as that levelled at species such as *C. obscurus* and *C. plumbeus* (e.g. McAuley et al. 2005, Cortés et al. 2006, Anon. 2011a, 2011b). However, the life-history characteristics of *C. brevipinna* suggest a similar vulnerability to overfishing and to slow intrinsic rates of population recovery (Branstetter 1987, Allen & Cliff 2000, Allen & Wintner 2002, Joung et al. 2005, White 2007, Capapé et al. 2003, Carlson & Baremore 2005). Furthering our understanding of global *C. brevipinna* populations, therefore, may be considered prudent.

Here we assess genetic structure and diversity in *C. brevipinna* using mitochondrial DNA (mtDNA) sequence data. We test a null hypothesis of genetic homogeneity throughout Australian and South African waters, and discuss the evolutionary history of the species in the region. We generate an estimate of scientific-observer accuracy in identifying *C. brevipinna* in an eastern Australian large-shark fishery, and also discuss the implications of our findings for fisheries management and conservation.

3.3 Materials & methods

3.3.1 Ethics Statement

Tissues were sampled from New South Wales (NSW) waters according to a protocol approved by the NSW Government Primary Industry (Fisheries) Animal Care and Ethics Research Authority (Permit ACEC REF 07/03 – CFC).

3.3.2 Sample collection

Shark tissues were collected from a range of locations in the southern Indo-Pacific (Figure 3.1) using a variety of fishery-dependent methods. From NSW waters, tissues were harvested

during 2007–2010 from landed catch by scientific observers on-board commercial shark-fishing vessels within the NSW OTLF. These samples were taken from individuals spanning the entire size range of the species (Figure 3.2). A small quantity (<2 g) of white muscle tissue was excised from each specimen, immediately preserved in 95 % reagent grade ethanol, and stored at room temperature. Additional samples, collected during 2000–2010, were obtained from more distant locations, including from the waters of north-western Northern Territory (NT), Gulf of Carpentaria (GoC) and Queensland (QLD) in northern Australia, as well as from the east coast of South Africa (Figure 3.1). Tissues from north-western NT, GoC and QLD were sampled from predominantly neonate and small-juvenile individuals from landed catch by observers within their respective commercial shark fisheries (Figure 3.2), and were preserved in 20 % dimethylsulphoxide (DMSO) solution. Fin-clip samples from South Africa, preserved in 100 % ethanol, were collected from adult and sub-adult sharks caught in the Kwazulu-Natal beach protection nets (Figure 3.2). For South African specimens, pre-caudal length (PCL) measurements were converted to total length (L_T) using the morphometric equation published in Allen and Wintner (2002). Additional samples were obtained from QLD and NSW waters by sampling sharks caught in government bather protection programs (Anon. 2006b, Reid et al. 2011).

3.3.3 DNA extraction, amplification & sequencing

To obtain mtDNA sequence data, total genomic DNA was extracted from 5 mg of tissue using a modified salting-out protocol (Sunnucks & Hales 1996). Samples were digested with 10 µl of Proteinase-K (10 $mg \cdot ml^{-1}$) in 580 µl of TNES [50 mM Tris.HCl (pH 7.5), 400 mM NaCl, 20 mM EDTA and 0.5 % SDS] by incubation overnight at 55 °C. Proteins were precipitated by adding 170 µl of 5 M NaCl followed by microcentrifugation at 14,000 rpm for 5 min.

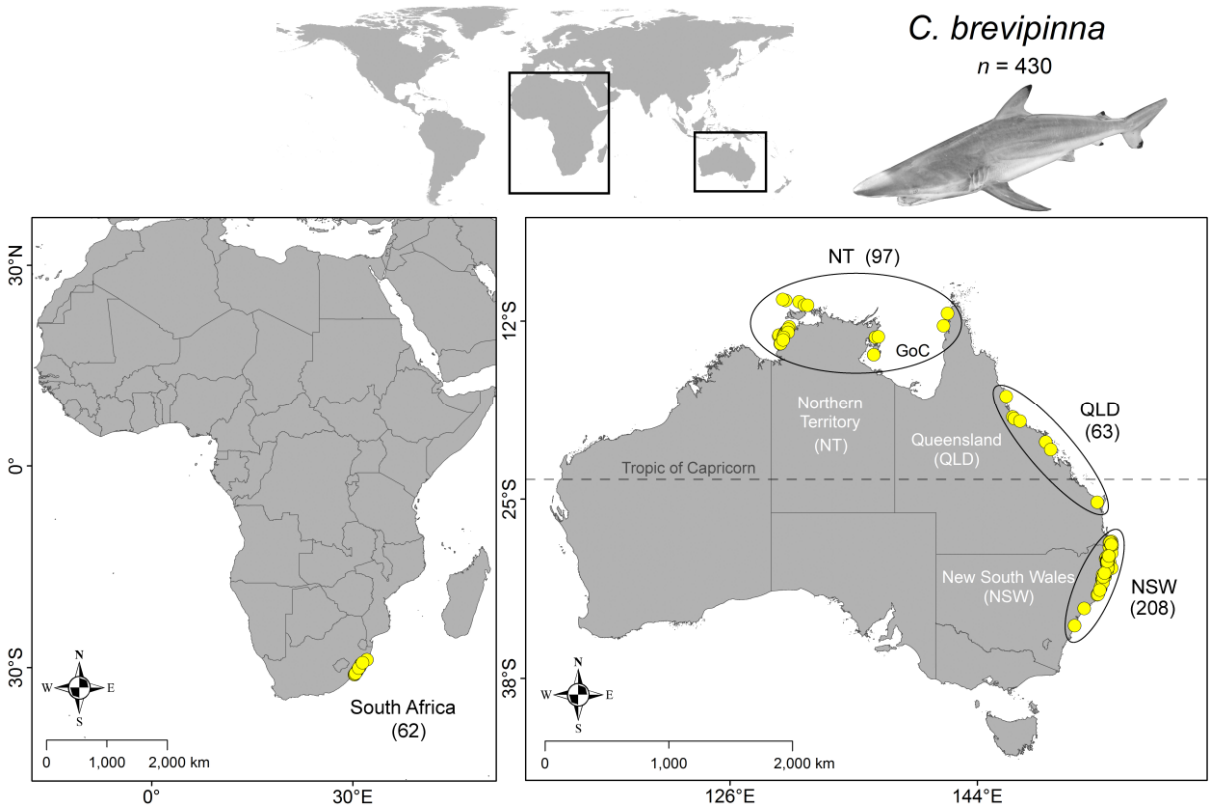

Figure 3.1 Collection locations for tissues included in genetic structure and diversity analyses. Sample numbers for each putative population are in parentheses. GoC = Gulf of Carpentaria.

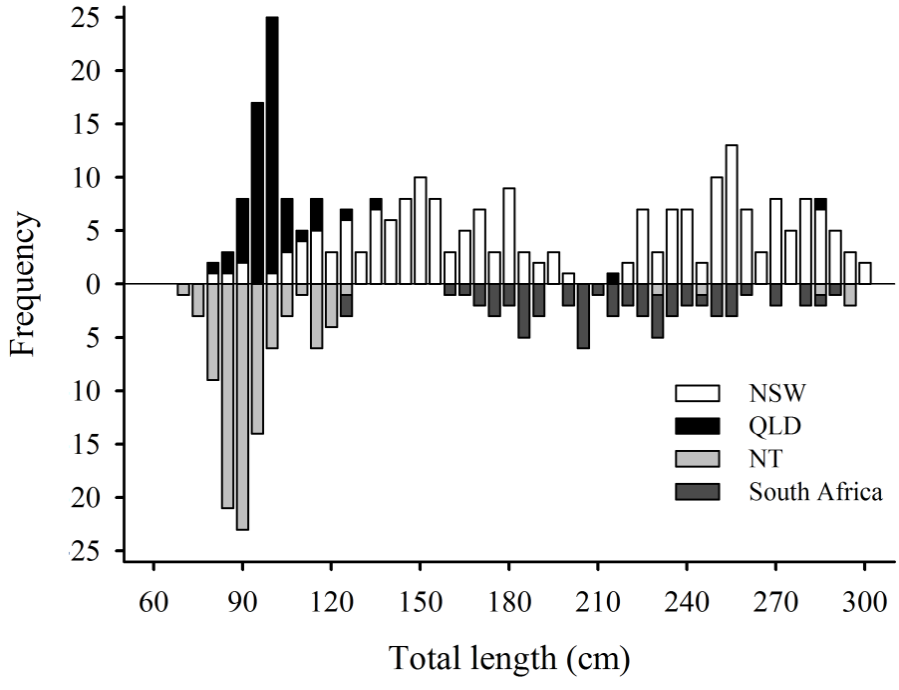

Figure 3.2 Length-frequency distribution of individuals from which tissues were sampled.

Supernatant (600 µl) was recovered into a fresh tube and the DNA precipitated by adding 600 µl of ice-cold 100 % ethanol. Tubes were stored at −20 °C for approximately 1 h. DNA was then recovered by microcentrifugation at 14,000 rpm for 15 min, and the ethanol decanted. The resulting DNA pellet was washed with 200 µl of 70 % ethanol, 100 mM sodium acetate solution, and microcentrifuged at 14,000 rpm for 3 min. Following decanting, all remaining ethanol was removed using a micropipette. DNA was air-dried, resuspended in 100 µl of TE buffer [10 mM Tris.HCl (pH 7.6) and 1 mM EDTA] and stored at −20 °C. DNA yield was checked on a 1.0 % agarose TBE (90 mM TRIS-borate and 2 mM EDTA) gel run at 110 V.

Polymerase Chain Reaction (PCR) was used to amplify the mitochondrial DNA NADH dehydrogenase subunit 4 (ND4) gene from all tissue samples. Reactions were carried out in 50 µl volumes containing 1 µl of DNA template, 1× GoTaq Colourless reaction buffer [consisting of 1.5 mM $MgCl_2$ and 200 µM deoxynucleoside triphosphates (dNTPs)] (Promega), 0.5 µl of RNase (1 $mg \cdot ml^{-1}$), and 0.5 µM of each of the primers ND4 (5’ CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC) (Arèvalo et al. 1994) and H12293-LEU (5’ TTG CAC CAA GAG TTT TTG GTT CCT AAG ACC) (Inoue 2001). Amplifications were performed in an Eppendorf ep gradient S Mastercycler (Eppendorf AG), using thermal cycling conditions consisting of an initial denaturation (94 °C for 3 min), followed by 35 cycles of 94 °C for 15 s, 60 °C for 30 s and 72 °C for 1 min, with a final extension step of 72 °C for 10 min, and soak/finish at 4 °C. PCR products were visualised on a 2.0 % agarose TBE gel, run at 110 V, and stained with GelRed (Biotium Inc.). PCR products were purified prior to sequencing using Exosap-IT (USB Corporation). Sequencing was performed with an Applied Biosystems 3130xl Genetic Analyzer 16-array capillary sequencer (Life Technologies). Sequencing reactions and analyses were carried out by the

Macquarie University (MQ) DNA Sequencing Facility using Big Dye Terminator reactions and the forward PCR primer only.

3.3.4 Sequence alignment & ID validation

Sequences were trimmed and edited manually. Edited sequences were entered into Biomanager (https://biomanager.info) and aligned using the ClustalW (accurate) algorithm (Thompson et al. 1994). GenBank reference sequences for *C. brevipinna* were available for the cytochrome oxidase I (CO1) gene, but not for ND4, prior to this study. Therefore, to validate that the study species had been correctly identified and also to determine the species identity of any misidentified individuals, randomly-selected representatives from each separate haplotype determined from the alignment output were amplified for the CO1 gene using the primers Fish F1 (5' TCA ACC AAC CAC AAA GAC ATT GGC AC) and Fish R1 (5' TAG ACT TCT GGG TGG CCA AAG AAT CA) (Ward et al. 2005). PCRs were carried out as above, with thermal cycling conditions consisting of an initial denaturation (95 °C for 5 min), followed by 30 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 1 min, with a final extension step of 72 °C for 7 min, and soak/finish at 4 °C. PCR products were purified and sequenced following the same protocol outlined for the ND4 locus. Resultant CO1 sequences were compared to reference sequences in GenBank for species recognition.

3.3.5 ND4 sequence analysis

To identify and characterise mitochondrial haplotypes, aligned *C. brevipinna* ND4 sequences were imported to Arlequin 3.5.1.2 (Excoffier & Lischer 2010). A sequence representing each haplotype was lodged in GenBank (Accession codes KF612545 – KF612581). The frequency of, and mutational steps between, haplotypes were assessed by generating statistical parsimony haplotype networks in TCS 1.21 using the default settings (Clement et al. 2000). Phylogenetic relationships among haplotypes were inferred using the

maximum likelihood method based on the Tamura-Nei model (Tamura & Nei 1993), and generated in MEGA 5 (Tamura et al. 2011) with 1,000 bootstrap replicates. The best-fitting model of nucleotide substitution, as offered by MEGA 5, was determined by likelihood ratio tests and calculations of Akaike and Bayesian Information Criteria performed in jModelTest 2.1.1 (Darriba et al. 2012). To assess the ability of the ND4 region to differentiate between carcharhinids, the phylogram was rooted with a range of morphologically similar species, as well as with two sphyrnid species as outgroups.

Genetic diversity indices were obtained with Arlequin using the Tamura-Nei substitution model (Tamura & Nei 1993), and included polymorphism statistics, number of haplotypes, haplotype diversity (h) and nucleotide diversity (π). Harpending's raggedness index (H_{RI}) was estimated from nucleotide mismatch distributions constructed in Arlequin under the sudden demographic expansion model with 20,000 bootstrap replicates (Harpending 1994). Tajima's *D* and Fu's *F* neutrality indices were also estimated in Arlequin, and are indicative of departures from mutation-drift equilibrium or patterns of selection (Tajima 1989, Fu 1997). In conjunction with H_{RI}, the latter two analyses can be used to determine if a population has undergone an expansion event (possibly following a genetic bottleneck). Mismatch distributions will be multi-modal (or ragged) in a stable population, where the generation of new mutations is offset by random drift, and uni-modal for expanding populations, where new mutations accumulate faster than their loss due to drift (Harpending 1994). For Tajima's *D* and Fu's *F*, signals of population expansion are denoted by significant negative test statistic values. Statistical significance was assessed here, following 20,000 simulated samples, at $\alpha = 0.05$ and $\alpha = 0.02$ for *D* and *F* values respectively (Fu 1997).

3.3.6 Population genetic structure

To test the null hypothesis of panmixia (genetic homogeneity) in Australian and South African waters for *C. brevipinna*, an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was implemented in Arlequin to evaluate the overall extent of net genetic subdivision between sample locations. We employed two *F*-statistic metrics of genetic divergence: Φ_{ST} (Excoffier et al. 1992) and F_{ST} (Wright 1965). While Φ_{ST} has been regarded as the superior metric on the basis of its incorporation of a measure of genetic distance between haplotypes, frequency-based F_{ST} has been proposed as potentially a more appropriate measure of genetic differentiation among locations where migration is theoretically occurring at a faster rate than mutation (Bird et al. 2011). Φ_{ST} was calculated via the computing of a distance matrix using the Tamura-Nei model (Tamura & Nei 1993) for estimation of genetic distance between sequences, while F_{ST} used haplotype frequencies only. AMOVA partitioned genetic variance among, and within, sample locations, and calculated overall Φ_{ST} and F_{ST} fixation indices. Genetic differentiation between each pair of locations was also measured by calculating pairwise Φ_{ST} and F_{ST} estimates. Statistical significance was determined following 20,000 permutations of the sequence data and, in the case of pairwise Φ_{ST} and F_{ST}, assessed at an initial critical significance level of $\alpha = 0.0083$ (adjusted from $\alpha = 0.05$) following sequential Bonferroni correction for six simultaneous comparisons (Holm 1979, Rice 1989). The AMOVA structure consisted of one group made up of the following four putative populations: NSW (n = 208), QLD (n = 63), NT (n = 97) and South Africa (n = 62) (Figure 3.1). The analysis outlined above is henceforth referred to as the 'original analysis'. Prior to conducting this large-scale AMOVA, we investigated the extent of genetic subdivision on a finer scale between GoC (n = 43) and north-western NT (n = 54) waters. This analysis indicated genetic homogeneity (fixation indices: $\Phi_{ST} = 0.00035$, $p > 0.39$; $F_{ST} = 0.00151$, $p >$

0.31), hence providing justification for pooling GoC and north-western NT samples to create one northern population termed 'NT'.

Carcharhinus brevipinna sample sizes were clearly biased towards NSW (Figure 3.1), where 208 samples were collected compared to 62, 63 and 97 samples from the other three locations. We evaluated the influence of this sampling bias on the *F*-statistics of pairwise population comparisons involving NSW via random re-sampling simulations. Ten thousand replicate random sample-sets of $n = 60$ (for comparison with QLD and South Africa, but not NT owing to its larger original sample size), $n = 100$ and $n = 150$ were selected without replacement from the NSW population, while QLD, NT and South African sample sizes were kept unchanged. Population pairwise Φ_{ST} and associated *p* values were generated for each replicate random sample-set in Arlequin using the batch processing function and permutation settings as outlined above. Resultant Φ_{ST} and *p* value distributions were plotted, and the likelihood of producing a result contradictory to that of the original analysis was calculated as either the proportion of *p* values ≤ 0.05 or > 0.05, depending on the result of the original analysis. That is, if the original pairwise *p* value was significant ($p \leq 0.05$), the likelihood of a contradictory result equals the absolute number of *p* values $> 0.05/10{,}000$.

The 'Isolation by Distance' (IBD) hypothesis was also tested to determine if inter-population genetic distances increased linearly with geographic distance. Genetic (Φ_{ST}) and geographic (km, by sea) distances between the four putative populations were calculated in GenAlEx (Peakall & Smouse 2012) and ArcMap 10.0 (ESRI), respectively. Pairwise genetic and geographic distance matrices were correlated using a Mantel test, with a test for a significant relationship by 9,999 random permutations, also implemented in GenAlEx.

3.3.7 Rarefaction analysis

To determine whether sample sizes adequately represented population genetic variability, rarefaction exact curves were generated to qualitatively assess the proportion of haplotypic diversity sampled at each of the four locations. The expected number of haplotypes found for a given sample number (from one to the total sample size obtained at each location) was calculated using the rarefaction formula of Hurlbert (1971), and executed in the statistical package R (2010). A trend towards an asymptotic relationship infers haplotype saturation, i.e. that the majority of the available genetic diversity was likely sampled at that location and that more intensive sampling is likely to yield few additional haplotypes. In contrast, a steep slope suggests that a large fraction of the available haplotype diversity remains unsampled.

3.4 Results

3.4.1 Fishery-observer accuracy in NSW waters

The ND4 gene region proved to be capable of distinguishing a range of morphologically-similar carcharhinids (Figure 3.3), as previously shown by Tillett et al. (2012a). Genetic validation was possible for a total of 190 sharks identified by scientific observers as *C. brevipinna* in the NSW OTLF from 2007–2010. Of these, 187 were genetically confirmed to be *C. brevipinna*, translating to an observer-accuracy estimate of 98.4 % for the identification of this species in the fishery (Table 3.1). Misidentified individuals ($n = 3$) comprised two *C. limbatus* and one *C. obscurus* (Table 3.1).

Table 3.1 Fishery-observer identification accuracy. Percentage (individual counts in parentheses) of each genetically-identified shark species from observer-identified *Carcharhinus brevipinna* in the New South Wales Ocean Trap and Line Fishery.

Genetic identification	Observer identified *C. brevipinna* ($n = 190$)
C. brevipinna	98.4 (187)
C. limbatus	1.1 (2)
C. obscurus	0.5 (1)

3.4.2 Genetic diversity & summary statistics

An 857 base pair mtDNA ND4 sequence was obtained for 430 *C. brevipinna* individuals collected from Australian and South African waters (Figure 3.1). A total of 37 haplotypes were defined, characterised by 41 polymorphic sites composed of 40 transitions and one transversion (see Supporting Information). A phylogenetic tree placed all haplotypes into a single, shallow clade (Figure 3.3).

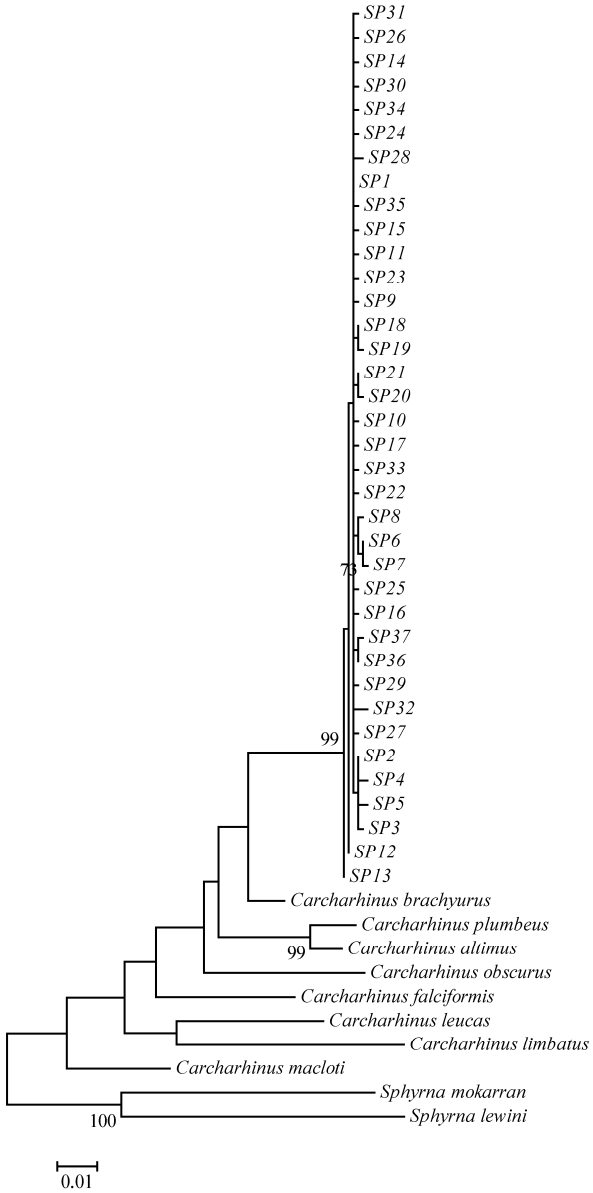

Figure 3.3 Maximum likelihood phylogenetic tree for *Carcharhinus brevipinna* haplotypes. Nodal bootstrap support is displayed where ≥ 70 %. Scale represents the proportion of polymorphic sites between haplotypes.

One haplotype (SP1) clearly dominated the sample set, and was found in all four populations in reasonably similar proportions (Table 3.2). The same number of haplotypes (*n* = 23) was found in NSW and NT waters, despite NSW having over double the sample size (Table 3.3). NSW exhibited six haplotypes endemic to the area, whereas NT displayed five. Almost identical sample sizes revealed 17 haplotypes from QLD waters and 11 from South African waters, with two unique haplotypes defined from each location (Table 3.3). Haplotype (*h*) and nucleotide (π) diversities were very similar, and high in the case of the former and low in the case of the latter, across three of the four putative populations (QLD, NT and South Africa; *h*, range 0.7279–0.7493; π, range 0.0015–0.0016) (Table 3.3). Comparatively lower diversity was observed in NSW waters ($h = 0.5984$, $\pi = 0.0010$). All mismatch distributions were consistent with the sudden population expansion model, with no significant deviation from a uni-modal distribution (H_{RI}, range 0.054–0.099) (Table 3.3). In support of this, all four putative populations displayed significant negative neutrality indices (*D*, range -2.245 – -1.506; *F*, range -23.626 – -4.464) (Table 3.3).

3.4.3 Rarefaction & optimum sample size

Rarefaction exact curves indicated trends towards asymptotic relationships for both the NSW and South African locations (Figure 3.4), despite markedly different sample sizes. This suggests that the majority of the haplotypic diversities available at these two locations were most likely sampled. Steeper slopes were observed from QLD and NT waters (Figure 3.4), suggestive that some proportion of the available genetic diversities remained unsampled. Optimum sample size for the adequate representation of levels of genetic variation present in a given *C. brevipinna* population appears to be site dependent.

Table 3.2 Haplotype relative frequencies observed from each sampling location.

Haplotype	Relative frequency				GenBank Accession Codes
	NSW (*n* = 208)	QLD (*n* = 63)	NT (*n* = 97)	South Africa (*n* = 62)	
SP1	0.625	0.492	0.505	0.468	KF612545
SP2	0.082	0.111	0.124	0.065	KF612546
SP3	0.005	0.032	0.010	–	KF612547
SP4	0.010	0.063	0.041	0.016	KF612548
SP5	–	–	0.010	–	KF612549
SP6	0.019	–	0.010	–	KF612550
SP7	–	–	–	0.065	KF612551
SP8	0.005	0.016	0.031	–	KF612552
SP9	–	–	–	0.032	KF612553
SP10	–	0.016	–	0.145	KF612554
SP11	–	–	0.010	0.016	KF612555
SP12	–	–	0.021	0.048	KF612556
SP13	–	–	0.010	0.016	KF612557
SP14	–	–	0.010	–	KF612558
SP15	–	–	0.010	–	KF612559
SP16	–	–	0.010	–	KF612560
SP17	0.019	0.016	0.021	0.032	KF612561
SP18	0.053	0.048	0.021	–	KF612562
SP19	–	–	0.010	–	KF612563
SP20	–	0.016	0.010	–	KF612564
SP21	0.038	–	0.041	–	KF612565
SP22	0.024	0.063	0.021	–	KF612566
SP23	0.005	–	0.010	–	KF612567
SP24	0.005	–	0.010	–	KF612568
SP25	–	0.016	–	–	KF612569
SP26	–	0.016	–	–	KF612570
SP27	0.005	0.016	–	–	KF612571
SP28	0.005	0.016	–	–	KF612572
SP29	0.019	0.016	0.010	–	KF612573
SP30	0.010	0.016	–	–	KF612574
SP31	0.010	–	–	–	KF612575
SP32	0.010	–	–	–	KF612576
SP33	0.014	–	–	–	KF612577
SP34	0.005	–	–	–	KF612578
SP35	0.005	–	–	–	KF612579
SP36	0.019	0.032	0.041	0.097	KF612580
SP37	0.010	–	–	–	KF612581

Table 3.3 Genetic diversity indices observed for *Carcharhinus brevipinna* sample locations in the southern Indo-Pacific.

Location	n [a]	n_H [b]	n_{Hq} [c]	h [d]	π [e]	H_{RI} [f]	D [g]	F [h]
NSW	208	23	6	0.5984 (± 0.040)	0.0010 (± 0.0008)	0.074	–2.245 ***	–23.626 ***
QLD	63	17	2	0.7424 (± 0.056)	0.0015 (± 0.0011)	0.057	–2.056 **	–13.080 ***
NT	97	23	5	0.7279 (± 0.047)	0.0015 (± 0.0010)	0.054	–2.163 **	–22.072 ***
South Africa	62	11	2	0.7493 (± 0.050)	0.0016 (± 0.0011)	0.099	–1.506 *	–4.464 *
Pooled	430	37	•	0.6770 (± 0.025)	0.0013 (± 0.0009)	0.064	–2.252 ***	–29.294 ***

[a] Sample size (n), [b] number of haplotypes (n_H), [c] number of unique haplotypes (n_{Hq}), [d] haplotype diversity (h), [e] nucleotide diversity (π), [f] Harpending's raggedness index (H_{RI}), [g] Tajima's (D) and [h] Fu's (F) tests of selective neutrality. Values in parentheses represent standard deviations (s.d.). (•), value not applicable. * denotes significance at the $p \leq 0.05$ level, ** $p \leq 0.01$, *** $p \leq 0.001$.

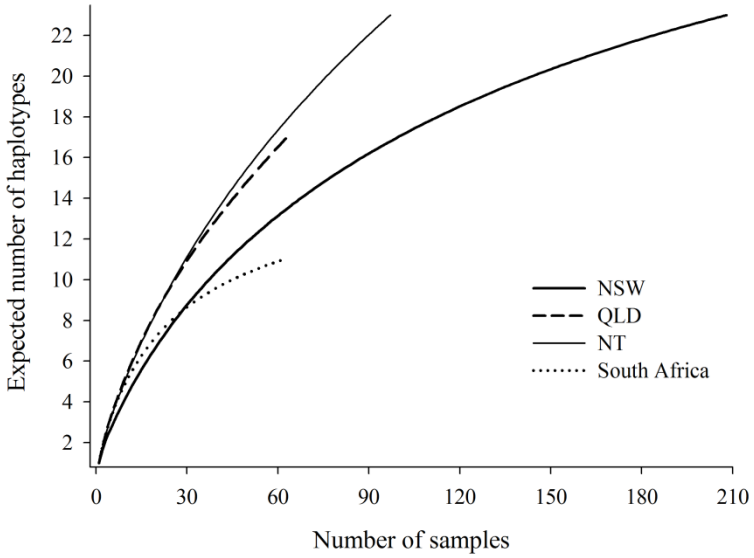

Figure 3.4 Rarefaction exact curves for sample locations.

3.4.4 Population genetic structure

The haplotype network incorporating the four putative populations was shallow and shaped in a distinct 'star-burst' pattern, characterised by one central haplotype (SP1) surrounded by an array of low, or lower, frequency variants (SP2–SP27) (Figure 3.5). A high degree of haplotype sharing was observed among the four geographically-distinct populations, with the

dominant haplotype (SP1) being common at each of the four locations and ~58 % (or n = 21 of n = 36) of lower frequency haplotypes being shared between two or more locations (Figure 3.5, Table 3.2).

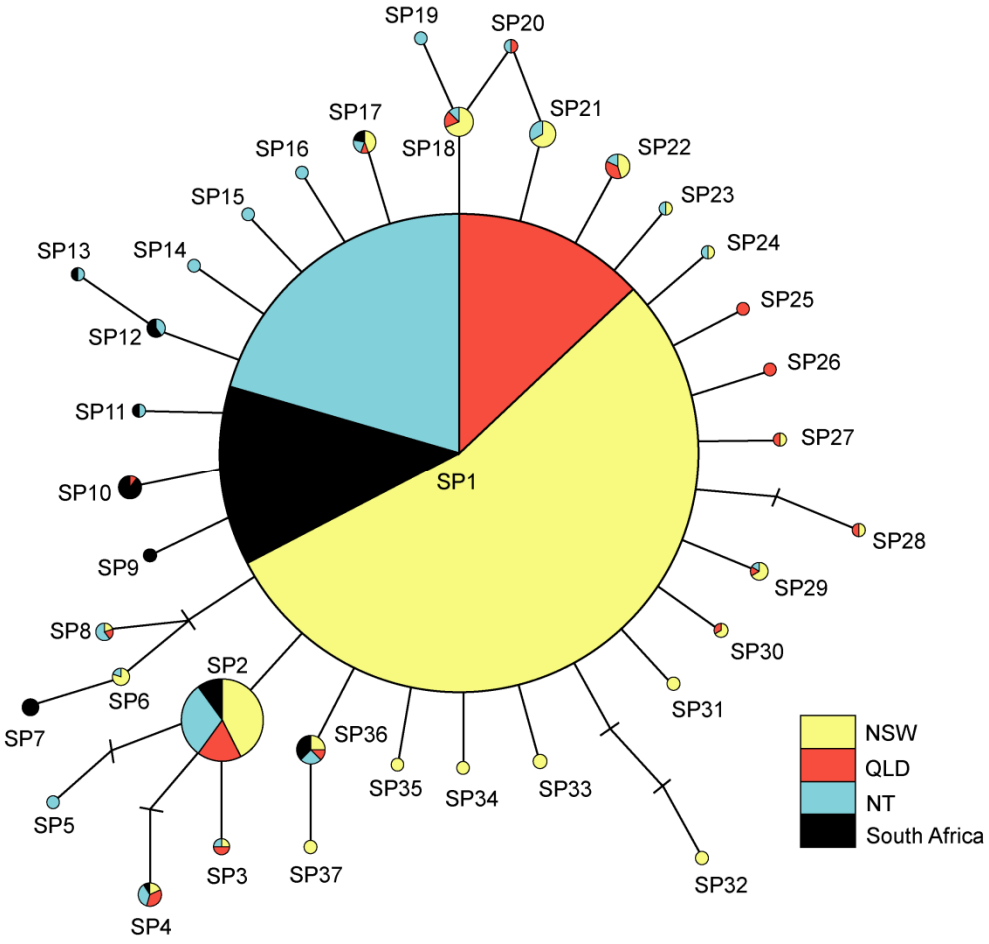

Figure 3.5 *Carcharhinus brevipinna* mitochondrial DNA ND4 haplotype network. Sizes of circles correspond to the number of individuals displaying each haplotype. Shading indicates the proportion observed from each of the four putative populations. (–) = mutational steps/missing haplotypes.

Despite this, AMOVA fixation indices detected significant levels of genetic differentiation between the four putative populations for both F-statistic metrics (Φ_{ST} = 0.01634, p = 0.0001; F_{ST} = 0.01493, p < 0.0035) (Table 3.4). We therefore reject the null hypothesis that *C. brevipinna* are panmictic in Australian and South African waters.

Table 3.4 AMOVA analyses of spatial genetic variation for *Carcharhinus brevipinna* from Australian and South African waters.

Source of variation	d.f.	Test statistic	Sum of squares	Variance components	Percentage of variation (%)
Among populations	3	Φ_{ST}	4.304	0.00916	1.63
	3	F_{ST}	2.475	0.00508	1.49
Within populations	426	Φ_{ST}	234.819	0.55122	98.37
	426	F_{ST}	142.742	0.33507	98.51
Fixation indices		$\Phi_{ST} = 0.01634$; $p = 0.00010$ (± 0.00007)			
		$F_{ST} = 0.01493$; $p = 0.00345$ (± 0.00041)			

Pairwise results, however, revealed some differences between the two measures of divergence. The Φ_{ST} metric detected genetic subdivision between South Africa and all Australian locations (pairwise Φ_{ST}, range 0.02714–0.03508; *p* value, range 0.0000–0.0013), with all three comparisons significant after Bonferroni correction (Table 3.5). Φ_{ST} also detected genetic differentiation, albeit weaker, between NSW and QLD waters (pairwise Φ_{ST} = 0.01328, $p < 0.016$) which was also significant after sequential Bonferroni adjustment, as well as some evidence for genetic subdivision between NSW and NT (pairwise Φ_{ST} = 0.00669) which was significant at $p < 0.05$ but not after Bonferroni correction (Table 3.5). In contrast, the haplotype-frequency based analysis indicated significant genetic differentiation between the NSW and South African locations only (pairwise $F_{ST} = 0.04056$, $p = 0.0008$) (Table 3.5). All other pairwise F_{ST} comparisons, with the exception of QLD vs NT, were only marginally non-significant (pairwise *p* value, range 0.0510–0.0845). The finding of genetic homogeneity between QLD and NT was concordant between both *F*-statistics. A strong positive relationship, with high goodness-of-fit ($r^2 = 0.86$), was observed between pairwise genetic and geographic distances for *C. brevipinna*. This relationship, being driven entirely by differences between Australian locations and South Africa, was not statistically supported by a mantel test ($p = 0.091$).

Table 3.5 Comparison of pairwise *F*-statistic values between putative populations. Observed Φ_{ST} values are below the diagonal and F_{ST} values are above diagonal, with *p* values in parentheses. Bold italics indicate values significant after sequential Bonferroni correction (initial $\alpha = 0.0083$). * Statistically significant at $p \leq 0.05$, but not following Bonferroni adjustment.

	NSW (*n* = 208)	QLD (*n* = 63)	NT (*n* = 97)	South Africa (*n* = 62)
NSW		0.01151 (0.0601)	0.00921 (0.0531)	***0.04056*** (0.0008)
QLD	***0.01328*** (0.0151)		–0.00704 (0.9099)	0.01306 (0.0845)
NT	0.00669 ***** (0.0387)	–0.00507 (0.8166)		0.01411 (0.0510)
South Africa	***0.03494*** (0.0000)	***0.03508*** (0.0009)	***0.02717*** (0.0013)	

Simulation was used to test the effect of a bias in the numbers of *C. brevipinna* sampled from NSW on the *F*-statistics analysis of pairwise population comparisons. Random re-samplings demonstrated an increasing likelihood of finding a non-significant result between NSW and QLD, and between NSW and NT, with decreasing NSW sample size (Figure 3.6). More specifically, for NSW vs QLD, 21.08 % of replicate pairwise comparisons where sample size was set to 150 for NSW (and left at 63 for QLD) did not provide statistical support for the original analysis, for which sample size was 208 for NSW and 63 for QLD. This increased to 48.29 % and 71.8 % as the NSW sample size was reduced further to 100 and 60, respectively. Considering NSW vs NT, the likelihood of producing a contradictory result to that of the original analysis was high as NSW sample size was reduced. Where sample size was set to 150 for NSW (and left at 97 for NT), 61.32 % of replicate pairwise comparisons did not provide statistical support for the original analysis, for which sample size was 208 for NSW and 97 for NT. This increased to 74 % when the NSW sample size was reduced to 100.

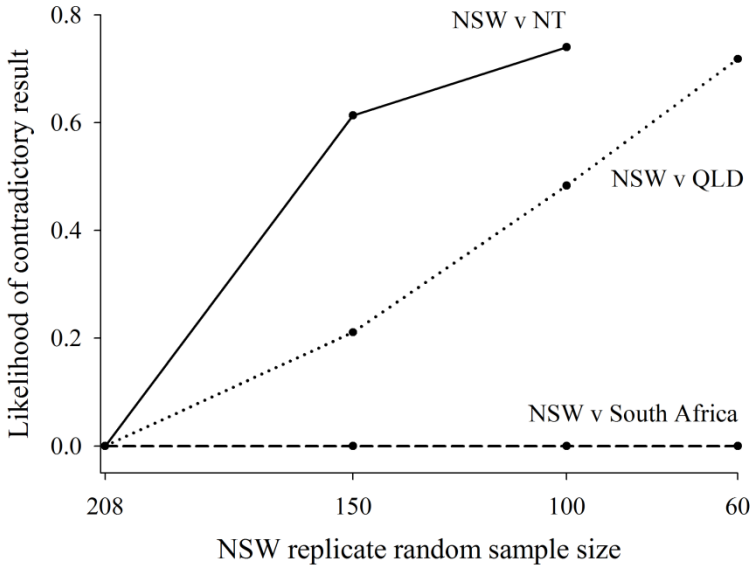

Figure 3.6 Likelihood of pairwise result contradicting that of the original analysis. Likelihoods computed based on 10,000 replicate random re-samples of the NSW population at varying sample sizes. Y-intercept represents original NSW population (n = 208).

Further illustrating this point, as NSW sample size was reduced, pairwise Φ_{ST} and p value distributions revealed increasing variability in conjunction with decreasing mean Φ_{ST} and increasing mean p value relative to the output of the original analysis (Figure 3.7). This pattern was observed for both sets of locations. In contrast, replicate pairwise comparisons between NSW and South Africa displayed an unchanging, and zero percent, likelihood of generating a different result to that of the original analysis as NSW sample size was altered (Figure 3.6).

3.5 Discussion & conclusions

3.5.1 Observer-identification accuracy in an east Australian shark fishery

Observer accuracy was high (98.4 %) in the identification of *C. brevipinna* in the NSW OTLF. This estimate is comparable to other target species within this same fishery; *C. obscurus* and *C. plumbeus* were correctly identified by fishery observers to accuracies of 96.6

% and 99.4 %, respectively (*Chapter 2*). Given the fundamental importance of accurate catch-composition data in fisheries (and species) management (Tillett et al. 2012a, Burgess et al. 2005, Field et al. 2009), this high level of accuracy in the recognition of the three most harvested shark species (by number) in the NSW OTLF (Macbeth et al. 2009) confirms the usefulness of fishery-observer data in the management of this eastern Australian large shark fishery.

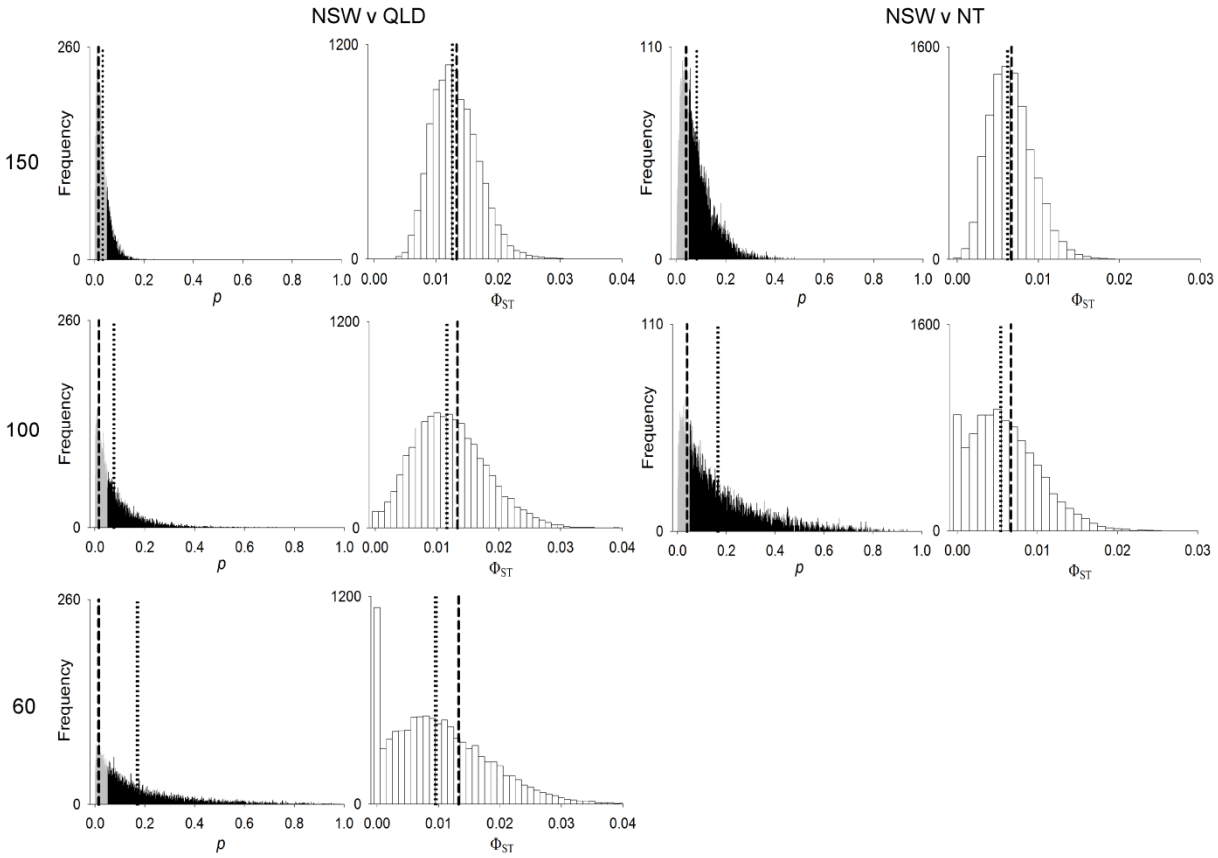

Figure 3.7 Pairwise Φ_{ST} and p value distributions following random re-sampling simulations. NSW versus QLD and NT pairwise distributions based on 10,000 replicate random re-samples of the NSW population at n = 150, 100 and 60. Grey and black zones on p value distributions represent $p \leq 0.05$ and $p > 0.05$ respectively. Dotted lines denote upper and lower 95 % confidence intervals around sample means. Dashed lines indicate pairwise Φ_{ST} and p values generated by the original analysis.

Our measure of observer accuracy (98.4 %) for *C. brevipinna* in the NSW OTLF was higher than that reported for the same species by Tillett et al. (2012a) in the Northern Territory Offshore Net and Line Fishery (NT ONLF), for which observer accuracy was estimated at 87.2 %. Higher identification accuracy in the NSW OTLF compared to the NT ONLF was not unexpected for this particular species given the difference in size class targeted by the two fisheries. The vast majority of the landed shark catch in the NSW OTLF is in the form of mature, adult individuals (Macbeth et al. 2009). In contrast, the NT ONLF targets predominantly neonate and small juvenile life stages, illustrated by the fact that all sharks identified as *C. brevipinna* by observers in the latter fishery were ≤ 1.2m L_T (Tillett et al. 2012a). Size-at-capture is important as *C. brevipinna* is characterised by diagnostic traits that become increasingly discernible as an individual grows larger, most notably tooth shape and fin pigmentation (Last & Stevens 2009). At small sizes, *C. brevipinna* can be difficult to distinguish from a range of other morphologically-similar carcharhinid species (Last & Stevens 2009).

3.5.2 Evolutionary history in the southern Indo-Pacific

The *C. brevipinna* haplotype network was distinctly star-shaped, characterised by a single dominant haplotype surrounded by a high number of low, or lower, frequency variants. This central, and presumably ancestral, haplotype was prominent in all three Australian sample locations, as well as off the coast of South Africa - evidence that Australian and South African waters share common ancestry in this species.

The pattern of genetic diversity observed here in *C. brevipinna* is indicative of a contemporary demographic expansion event having occurred throughout the southern Indo-Pacific. This hypothesis is supported by a range of evidence: the distinctly ‘star-burst’ haplotype network denoted by numerous low-frequency mutations, mismatch distributions

and neutrality test statistics suggesting strong departures from mutation-drift equilibrium for all four putative populations and the observed combination of generally high haplotype and low nucleotide diversities (Tajima 1989, Fu 1997, Grant & Bowen 1998, Ramos-Onsins & Rozas 2002, Ramírez-Soriano et al. 2008). Attempts at dating this population expansion event were abandoned in the absence of mutation-rate estimates for ND4 in elasmobranchs.

It must be noted here, however, that spatial sample coverage in the present study was limited to only a very small area of this species' global distribution range, which includes much of the world's tropical and warm-temperate continental shelf waters (Last & Stevens 2009). Therefore, in the absence of genetic analysis of samples representative of the entire distribution of the species, we are unable to determine whether or not this rapid population growth was a worldwide event or was restricted to the southern Indo-Pacific.

Signals of population expansion as strong as that reported here in *C. brevipinna* is unprecedented among sharks, with comparable signals more commonly associated with taxa such as humans (Excoffier 1990) and teleost fishes (e.g. Thacker 2004, Díaz-Jaimes et al. 2006, Broderick et al. 2011). Evidence for population expansion has, however, been presented for some shark species through analyses of mismatch distributions (Duncan et al. 2006, Hoelzel et al. 2006), star-like haplotype networks (Ovenden et al. 2011, Karl et al. 2012, Naylor et al. 2012), or combinations of the latter two supported by neutrality indices (Pereyra et al. 2010, Veríssimo et al. 2010).

3.5.3 Contemporary genetic structuring

This study marks the first dedicated assessment of genetic structure in *C. brevipinna*. The application of two metrics of genetic divergence (Φ_{ST} and F_{ST}) demonstrated that population genetic findings can be dependent on the *F*-statistic employed - especially pertinent where subdivision is at the margins of statistical significance (Broderick et al. 2011). We therefore

encourage the concurrent use of both metrics as standard practice in population genetic studies.

With this in mind, genetic differentiation was detected over a broad spatial scale between Australian and South African waters. This finding based on mtDNA was not unexpected and, being consistent with a range of other shark population genetic studies (Pardini et al. 2001, Duncan et al. 2006, Ahonen et al. 2009, Chabot & Allen 2009, Portnoy et al. 2010, Benavides et al. 2011a, 2011b, Daly-Engel et al. 2012), re-iterates that large oceanic expanses (in this case the Indian Ocean) represent robust barriers to contemporary gene flow in coastal shark species.

Evidence for genetic subdivision, albeit weak, was also detected over finer spatial scales within Australian waters, i.e. between NSW and both QLD and, to a lesser degree, NT. Genetic homogeneity was observed between QLD and NT waters. These results tentatively suggest that gene flow is restricted to some degree along Australia's eastern continental margin as well as between the south-eastern and northern coastlines, and that gene flow is unencumbered between north and north-eastern Australian waters. These findings were somewhat unexpected given *C. brevipinna*'s potential for active dispersal. That said, however, genetic differentiation has previously been detected in similar and related shark species over comparable geographic scales in Australian waters (Morgan et al. 2011, Blower et al. 2012, Tillett et al. 2012b, 2012c), as well as those of the Gulf of Mexico and north-western Atlantic (Keeney et al. 2005, Karl et al. 2011).

Reproductive philopatry, or the fidelity of gravid females to nursery areas, is typically invoked to explain fine-scale genetic structuring (based on maternally-inherited mtDNA) in the absence of barriers to dispersal for highly-vagile sharks (Keeney et al. 2005, Schultz et al. 2008, Karl et al. 2011, Blower et al. 2012, Portnoy & Heist 2012, Tillett et al. 2012c). Confidently discerning this sex-biased behavioural trait, however, is complex and relies on a

robust experimental design involving the exclusive sampling of neonates, or adult females at time of parturition rather than during dispersal, from spatially discrete areas (Keeney et al. 2005, Dudgeon et al. 2012). The collection of tissues in the present study was generally reliant on both spatial and temporal opportunistic sampling, rather than according to a dedicated experimental design. Nevertheless, tissues from NT and QLD were almost exclusively sampled from neonates and small juveniles, with length-frequency modes at 90 cm and 95–100 cm L_T respectively (Figure 3.2). While it is conceivable that the fine-scale genetic structuring observed in this study reflects signs of reproductive philopatry, the only meaningful test of this hypothesis would be a comparison of the NT and QLD locations between which our data failed to detect genetic differentiation.

Consideration of our results in light of those by Ovenden et al. (2011), however, would suggest that an affinity for nearshore habitat for nursery purposes in *C. brevipinna* has influenced our findings of fine-scale genetic differentiation to some degree. In their study, Ovenden et al. (2011) failed to detect evidence for genetic subdivision along Australia's east coast in milk sharks (*Rhizoprionodon acutus*) using ND4 sequence data. *Rhizoprionodon acutus*, a considerably smaller-bodied and presumably less-vagile species than *C. brevipinna*, conforms to a population model characterised by permanent habitation of nearshore waters without the use of discrete nursery areas (Knip et al. 2010). In contrast, the exclusive use of nearshore habitat by *C. brevipinna* for parturition and juvenile development is well documented (Castro 1993, Carlson & Brusher 1999, Thorpe et al. 2004, White & Potter 2004, Reid et al. 2011). Differing life-cycles denoted by varying usage of nearshore habitat, therefore, may account for these contrasting genetic structures observed along Australia's east coast.

Alternatively, genetic differentiation between NSW and NT may be a relict signature of repeated periods of temporary isolation due to the rise and fall of the Torres Strait land bridge

caused by fluctuating sea levels during the Pleistocene epoch (Voris 2000, Lambeck et al. 2002). This physical, yet temporary, barrier to movement (and hence gene flow) in marine taxa between the east coast and areas west of the Cape York Peninsula was hypothesised to account for contemporary genetic subdivision in pigeye sharks (*Carcharhinus amboinensis*) (Tillett et al. 2012b) which, like *C. brevipinna*, have a distribution restricted to northern regions in Australian waters (Last & Stevens 2009). Under this hypothesis, however, one would anticipate a similar level of genetic differentiation between QLD and NT, rather than genetic homogeneity as observed.

Similarly, a marked change in marine environment coinciding with the Tropic of Capricorn (Figure 3.1) represents an alternative hypothesis explaining restricted contemporary gene flow between south-eastern and more northern Australian waters (Morgan et al. 2011). This latitudinal line discretely separates the NSW population from both QLD and NT populations (with the exception of one individual from southern QLD waters), and delineates a shift from temperate and subtropical continental shelf waters, rocky coastline and drowned river valleys to a largely reef and lagoon-dominated tropical ecosystem.

3.5.4 Project limitations

This study was subject to a range of limitations requiring careful consideration. To begin with, very low values for both Φ_{ST} and F_{ST} metrics (resulting from high incidence of haplotype sharing of both ancestral and recently derived haplotypes among all four putative populations, coupled with generally shallow divergence between mutational variants) is suggestive of a slow rate of mutation in the ND4 gene region. This raises considerable doubt as to the ability of ND4 to effectively discriminate population structure in *C. brevipinna*. For example, pairwise *F*-statistic estimates involving the South African population were demonstrably low in the present study (range, 0.01306–0.04056) compared to others reporting

genetic differentiation in sharks over comparable spatial scales (range, 0.18–0.991) (Table 3.6). Given that these previous studies were all based on analysis of a different mitochondrial locus (i.e. the control region), a slower rate of mutation in the ND4 region may account for the comparatively low *F*-statistics observed here. However, a hypothesis based on low ND4 mutation rate is challenged by the findings of both Dudgeon et al. (2009) and Ovenden et al. (2010) who demonstrated that for *C. limbatus*, Australian blacktip (*Carcharhinus tilstoni*) and zebra (*Stegostoma fasciatum*) sharks, ND4 was the most polymorphic of a range of mtDNA markers, including the control region. Alternatively, therefore, low *F*-statistic values associated with observed genetic structuring between Australia and South Africa, as well as within Australian waters, may reflect continued low-level gene flow, or a recent cessation of gene exchange, between subdivided locations. Until the relative mutation rates of ND4 and CR are determined for *C. brevipinna*, however, or this study is reassessed via sequencing of CR, it is impossible to confidently support or refute the abovementioned hypotheses. Moreover, this issue emphasises the limitations inherent in the analysis of only one mtDNA locus.

The clear bias in sample sizes weighted towards the NSW population represents another major limitation of this study. Random-resampling simulations provided some evidence that the detections of significant genetic differentiation within Australian waters (i.e. between NSW and QLD, and NSW and NT) were driven in large part by this bias. Replicate pairwise comparisons for both sets of locations indicated an increasing likelihood of finding a non-significant result as the NSW sample size decreased towards a more balanced analysis. This either serves to emphasise the weak nature of the observed fine-scale genetic subdivisions within Australian waters, or draw their actual existence into question. Conversely, replicate pairwise comparison between NSW and South Africa returned a significant difference independent of the NSW sample size, hence reinforcing the strength of the genetic

subdivision between the latter two regions, and indicating that the original analysis was robust to the bias in sample size in this instance.

Table 3.6 Mitochondrial divergence metrics for population pairwise comparisons involving Australia and South Africa. CR = control region, ND4 = NADH dehydrogenase subunit 4. AUS = Australia (general), EAUS = eastern Australia, NEAUS = north-eastern Australia, WAUS = western Australia, SAUS = southern Australia, SA = South Africa.

Pairwise comparison	Species	Gene	F_{ST}	Φ_{ST}	Reference
AUS v SA	*Carcharhinus brachyurus*	CR		0.97	Benavides et al. 2011a
	Carcharhinus obscurus	CR		0.18	Benavides et al. 2011b
	Carcharodon carcharias	CR	0.81		Pardini et al. 2001
	Carcharhinus brevipinna	ND4		0.03216	Present study
EAUS v SA	*Carcharias taurus*	CR	0.813		Ahonen et al. 2009
	Carcharhinus brevipinna	ND4	0.04056	0.03494	Present study
NEAUS v SA	*Carcharhinus plumbeus*	CR		0.588	Portnoy et al. 2010
	Carcharhinus brevipinna	ND4	0.01306	0.03508	Present study
WAUS v SA	*Carcharias taurus*	CR	0.676		Ahonen et al. 2009
	Carcharhinus plumbeus	CR		0.6165	Portnoy et al. 2010
	Sphyrna lewini	CR		0.991	Duncan et al. 2006
	Sphyrna lewini	CR	0.45		Daly-Engel et al. 2012
SAUS v SA	*Galeorhinus galeus*	CR		0.34	Chabot & Allen 2009

Rarefaction analysis added further uncertainty regarding the reliability of our fine-scale findings reported in the present study. NSW and South Africa were the only two locations at which adequate levels of the available genetic diversities were likely sampled, hence confirming the robustness of the comparison between these two putative populations. In contrast, a proportion of the available diversity appeared to have remained unsampled from QLD and NT, suggesting that findings emanating from comparisons involving the latter two locations should be treated with some degree of caution. Rarefaction curves demonstrated that the optimum sample size required to accurately represent levels of haplotypic variation, and in turn to confidently discern haplotype relative frequencies, within any given *C. brevipinna*

population is site dependent. For Australian locations, sample sizes in excess of 100 were required for robust comparisons, whereas a sample size of ~60 appeared sufficient for South African waters.

3.5.5 Implications for management & future direction

The generally high genetic diversity reported here in *C. brevipinna* is cause for optimism when considering the management and conservation of this commercially-targeted species in southern Indo-Pacific waters. *Carcharhinus brevipinna* exhibited high haplotype numbers and similar or high haplotypic diversity (n_H = 23, h = 0.5984, n = 208) compared to *C. obscurus* (n_H = 12, h = 0.5224, n = 301) and *C. plumbeus* (n_H = 11, h = 0.2826, n = 440), two closely-related species, off Australia's east coast (*Chapter 2*). Comparatively high haplotype numbers implies that *C. brevipinna* may display a greater resilience to a loss of genetic diversity, as a result of high-intensity fishing pressure, than these other commercially-targeted shark species in Australian waters.

The lower genetic diversity observed in *C. brevipinna* from the south-eastern zone (h = 0.5984), compared to QLD (h = 0.7424) and NT (h = 0.7279), may be accounted for by NSW representing sampling of the species' southern-most distribution limit (Last & Stevens 2009). Range limits are associated with extreme and/or unstable environmental conditions, and have been hypothesised to result in low population density, increased genetic drift and inbreeding and, consequently, lower genetic diversity [e.g. Arnaud-Haond et al. 2006, Lind et al. 2007). Alternatively, lower genetic diversity in NSW may be a consequence of greater harvest pressure in the region. This hypothesis, however, is difficult to support given the absence of robust data permitting a direct comparison of historical harvest levels of *C. brevipinna* between NSW, QLD and NT, as well as a lack of knowledge pertaining to original population sizes and periods of time required to affect quantifiable reductions in genetic diversity.

Our genetic structure results indicate the delineation of two management units for *C. brevipinna* in the southern Indo-Pacific – Australia and South Africa. The most appropriate boundary between these management units, however, is unknown and would require more detailed spatial sampling within the Indian Ocean basin. Our data also suggest, albeit tentatively, two management units within Australian waters – south-eastern (NSW) and northern (QLD and NT) Australia. This implies that, in the event of a population collapse in south-eastern Australia, recovery of genetic diversity would rely largely on reproduction by surviving local individuals in NSW waters. Currently, each Australian state is independently responsible for the management of shark fishing operations occurring within its respective waters, with little to no collaboration across jurisdictional borders. Our results suggest that the independent management of NSW and QLD *C. brevipinna* populations is perhaps appropriate, but that cooperation between QLD and NT would be prudent.

In light of the limitations of the present study, however, we recommend this work be considered as a starting point for evaluations of genetic structure in this commercially-important species, rather than a study upon which definitive management decisions are made. Moreover, we strongly urge future studies to focus on achieving greater population structure resolution via more extensive sampling within Australian waters, as well as throughout this species' global distribution range, in conjunction with analysis of nuclear and/or additional mitochondrial markers. Such studies, conducted in association with active tagging and tracking, would assist with more robust allocations of management units, and hence the sustainable exploitation of this targeted species.

3.6 Acknowledgements

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3.7 Supporting Information - Polymorphic sites for mitochondrial DNA ND4 haplotypes defined for *Carcharhinus brevipinna*.

Haplotype	Nucleotide polymorphism position (1–857)																																								
	54	55	93	106	150	160	168	189	198	216	243	257	289	291	315	326	327	357	358	366	386	426	438	501	514	558	576	582	596	604	642	654	663	736	759	774	804	813	815	816	822
SP1	T	A	T	T	A	T	T	T	G	C	T	T	G	T	C	T	T	G	A	T	C	T	C	C	G	T	T	C	G	C	A	A	T	G	C	T	T	G	G	C	G
SP2	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SP3	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
SP4	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
SP5	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SP6	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
SP7	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.
SP8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
SP9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
SP10	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SP11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
SP12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
SP13	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
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(.) indicates the same nucleotide as in haplotype SP1.

CHAPTER 4. Micro-Computed Tomography: an Alternative Method for Shark Ageing

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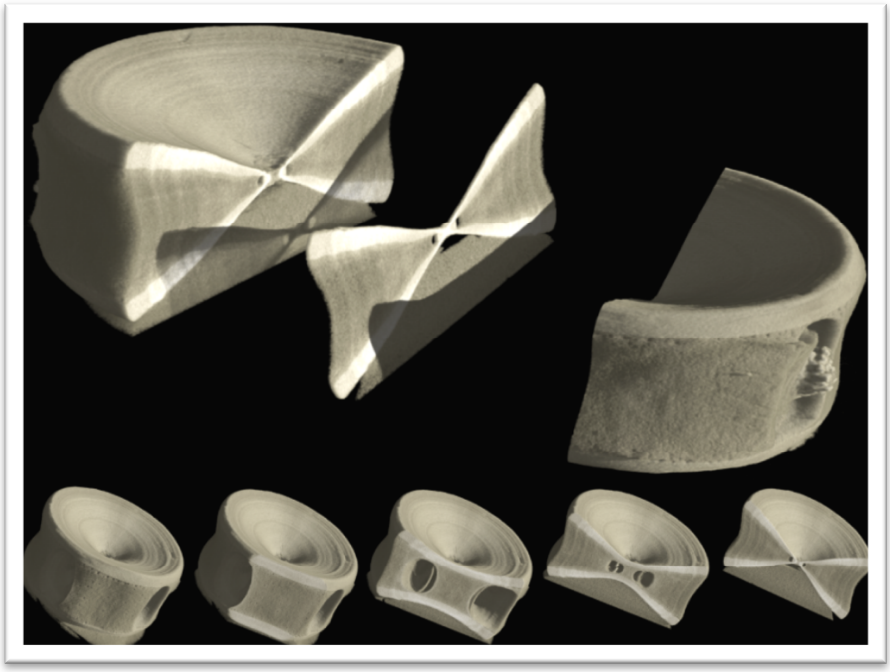

Plate 5. Virtual sectioning of a shark vertebra using microCT technology.

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4.1 Abstract

Micro-computed tomography (microCT) produced 3D reconstructions of shark (*Carcharhinus brevipinna*) vertebrae that could be virtually sectioned along any desired plane, and upon which growth bands were readily visible. When compared to manual sectioning, it proved to be a valid and repeatable means of ageing and offers several distinct advantages over other ageing methods.

4.2 Introduction

Increases in commercial fishing effort targeting sharks have prompted worldwide concern regarding the status of shark stocks, and have highlighted the need for sustainable exploitation through appropriate fishery management (Barker & Schluessel 2005, Baum & Blanchard 2010, Ferretti et al. 2010). Growth rates, natural mortality rates and longevity, and hence the resilience of shark stocks to various levels of fishing mortality, can all be estimated using age and size data. Accurate methods of shark ageing are, therefore, essential for comprehensive assessment and management of exploited shark populations.

Age determination in sharks is most commonly achieved via analysis of growth bands in vertebral centra using a range of specific methods (Cailliet et al. 1983, Cailliet 1990). Techniques such as X-ray imaging (Liu et al. 1999), centrum surface micro-topography (Carlson & Parsons 1997) and staining (Wintner & Cliff 1995, Officer et al. 1996) have been applied to derive age estimates using whole vertebrae. While the suitability of whole vertebrae has been demonstrated for the ageing of young sharks (MacNeil & Campana 2002), accuracy in the cases of older individuals is limited by: (1) difficulties in resolving tightly grouped banding on the outer margins of vertebrae; (2) obscuring of growth bands on

opposing halves due to vertebral geometry; and, (3) variability in birthmark clarity (MacNeil & Campana 2002, Goldman 2004, Cailliet & Goldman 2004). Consequently, the use of whole vertebrae for verification of growth band periodicity via marginal increment and centrum edge analyses is prone to inaccuracy, particularly given the need for precise characterisation and measurement of the critical areas of the centrum outer margin (Cailliet et al. 2006).

In light of these limitations, the analysis of sagittally cut vertebral sections (generally < 0.6 mm thick) has underpinned the majority of shark ageing studies to date. Unenhanced sections have typically produced the best readability across a range of shark species (Wintner et al. 2002, Carlson & Baremore 2005, Carlson et al. 2006, McAuley et al. 2006). Techniques such as calcium-binding stains (Piercy et al. 2007), X-ray micro-radiography (Simpfendorfer et al. 2002, Joung et al. 2008), submersion in ethanol (Bishop et al. 2006), histology (Natanson et al. 1995) and metal substitution (Gelsleichter et al. 1998) have been used, however, in attempts to enhance growth band clarity. Despite its widespread use and acceptance as the preferred method of shark ageing (Goldman 2004), manually obtaining sagittal sections from vertebral centra is a destructive sampling process and is vulnerable to the inherent variability in section quality associated with manual processing.

This study aimed to assess the use of micro-computed tomography (microCT) as a valid and repeatable alternative technique for age determination in a species of carcharhinid. MicroCT utilises X-ray technology to produce image stack reconstructions of 3D objects from which virtual sections can be visualised and extracted at any orientation. The suitability of the microCT method was thus assessed via direct comparisons between manually cut sagittal sections and three-dimensional virtual sections imaged from whole vertebrae, across a range of sizes and ages of shark.

4.3 Materials & methods

The spinner shark *Carcharhinus brevipinna* (Müller & Henle 1839), a species distributed widely throughout warm temperate and tropical shelf waters of the world (Last & Stevens 2009) and caught commercially in coastal waters of eastern Australia, was the fish studied. A total pool of 166 *C. brevipinna* was separated according to three vertebral diameter size classes: 12-16, 20-24 and 28-32 mm (measured using digital callipers). These vertebral size classes were chosen to correspond with two vertebrae diameter frequency histogram modes from observed commercial catch data from 2008 to 2009 (WG Macbeth unpublished data). For each size class, vertebrae samples from eight individuals were randomly selected, providing a total of 24 individuals for assessment. The selected individuals ranged in size from 132 to 257 cm total length (L_T) and had a male:female ratio of 1.4:1.

From each *C. brevipinna*, a section of three to five vertebrae was sampled from the cervical region of the vertebral column (i.e. anterior to the first dorsal fin), stored on ice and then frozen upon return to the laboratory. In preparation for ageing, vertebrae samples were thawed, manually cleaned of excess soft tissue, separated into individual centra and soaked in a 5 % sodium hypochlorite solution until all remaining soft tissue had been removed. Soak time varied from 15 to 45 min depending on the size of the centra. Cleaned vertebrae were rinsed thoroughly in tap water and then stored in 70 % ethanol.

One vertebra from each *C. brevipinna* was chosen at random, removed from the alcohol and air-dried in preparation for scanning. Specimens were scanned using an Xradia (www.xradia.com) MicroXCT-400 X-ray micro-tomography system. The scanning system was set to a source energy of 120 keV, with a flux of 83 µA for all scans. To provide some phase enhancement to the resulting tomographic projections, the source and ×0.5 scintillator or objective were set at 150 and 200 mm from the specimen, respectively. This scanning

geometry resulted in a pixel size of 24.41 μm with the cooled CCD camera being used in its binning 2 mode, and tomographic projection images of 1024 × 1024 with a field of view of 25 × 25 mm. The camera exposure was set at 1.0 s and a total of 360 projection images were obtained during each scan of *c.* 42 min. All projection images were at 16 bit grey-scale depth and the resulting raw X-ray projection file was 4.1 GB in size.

Projection image data sets were reconstructed into axial-slice image stacks using a filtered back-projection algorithm implemented in graphical processing unit (GPU) hardware and supplied with the scanner. Corrections were made for rotational misalignments (i.e. centre shift), beam-hardening and ring artefacts. The resulting reconstructed image stacks were of variable thickness depending on the size of the vertebrae: 400 slices for small, 512 slices for medium and 670 slices for the large. Average reconstruction times were < 5 min for all the specimens.

The data visualisation software VG Studio Max 1.2 (Volume Graphics; www.volumegraphics.com) was used to visualise the axial slice stacks in full 3D context. This software permitted complete 3D visualisation and facilitated extraction of virtual sections at any orientation through the specimen using digital clipping planes. For quantitative ageing assessment, virtual sections clipped along the sagittal plane to include the vertebral focus were extracted from all vertebral specimens.

Following microCT scanning, the same vertebral centra were sagittally sectioned to 0.5-0.6 mm thickness using an Isomet low-speed diamond blade saw (www.buehler.com). Sections were fixed to a glass slide with waxed resin and examined under reflected light using an Olympus SZ dissecting microscope fitted with digital camera (http://microscope.olympus-global.com/).

Growth bands were counted on microCT virtual sections and manually cut sections on two independent occasions (readings 1 and 2) by one reader without prior knowledge of the size of each individual *C. brevipinna*. A growth band was defined as a band-pair, comprising one opaque and one translucent band (Cailliet et al. 2006). For the purposes of this study, the term age count is used to denote estimates of age based on the assumption of annual band-pair deposition in the absence of age validation for this species. Age counts were derived by counting fully formed translucent bands occurring after the birthmark; the latter being denoted by an angle change on the centrum face (Goldman 2004). The readability of each microCT virtual and manually cut section was scored according to the following definitions: 1, all growth bands well defined and visible; 2, almost all bands visible, clear interpretation possible; 3, most bands visible, interpretation reliable to within ± 1; 4, bands visible, majority difficult to interpret; 5, unreadable.

A combination of methods was used to evaluate bias and precision in age counts between-reading and between-method (Cailliet & Goldman 2004). Bias was investigated using age-bias plots and Bowker's test of symmetry to determine whether observed count differences were systematic or due to random error (Hoenig et al. 1995, Campana 2001). Precision estimates were calculated using the coefficient of variation (c.v.) (Chang 1982). Age counts obtained from reading 1 were used for between-method analyses.

4.4 Results

MicroCT scanning produced high-resolution, 3D images representative of the four vertebral ageing templates employed in the literature: whole vertebrae, radiograph, half vertebrae, and sagittal section (Figure 4.1). The quality and resolution of microCT output were sufficiently high such that growth bands were visible for each of the four image types.

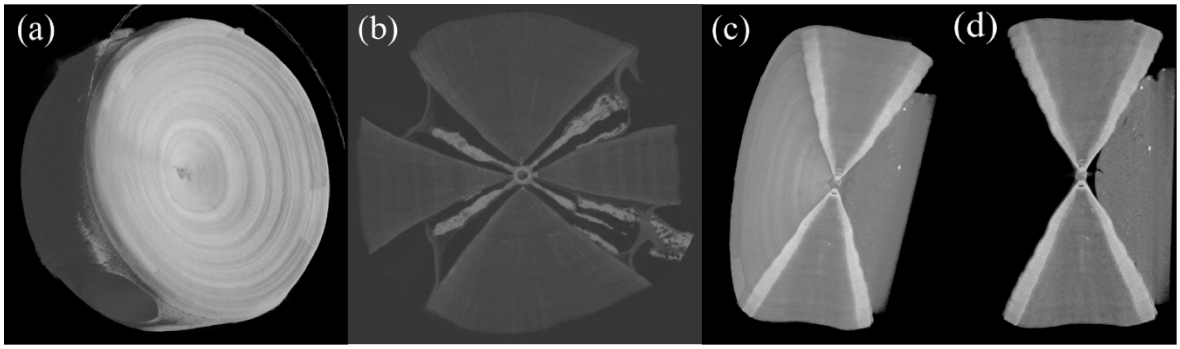

Figure 4.1 Reconstructed 3D virtual images of (a) a whole vertebra, (b) a radiograph, (c) a half vertebra and (d) a sagittal section of *Carcharhinus brevipinna* following microCT scanning of one vertebra.

All microCT virtual sections had discernible growth bands extending along the corpus calcareum from the birthmark to the centrum edge that were directly comparable to those on manually cut sections (Figure 4.2).

Growth band clarity was similar between methods, with mean ± S.E. readability for microCT and manual sections scored as 2.6 ± 0.1 and 2.8 ± 0.1, respectively.

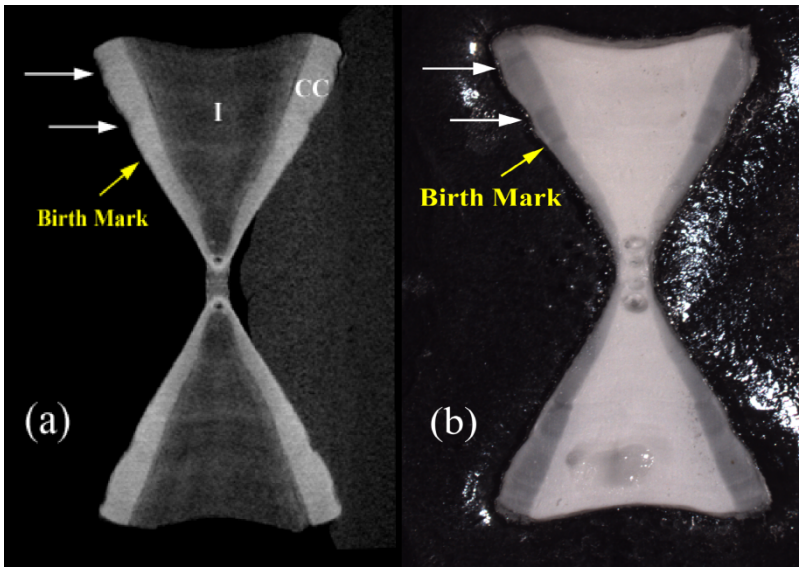

Figure 4.2 Visual comparison of a (a) microCT virtual section and (b) manually cut section from the same vertebra of *Carcharhinus brevipinna*. White arrows, fully formed translucent growth bands. I, intermedialia; CC, corpus calcareum.

Age-bias plots and Bowker's test of symmetry identified no systematic between-reading bias in age counts for manually cut sections ($\chi^2 = 9.33$, df = 9, $p > 0.05$) and microCT virtual sections ($\chi^2 = 9$, df = 10, $p > 0.05$) (Figure 4.3a, b) or between-method bias ($\chi^2 = 13$, df = 12, $p > 0.05$) (Figure 4.3c) across the age range 2-19 years. Precision estimates were considered acceptable (c.v. values < 11) for all three comparisons (Figure 4.3) (Campana 2001).

4.5 Discussion

While microCT is already an established technology for imaging mineralised animal tissues (Neues & Epple 2008), this study marks its first application to the ageing of elasmobranchs. MicroCT-generated sections provided comparable and repeatable age counts relative to manually produced sections across a wide age range of *C. brevipinna*. The microCT method, like manual sectioning, is capable of resolving tight banding on the centrum outer edge, a critical factor in the accurate ageing of older individuals and calculation of marginal increment ratios. In the case of larger vertebrae, microCT was observed to improve growth band resolution in the intermedialia, particularly near to the centrum edge, when compared to manual sections, although this coincided with comparably reduced readability along the corpus calcareum (see Figure 4.2 for vertebral zone locations).

This research identified several distinct advantages of microCT over manual sectioning. First, it is a non-destructive technique that can provide a reliable age count without affecting the structural integrity of the vertebral sample. While it therefore permits unlimited multiple virtual sectioning from unlimited angles and perspectives, researchers would need to maintain consistency with respect to the angle and perspective used among vertebrae when ageing individuals of a particular species. This method also permits an archive of the intact vertebrae should novel vertebral analysis techniques involving whole vertebrae be developed in the

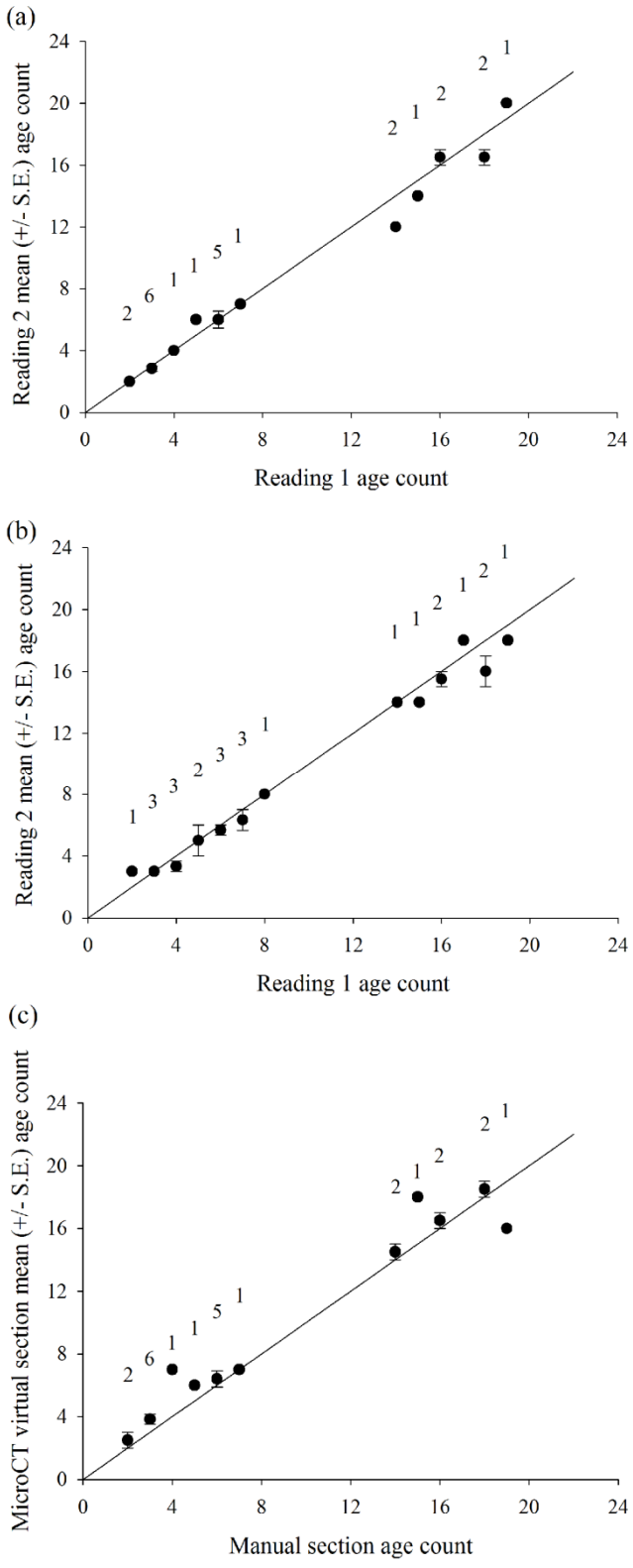

Figure 4.3 Age-bias plots of *Carcharhinus brevipinna* vertebral age counts from (a) two independent readings of manually cut sections (c.v. = 5.58), (b) two independent readings of microCT virtual sections (c.v. = 7.00) and (c) two independent methods (c.v. = 10.7). Sample sizes and one-to-one equivalence lines are shown.

future. Second, variables inherent to manual processing such as section width and location are eliminated, as the digital sectioning of the virtual vertebra can be precisely specified at the desired width or location. Third, microCT eliminates the need to adjust light source and light angle during reading, a potential source of between-reader variability. Finally, although cleaned prior to scanning in this study, the low intrinsic X-ray contrast of non-mineralised tissues (Metscher 2009) means that vertebral samples can be scanned in an uncleaned state without affecting the quality or resolution of the microCT output, hence substantially reducing sample processing time.

Owing to limited financial resources, this research was performed on only 24 vertebral samples from only one carcharhinid species. Optimal ageing methods can, however, be species-specific (Cailliet & Goldman 2004), and so a more robust methodology for evaluating any given shark ageing technique would encompass larger sample sizes and, ideally, more than one species. The nature of the microCT method is such that a longer scan-time (or greater number of projection images) translates to higher-quality output, but at correspondingly higher cost. During this study a compromise was reached between scan time (and therefore cost) and data quality resulting in growth band clarity being comparable to manually prepared sections. Employment of a longer scan time per vertebral sample would, however, probably have improved virtual section readability.

4.6 Acknowledgements

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CHAPTER 5. Age and Growth Parameters for Three Heavily Exploited Shark Species off Temperate Eastern Australia

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Plate 6. A mature male, 307 cm L_T dusky shark (*Carcharhinus obscurus*) captured during commercial shark-fishing activities in eastern Australian waters and aged in the present study at 17 years.

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5.1 Abstract

The removal of large predatory sharks from the world's oceans poses profound threats to marine community structure and species conservation. Effective management of exploited shark stocks requires a sound understanding of the life histories of target species. Here we provide the first assessment of age and growth for *Carcharhinus brevipinna* in Australian waters, and for *Carcharhinus obscurus* and *Carcharhinus plumbeus* in eastern Australian waters, based on interpretations of vertebral growth bands. In doing so, we provide arguably among the most robust growth parameters to date for the abovementioned taxa on the bases of genetic validation and sample size and distribution, but acknowledge equally a range of limitations – most notably those associated with vertebral ageing and our lack of age validation. Comparatively, the three species displayed both contrasts and consistencies in their growth characteristics off Australia's south-east coast. For all three sharks, rates of growth were greatest in the years immediately after birth, males grew more rapidly than females in the juvenile phase, and females were observed to grow larger, live longer and were generally larger at any given age. Longevity and all modelled growth parameters (L_∞, k and L_0), however, differed among the three species, and appeared to challenge the findings for conspecific populations in other parts of the world. The validity of these latter comparisons is, however, compromised by a range of confounding factors. Nevertheless, we provide the least conservative k estimates for *C. obscurus* and *C. plumbeus* of those previously reported, and extend maximum age estimates for *C. brevipinna*. In this way, our results have important implications for the assessment of natural mortality, productivity, and hence resilience to stock depletion, in these species in south-eastern Australian waters.

5.2 Introduction

Apex predators play a fundamental role in regulating species abundance and community structure in ecosystems (Ritchie & Johnson 2009). The removal of such organisms, via natural or anthropogenic causes, can induce profound and complicated cascading impacts on lower trophic levels – as has been demonstrated in terrestrial (Moreno et al. 2006, Beschta & Ripple 2009, Wallach et al. 2010) and marine environments (Myers et al. 2007, Baum & Worm 2009). Biological traits such as slow growth rate, long life span, late onset of maturity and low reproductive output render many apex predators vulnerable to rapid population decline and slow rates of recovery (Musick 1999, Purvis et al. 2000, Webb et al. 2002, Field et al. 2009). This is exemplified for oceanic species such as sharks, where continued overexploitation has led to the depletion of virgin stocks in many parts of the world (e.g. Baum et al. 2003, Ferretti et al. 2008). While levels of decline are highly debatable (Burgess et al. 2005), there is nevertheless widespread consensus regarding the need for effective shark fishery management and conservation (Barker & Schluessel 2005).

Dusky (*Carcharhinus obscurus*), spinner (*Carcharhinus brevipinna*) and sandbar (*Carcharhinus plumbeus*) sharks are three large-medium sized carcharhinids found throughout much of the world's tropical and warm-temperate coastal and continental-shelf waters (Last & Stevens 2009). Highly sought-after for their fins (Clarke et al. 2006), all three species are important components of commercial and artisanal catches in multi-species shark fisheries across the globe (e.g. Amorim et al. 1998, Castillo-Géniz et al. 1998, McVean et al. 2006, Henderson et al. 2007, White 2007, Morgan et al. 2009, Manojkumar et al. 2012). Recreational catches and rates of by-catch in non-target fisheries are also suspected to be substantial but, as for most shark species, they remain largely unquantified (Bonfil 1994).

Carcharhinus obscurus, *C. brevipinna* and *C. plumbeus* are highly vulnerable to overfishing and human-induced habitat alteration due to their life-history traits (e.g.

Simpfendorfer et al. 2002, Capapé et al. 2003, Carlson & Baremore 2005, Dudley et al. 2005, McAuley et al. 2006, Baremore & Hale 2012, *Chapter 6*), susceptibility to multiple harvest methods and utilisation of inshore nursery habitat for neonate and juvenile development (e.g. Thorpe et al. 2004, Conrath & Musick 2007, Taylor & Bennett 2013). Consequently, the sustainability of targeted fishing activities exploiting *C. obscurus* and *C. plumbeus* in particular has been subject to considerable scrutiny in recent years (e.g. Sminkey & Musick 1996, McAuley et al. 2005, 2007a, Cortés et al. 2006, Romine et al. 2009, Anon. 2011a, 2011b), resulting in global IUCN classifications of 'vulnerable' for both species (Musick et al. 2009a, 2009b). Some populations have experienced greater levels of fishing mortality than others. In the NW Atlantic, for example, *C. obscurus* is regionally listed as 'endangered' (Musick et al. 2009a), and declines of up to 64-99 % in *C. obscurus* and *C. plumbeus* stocks are purported (Cortés et al. 2006, Myers et al. 2007, Baum & Blanchard 2010). Comparatively, *C. brevipinna* is considered of less conservation concern in spite of similar life-history traits, and is globally IUCN listed as 'near threatened' (Burgess 2009).

In Australian waters, the three study species are actively targeted along the eastern, northern and western coastlines, as well as the southern coastline in the case of *C. obscurus*, with capture typically via demersal longlines, demersal and pelagic gillnets, and handlines (Simpfendorfer & Donohue 1998, Macbeth et al. 2009, Harry et al. 2011a, Tillett et al. 2012a, Rogers et al. 2013). Dramatic increases in commercial catches of these species have been reported from Australia over recent decades. For example, a six-fold increase in landings of *C. obscurus* [~100 to 600 tonnes (t)] and a four-fold increase in landings of *C. plumbeus* (~100 to 415 t) were reported from Western Australian waters between 1980 and 1990, and 1995 and 2004, respectively (McAuley 2006a, 2006b). Despite extensive management measures having been implemented in this region (Simpfendorfer & Donohue 1998), underestimation of both species' vulnerability to fishing mortality failed to halt unsustainable

fishing levels and declining stocks (McAuley et al. 2007a). Off Australia's south-eastern seaboard, a three-fold increase in total shark catch (152 to 457 t) was recorded between 2005 and 2007 by the New South Wales Ocean Trap and Line Fishery (NSW OTLF), where *C. plumbeus*, *C. obscurus* and *C. brevipinna* were the three most abundantly caught species respectively (Macbeth et al. 2009). During this time, shark fishing associated with the NSW OTLF was managed by input controls limiting the number of potential participants but was not subject to restrictions on the volume of catch able to be taken, highlighting the urgent need for assessment of shark exploitation and management arrangements off Australia's south-east coast.

Effective management of exploited shark populations requires a sound understanding of the life history of target species. For example, robust estimates of age provide a basis for determining other pertinent parameters such as longevity, growth rate, natural mortality, and hence resilience to various levels of fishing pressure (Goldman 2004). Cosmopolitan distributions and commercial importance have led to numerous ageing studies on *C. obscurus*, *C. brevipinna* and *C. plumbeus*. Age and growth parameters are available for all three species from the Indian Ocean and NW Atlantic (Casey et al. 1985, Branstetter 1987, Casey & Natanson 1992, Natanson et al. 1995, Sminkey & Musick 1995, Natanson & Kohler 1996, Allen & Wintner 2002, Carlson & Baremore 2005, McAuley et al. 2006, Hale & Baremore 2010) as well as from the W Pacific for *C. brevipinna* and *C. plumbeus* (Joung et al. 2004, 2005), and central Pacific for *C. plumbeus* (Romine et al. 2006). In Australian waters, validated age and growth studies have been conducted on *C. obscurus* (Simpfendorfer et al. 2002) and *C. plumbeus* (McAuley et al. 2006) off the west coast. While the propensity for vertebrae to underestimate age in large adult sharks is purported (Francis et al. 2007, Andrews et al. 2011), the abovementioned studies revealed all three to be long lived species, exhibiting generally slow rates of growth and conforming to the patterns outlined by Cortés et al. (2000)

– i.e. initially faster growth in males than females; females growing older and to larger sizes than males; and growth rates for both sexes fastest during the juvenile stage.

Although the growth dynamics of *C. obscurus*, *C. plumbeus* and *C. brevipinna* have been widely documented across much of their respective distribution ranges, many such studies report biologically unrealistic growth parameters. Most notably, estimates of theoretical asymptotic length (L_∞) are typically overestimated, translating to underestimates of the growth coefficient (k). Inaccuracies such as these have profound implications for demographic analyses and population models, and generally stem from sampling biases. Nonetheless, life-history characteristics have been reported to vary among conspecific shark populations (Lombardi-Carlson et al. 2003, Driggers et al. 2004, Cope et al. 2006, Harry et al. 2011b). Accurate age and growth parameters specific to both geographically- and genetically-distinct populations, therefore, are critical for informed regional fishery management.

In south-eastern Australian waters, life-history information on the three study species (and all exploited carcharhinids for that matter) is currently undefined. The objective of the present study, therefore, was to provide the first detailed assessment of the age and growth of *C. brevipinna* in Australian waters, and of *C. obscurus* and *C. plumbeus* in eastern Australian waters, based on interpretations of vertebral growth bands.

5.3 Methods

5.3.1 Sample collection and genetic validation

Vertebrae samples were collected between November 2007 and September 2010 by scientific-observers on-board commercial shark-fishing vessels operating off Australia's New South Wales (NSW) coast between Tweed Heads (28° 4' S) and Sydney (34° 3' S) (Figure 5.1). All animals were sexed and recorded for total (L_T), fork (L_F) and pre-caudal lengths (L_{PC}) to the nearest centimetre (cm).

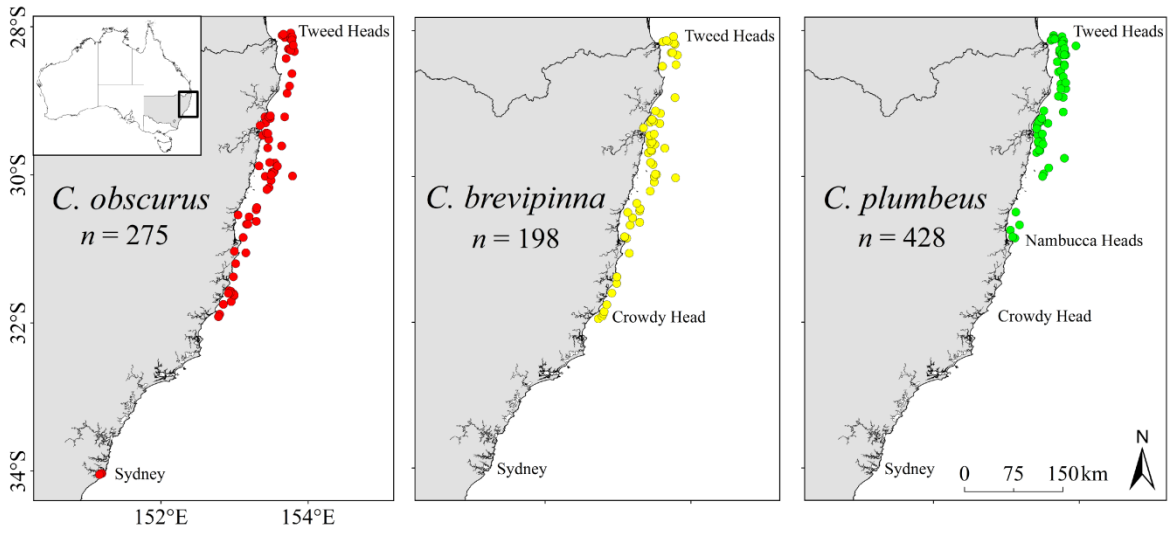

Figure 5.1 Study area and capture location for individual sharks aged.

Owing to the morphological similarities among carcharhinids, a small quantity (< 2 g) of white muscle tissue was collected from each individual and tested, using mitochondrial DNA, to validate species identity. Vertebrae and data associated with misidentified individuals were excluded from analyses.

Morphometric relationships between L_T, L_F and L_{PC} were determined using linear regression analyses, with male and female relationships statistically compared using analyses of co-variance (ANCOVA) (Table 5.1).

Table 5.1 Morphometric relationships (cm) for *Carcharhinus obscurus*, *Carcharhinus brevipinna* and *Carcharhinus plumbeus* in NSW waters. ANCOVA revealed no statistical difference between male and female length relationships for any of the species, thus regression equations represent combined sexes. All relationships were linear and highly significant ($p < 0.001$). L_T, L_F and L_{PC} denote total, fork and pre-caudal lengths, respectively.

Species	Equation	*n*	r^2	ANCOVA		
				F	df	*p*
C. obscurus	$L_T = 1.305 \cdot (L_{PC}) + 8.021$	255	0.99	0.086	253	0.770
	$L_T = 1.203 \cdot (L_F) + 4.226$	236	0.99	0.004	234	0.951
C. brevipinna	$L_T = 1.286 \cdot (L_{PC}) + 6.208$	183	0.99	0.668	181	0.415
	$L_T = 1.188 \cdot (L_F) + 3.519$	191	0.99	1.178	189	0.279
C. plumbeus	$L_T = 1.316 \cdot (L_{PC}) + 4.566$	424	0.98	0.406	422	0.525
	$L_T = 1.206 \cdot (L_F) + 2.747$	427	0.98	0.820	425	0.366

5.3.2 Vertebrae preparation & ageing protocol

A section of 3-5 vertebrae was sampled from the cervical region of the vertebral column (i.e. anterior to the first dorsal fin) of each shark, stored on ice, and frozen upon return to the laboratory. In preparation for ageing, vertebrae samples were thawed, manually cleaned of excess soft tissue, separated into individual centra, and soaked in a 5 % sodium hypochlorite solution (bleach) until all remaining soft tissue had been removed. Soak time varied from 15-45 min depending on the size of the centra. Cleaned vertebrae were rinsed thoroughly in tap water and stored in 70 % ethanol. One vertebra from each shark was chosen at random, removed from the alcohol and air-dried in preparation for sectioning. Centra were sagittally sectioned through the focus to 0.5-0.6 mm thickness using an Isomet low-speed diamond-blade saw.

To determine the best vertebra preparation method, trials were conducted comparing unstained sections to sections stained with alizarin red and crystal violet. MicroCT scanning was also investigated as an alternative visualisation technique (Geraghty et al. 2012 – *Chapter 4*). All four methods produced comparable section readability, however neither method noticeably enhancing growth-band clarity relative to the other. For practicality, unstained sections were employed for ageing analysis.

Unstained sagittal sections were fixed to a glass slide with waxed resin, and examined under reflected light on a dark background using an Olympus SZ dissecting microscope fitted with digital camera. Growth bands were counted by two independent readers (Reader 1 and Reader 2) without prior knowledge of the size, sex or date of capture of the subject. Reader 1 was experienced in shark ageing methods and interpretation, while Reader 2 was relatively inexperienced. Digital images were taken of each vertebral section, and growth bands were independently marked by both readers using ImageJ. Archived images of both readers' ageing interpretations permitted accurate review. A growth band was defined as a band-pair,

comprising one opaque and one translucent band (Cailliet et al. 2006). For the purpose of this study, the term *age count* is used to denote estimates of age based on annual band-pair deposition; the latter having been validated for *C. obscurus* (Simpfendorfer et al. 2002) and *C. plumbeus* (McAuley et al. 2006) in Australian waters, but has been assumed here for *C. brevipinna* in the absence of age validation for this species. Age counts were derived by counting fully-formed translucent bands along the corpus calcareum occurring after the birth mark; the latter being denoted by an angle change on the centrum face (Goldman 2004) (Figure 5.2). The readability of each vertebral section was scored according to the following definitions: 5, all growth bands well defined and visible; 4, almost all bands visible, clear interpretation possible; 3, most bands visible, interpretation reliable to within ± 1; 2, bands visible, majority difficult to interpret; 1, unreadable. All sections deemed unreadable were excluded from further analyses. Age counts in agreement between readers were adopted as the final age count for those vertebral sections. For all sections where there was disagreement between readers, a final age count was decided upon by the more experienced reader (Reader 1) following an interactive review and evaluation of both readers' interpretations.

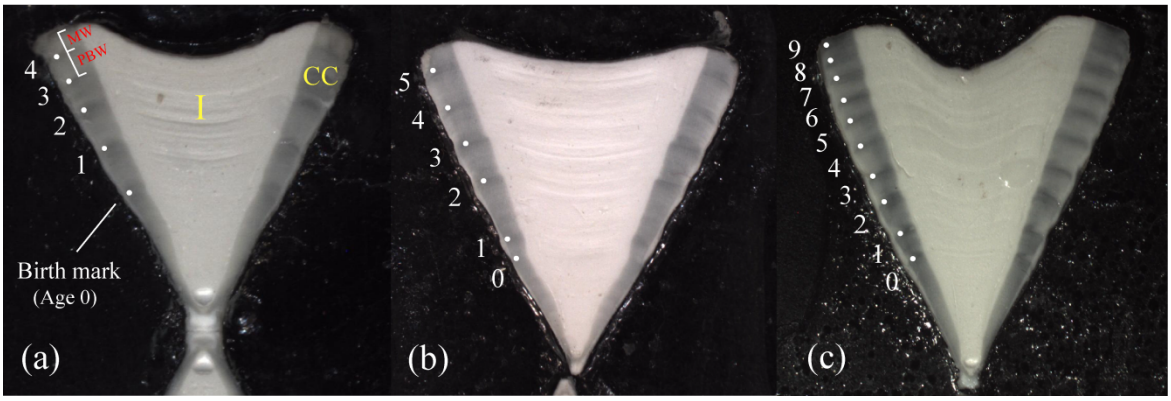

Figure 5.2 Unstained sagittal sections from a (a) 4+ year old, 145 cm total length (L_T) male *Carcharhinus plumbeus*; (b) 5+ year old, 176 cm L_T female *Carcharhinus brevipinna*; and, (c) 9+ year old, 245 cm L_T female *Carcharhinus obscurus*. Fully-formed translucent bands occurring after the birth mark are marked with white dots. All three sections were scored a readability of 5. I, intermedialia; CC, corpus calcareum; MW, margin width; PBW, previous band width.

5.3.3 Between-reader bias and precision

A combination of methods was used to evaluate bias and precision in age counts between readers (Cailliet & Goldman 2004). Bias was investigated using age-bias plots and Bowker's test of symmetry to determine whether observed count differences were systematic or due to random error (Campana et al. 1995, Hoenig et al. 1995, Campana 2001). Inter-reader precision estimates were calculated using the co-efficient of variation (c.v.) (Chang 1982) and percentage agreement (PA) (Goldman 2004).

5.3.4 Growth modelling

The von Bertalanffy growth function (von Bertalanffy 1983) has been the model most applied for describing growth in elasmobranchs (Cailliet & Goldman 2004), however studies comparing the performance of multiple models have demonstrated others to be more appropriate in some shark species (Carlson & Baremore 2005, Natanson et al. 2006, Barreto et al. 2011). Six candidate models, therefore, were fitted to observed length-at-age data for each species. Modified, three-parameter forms of the von Bertalanffy (VB-3), Gompertz (GOM-3) and logistic (LOGI-3) growth models were given by the following equations, where L_a is observed length at age a and L_0 (length-at-birth), L_∞ (theoretical asymptotic length) and k (growth coefficient) are fitted parameters:

(VB-3) $$L_a = L_0 + (L_\infty - L_0)\left(1 - e^{(-ka)}\right)$$ (Simpfendorfer et al. 2000)

(GOM-3) $$L_a = L_0 \left(e^{\ln\left(\frac{L_\infty}{L_o}\right)\left(1-e^{(-ka)}\right)}\right)$$ (Braccini et al. 2007)

(LOGI-3) $$L_a = \frac{L_\infty \cdot L_0 \cdot e^{(ka)}}{L_\infty + L_0\left(e^{(ka)}-1\right)}$$ (Thorson & Simpfendorfer 2009)

Two-parameter versions of the above equations were also computed (VB-2, GOM-2 & LOGI-2) by substituting L_0 for a fixed length-at-birth value. Empirical L_0 values for each species were estimated to be between the largest observed embryos and the smallest free-swimming individuals encountered during this study: 94 cm L_T for *C. obscurus*, 80.5 cm L_T for *C. brevipinna* and 71 cm L_T for *C. plumbeus* (*Chapter 6*). Models were fitted using the method of non-linear least squares in the statistical package R (R Development Core Team 2010). Please note, the parameter L_∞ is common to all models, however k and fitted (as opposed to empirical) L_0 are not directly comparable between growth-model families.

A multi-model inference (MMI) information-theoretical approach was used to determine the most appropriate growth model for each species (Burnham & Anderson 2001, Katsanevakis & Maravelias 2008, Harry et al. 2011b). Model performance was evaluated using Akaike's information criteria (AIC), with the best-fit model displaying the lowest AIC value. AIC differences were calculated as $\Delta_i = y_i - x\text{min}$ and used to rank the support of the remaining models (i = 1-6) relative to the best model. Models with Δ of 0-2 had substantial support; models with Δ of 4-7 had considerably less support; models with Δ > 10 essentially no support (Burnham & Anderson 2002). Akaike weights (w_i) were calculated as the weight of evidence in favour of a model being the best in the set of candidate models (Burnham & Anderson 2002). The 95 % confidence intervals (C.I.) around the best-fit parameter estimates were derived from 10,000 re-sampled data sets.

5.3.5 Growth-band periodicity

Verification of growth-band periodicity was achieved via marginal increment analysis. Only sections displaying clearly defined, unambiguous growth bands on the centrum outer margin were included. Marginal increment ratios (MIR) were calculated using the following equation, with means (± S.E.) subsequently plotted against month:

$$MIR = {}^{MW}/_{PBW}$$ (Conrath et al. 2002);

where MW = margin width and PBW = previous band pair width (see Figure 5.2).

5.4 Results

5.4.1 Carcharhinus obscurus

Carcharhinus obscurus was caught along the NSW coast between Tweed Heads and Sydney (Figure 5.1). Vertebrae from 275 genetically-confirmed individuals, ranging in size from 92-386 cm L_T, were sectioned and read. Specimens sampled for both sexes were predominantly large (> 270 cm L_T), although some small individuals were also obtained (Figure 5.3a).

Vertebral growth-band readability was generally high in individuals ≤ 270 cm L_T and comparatively low in individuals > 270 cm L_T (Figure 5.4a). Overall mean (± S.E.) readability was moderate (2.6 ± 0.05). Eighteen sections were deemed unreadable and were excluded from further analyses. Growth was therefore examined using observed length-at-age data from 257 individuals (126 females and 131 males), with lengths ranging from 99-386 cm L_T for females and 92-356 cm L_T for males.

An age-bias plot and Bowker's test of symmetry identified no systematic bias in age counts between Reader 1 and 2 ($\chi^2 = 80.5$, df = 68, $p > 0.05$) (Figure 5.5a). Overall inter-reader precision was high (c.v. = 7.48) (Campana 2001), despite percentage agreement (PA) being < 30 % (Figure 5.5a, Supplementary material A). Agreement with the final age count was 72.4 % for Reader 1 and 37.0 % for Reader 2.

Marginal increment analysis provided evidence for annual band-pair deposition commencing in mid-winter. Marginal increment ratios peaked in autumn (March-May) and

remained high in early winter (June), but were comparatively small in late-winter (August) and spring (September-November) (Figure 5.6a).

All six growth models provided good fits of the observed length-at-age data for both sexes (Figure 5.7a). Statistically, the three-parameter von Bertalanffy (VB-3) growth function was the best model for describing female *C. obscurus* growth in NSW waters, with L_∞, k and L_0 estimated at 365.03 cm L_T, 0.083 and 107.03 cm L_T, respectively (Table 5.2a). The two-parameter von Bertalanffy (VB-2) model was considered the best for describing male growth, with L_0 fixed at 94 cm L_T and L_∞ and k estimated at 336.28 cm L_T and 0.108, respectively (Table 5.2a).

Observed mean length-at-age varied between sexes (Supplementary material B). At most ages, females were larger than males. Predicted length-at-age, however, suggested less contrast between males and females, with both sexes similar in size for the first 17 years of life (Figure 5.8, Supplementary material B). Females and males displayed similar longevities, with the oldest observed *C. obscurus* a 359 cm L_T female aged at 33 years and the oldest observed male a 347 cm L_T individual aged at 32 years (Figures 5.7a, 5.8).

Analysis of modelled yearly growth increments suggested males grow at a faster rate than females for the first eight years of life, after which females grow faster than males (Figure 5.9, Supplementary material B). For both sexes, growth was greatest in the first year following birth (Figure 5.9, Supplementary material B).

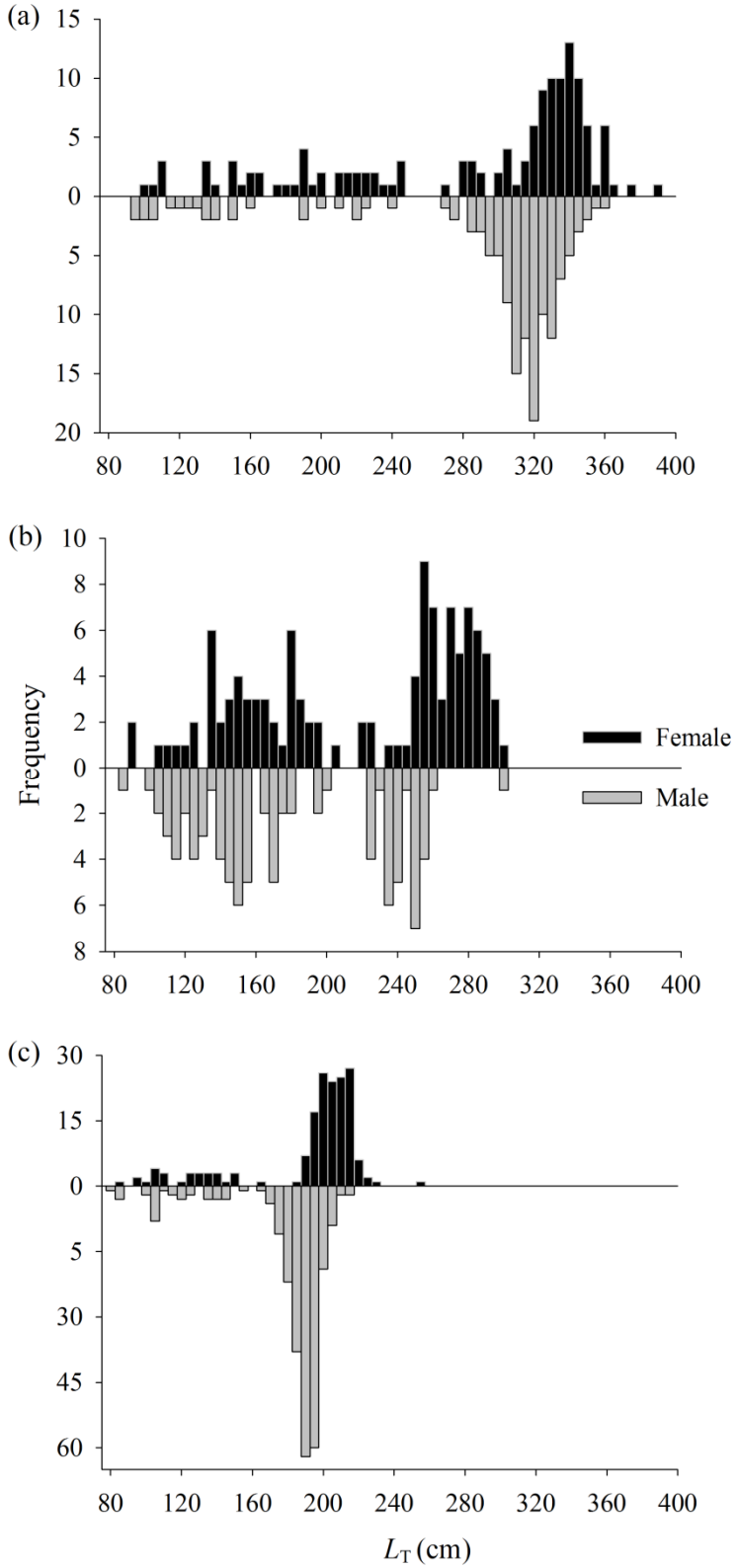

Figure 5.3 Length-frequency distributions, demonstrating differences in attainable size, of (a) *Carcharhinus obscurus* (*n* = 275), (b) *Carcharhinus brevipinna* (*n* = 198) and (c) *Carcharhinus plumbeus* (*n* = 428) specimens aged via vertebral analysis.

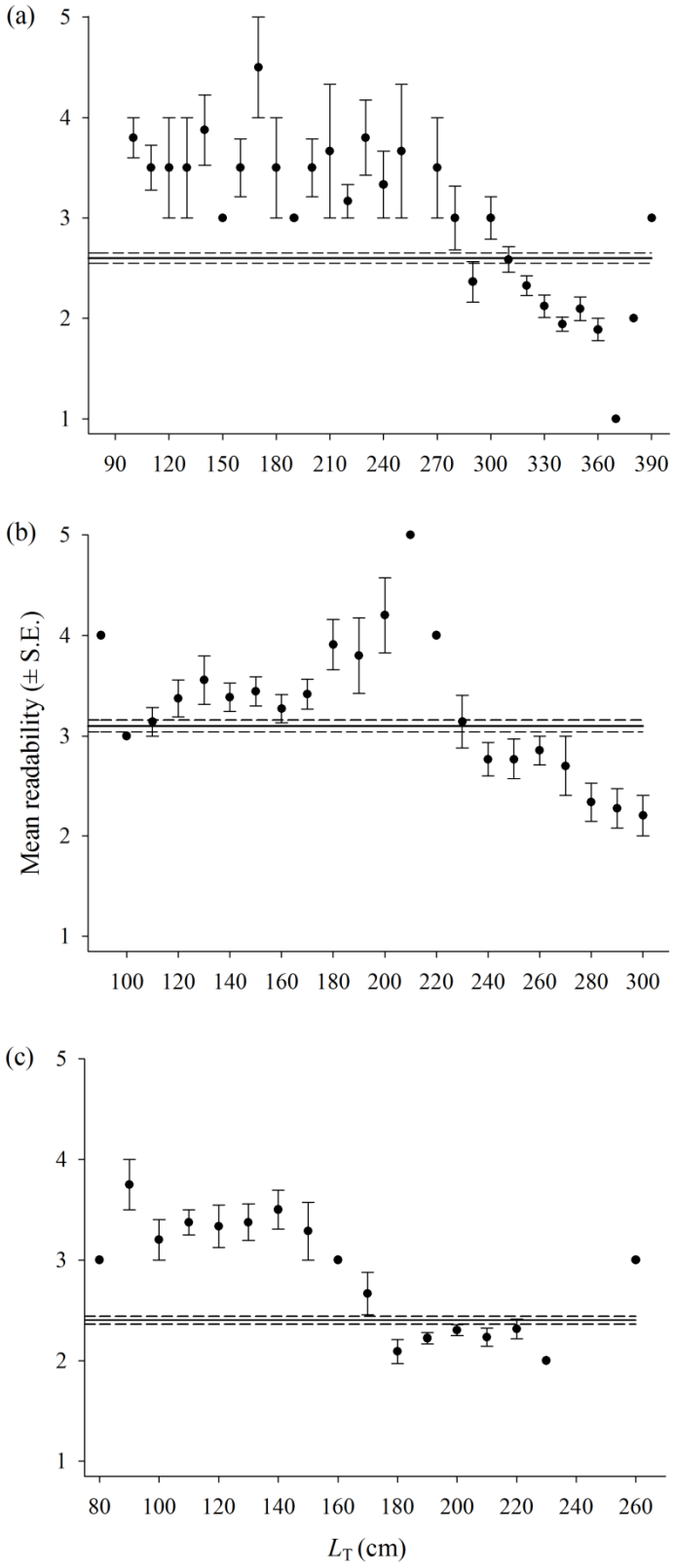

Figure 5.4 Mean readability (± S.E.) by total length (L_T) for (a) *Carcharhinus obscurus* (*n* = 275), (b) *Carcharhinus brevipinna* (*n* = 198) and (c) *Carcharhinus plumbeus* (*n* = 428). Solid and dashed lines represent overall mean readability and upper and lower standard errors, respectively.

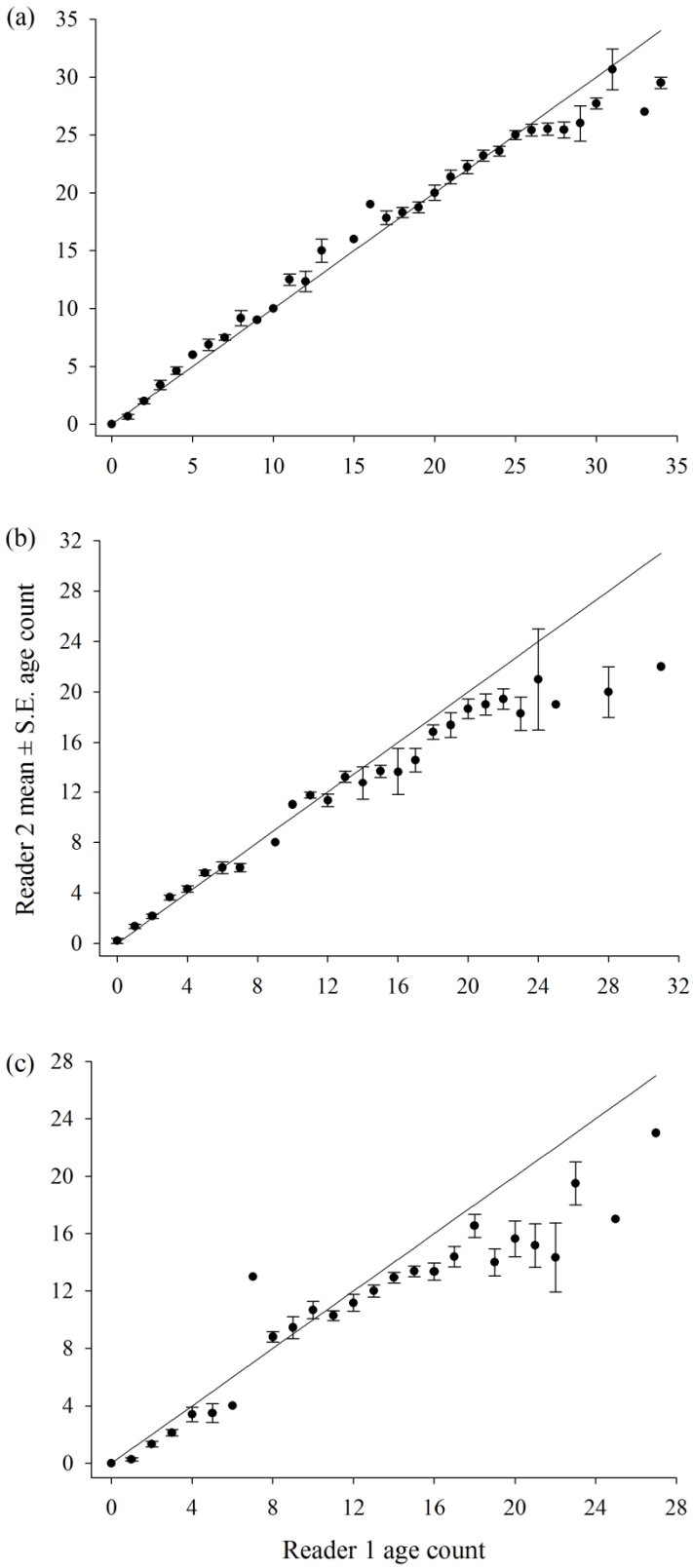

Figure 5.5 Between-reader age-bias plots of (a) *Carcharhinus obscurus* (*n* = 257, c.v. = 7.48), (b) *Carcharhinus brevipinna* (*n* = 195, c.v. = 12.6) and (c) *Carcharhinus plumbeus* (*n* = 393, c.v. = 19.8) vertebral age counts. One-to-one equivalence lines are shown.

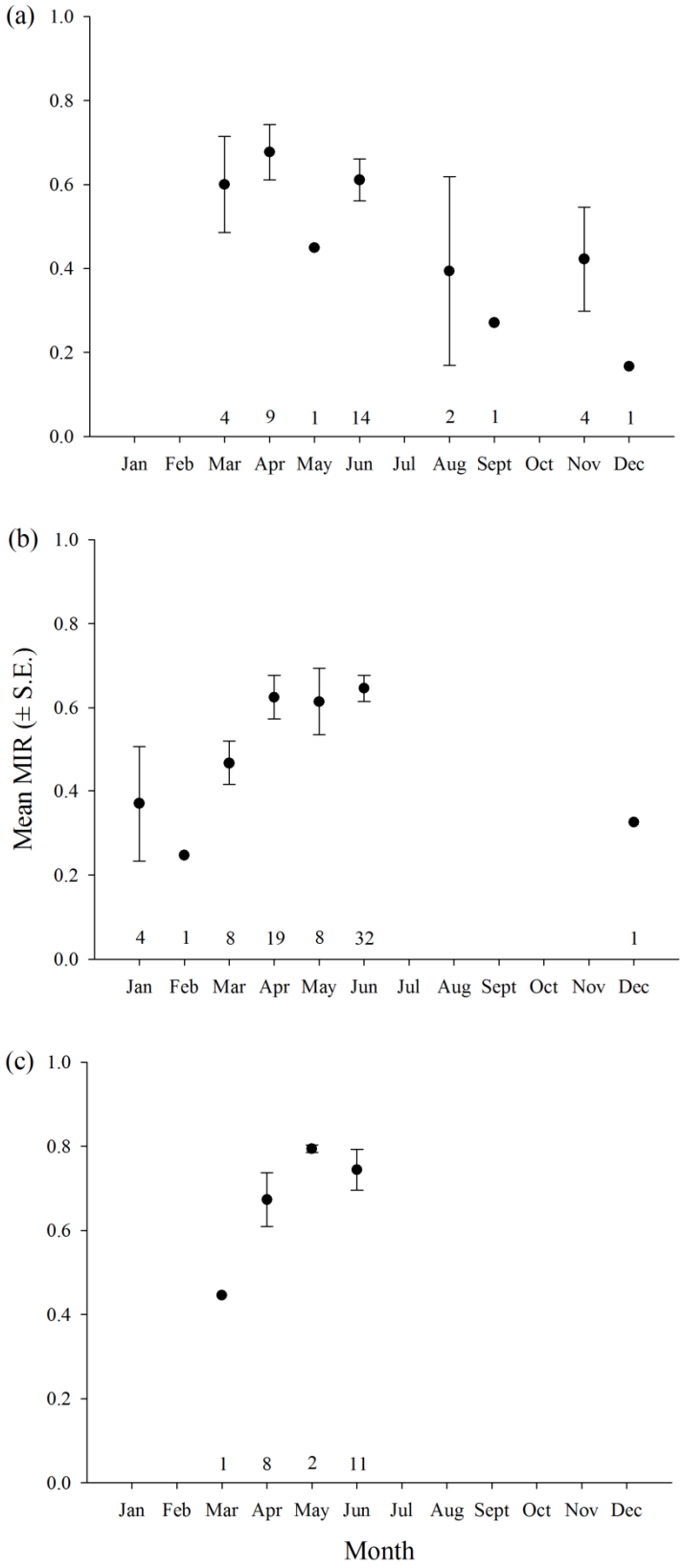

Figure 5.6 Monthly mean marginal increment ratios (MIR, ± S.E.) for (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus* in New South Wales waters. Monthly sample sizes are shown.

Table 5.2 Summary of fitted parameter values (with 95 % C.I.) and Akaike's Information Criteria results from six candidate models describing (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus* growth in New South Wales waters. Parameters are asymptotic total length (L_∞, cm L_T), total length-at-birth (L_0, cm L_T) [fixed for 2-parameter models at (a) 94 cm L_T for *C. obscurus*, (b) 80.5 cm L_T for *C. brevipinna* and (c) 71 cm L_T for *C. plumbeus*] and growth coefficient (k). Akaike's Information Criteria values (AIC), Akaike differences (Δ) and Akaike weights (w) show the relative support for each model. R.S.E. = residual standard error. The 'best-fit' model for each sex, as determined by AIC, is bolded.

(a) *Carcharhinus obscurus*

	Model	L_∞	L_0	k	AIC	Δ	w	R.S.E.
Females (n = 126)	**VB-3**	**365.03 (354.99, 377.68)**	**107.03 (97.70, 115.98)**	**0.083 (0.071, 0.095)**	**1037.14**	**0.00**	**86.41**	**14.54**
	VB-2	357.16 (350.02, 365.23)	94	0.095 (0.086, 0.103)	1042.92	5.78	4.80	14.94
	GOM-3	350.11 (343.50, 358.45)	114.02 (106.06, 121.73)	0.124 (0.109, 0.139)	1041.75	4.60	8.65	14.81
	GOM-2	341.64 (336.73, 346.89)	94	0.155 (0.144, 0.165)	1061.35	24.21	0.00	16.07
	LOGI-3	342.96 (337.62, 349.20)	119.94 (112.34, 127.29)	0.165 (0.148, 0.184)	1050.04	12.90	0.14	15.31
	LOGI-2	334.86 (330.49, 339.33)	94	0.226 (0.213, 0.240)	1086.03	48.89	0.00	17.72
Males (n = 131)	VB-3	338.15 (329.68, 349.89)	98.22 (89.52, 106.69)	0.104 (0.087, 0.121)	1078.36	1.04	20.92	14.56
	VB-2	**336.28 (329.19, 345.50)**	**94**	**0.108 (0.095, 0.123)**	**1077.32**	**0.00**	**35.28**	**14.55**
	GOM-3	327.52 (322.00, 334.77)	102.75 (94.77, 110.43)	0.153 (0.133, 0.175)	1077.54	0.22	31.59	14.51
	GOM-2	324.63 (320.31, 329.93)	94	0.168 (0.152, 0.185)	1080.28	2.96	8.05	14.72
	LOGI-3	322.23 (317.98, 327.64)	106.84 (98.97, 114.20)	0.205 (0.179, 0.233)	1081.62	4.30	4.10	14.74
	LOGI-2	319.07 (315.64, 322.96)	94	0.241 (0.220, 0.263)	1090.14	12.82	0.06	15.28

(b) *Carcharhinus brevipinna*

	Model	L_∞	L_0	k	AIC	Δ	w	R.S.E.
Females (n = 110)	VB-3	296.04 (288.18, 305.36)	89.06 (81.22, 96.39)	0.113 (0.098, 0.127)	858.90	9.67	0.54	11.74
	VB-2	291.70 (285.40, 298.35)	80.5	0.124 (0.115, 0.134)	861.74	12.51	0.13	11.94
	GOM-3	286.57 (280.87, 293.00)	95.97 (89.58, 102.10)	0.162 (0.145, 0.180)	850.76	1.53	31.54	11.31
	GOM-2	280.15 (275.56, 284.78)	80.5	0.198 (0.186, 0.210)	870.78	21.55	0.00	12.44
	LOGI-3	**281.63 (276.85, 286.79)**	**101.43 (95.87, 106.76)**	**0.212 (0.192, 0.233)**	**849.23**	**0.00**	**67.79**	**11.23**
	LOGI-2	274.12 (269.94, 278.32)	80.5	0.287 (0.271, 0.302)	893.10	43.88	0.00	13.77
Males (n = 85)	VB-3	257.24 (250.23, 266.52)	85.67 (77.78, 93.92)	0.145 (0.122, 0.170)	651.09	0.44	30.51	10.83
	VB-2	**254.67 (249.07, 261.30)**	**80.5**	**0.158 (0.145, 0.172)**	**650.65**	**0.00**	**37.93**	**10.86**
	GOM-3	250.31 (245.11, 256.93)	90.31 (83.84, 97.46)	0.210 (0.180, 0.241)	651.65	1.00	23.06	10.86
	GOM-2	247.01 (242.55, 252.21)	80.5	0.248 (0.232, 0.265)	656.93	6.28	1.64	11.27
	LOGI-3	246.91 (242.58, 252.31)	93.98 (87.96, 100.37)	0.277 (0.241, 0.316)	654.08	3.42	6.85	11.02
	LOGI-2	243.66 (239.46, 248.37)	80.5	0.355 (0.336, 0.377)	667.93	17.28	0.01	12.02

Table 5.2 cont.

(c) *Carcharhinus plumbeus*

	Model	L_∞	L_0	k	AIC	Δ	w	R.S.E.
Females (n = 156)	VB-3	214.59 (210.24, 220.75)	79.45 (71.33, 87.33)	0.159 (0.131, 0.189)	1148.92	3.20	9.85	9.46
	VB-2	211.80 (208.87, 215.20)	71	0.182 (0.164, 0.201)	1151.21	5.49	3.13	9.56
	GOM-3	211.27 (207.85, 215.91)	84.60 (77.87, 91.16)	0.206 (0.174, 0.243)	1146.20	0.48	38.26	9.38
	GOM-2	207.54 (205.40, 209.98)	71	0.266 (0.243, 0.290)	1158.86	13.14	0.07	9.80
	LOGI-3	**209.27 (206.38, 213.09)**	**88.42 (82.50, 94.19)**	**0.253 (0.216, 0.297)**	**1145.72**	**0.00**	**48.70**	**9.37**
	LOGI-2	205.47 (203.64, 207.45)	71	0.369 (0.341, 0.401)	1171.18	25.46	0.00	10.20
Males (n = 237)	VB-3	195.34 (193.15, 197.99)	80.27 (75.58, 84.75)	0.214 (0.191, 0.238)	1607.72	7.51	1.37	7.12
	VB-2	193.50 (191.78, 195.38)	71	0.244 (0.226, 0.264)	1621.22	21.00	0.00	7.34
	GOM-3	193.12 (191.36, 195.12)	83.15 (79.01, 87.05)	0.273 (0.246, 0.302)	1600.96	0.74	40.28	7.02
	GOM-2	190.93 (189.62, 192.31)	71	0.337 (0.314, 0.361)	1629.97	29.75	0.00	7.47
	LOGI-3	**191.74 (190.22, 193.50)**	**85.75 (81.88, 89.44)**	**0.332 (0.301, 0.367)**	**1600.22**	**0.00**	**58.35**	**7.00**
	LOGI-2	189.43 (188.28, 190.63)	71	0.451 (0.422, 0.482)	1646.96	46.74	0.00	7.75

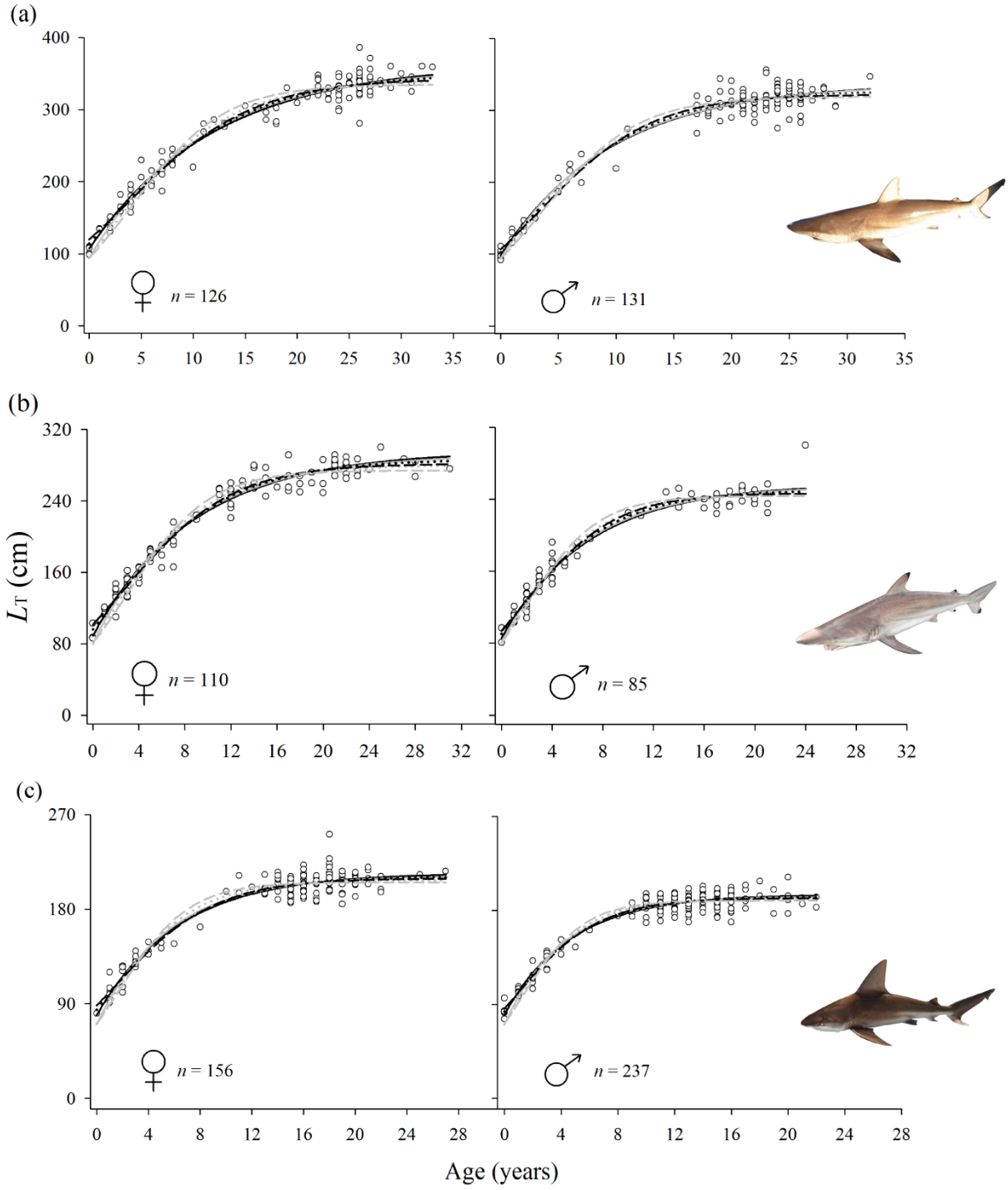

Figure 5.7 Observed total (L_T) length-at-age for (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus* in New South Wales waters as determined by vertebral analysis. Fitted candidate growth curves: —— VB-3; —— VB-2; ·····GOM-3; ·····GOM-2; – – – LOGI-3; – – – LOGI-2.

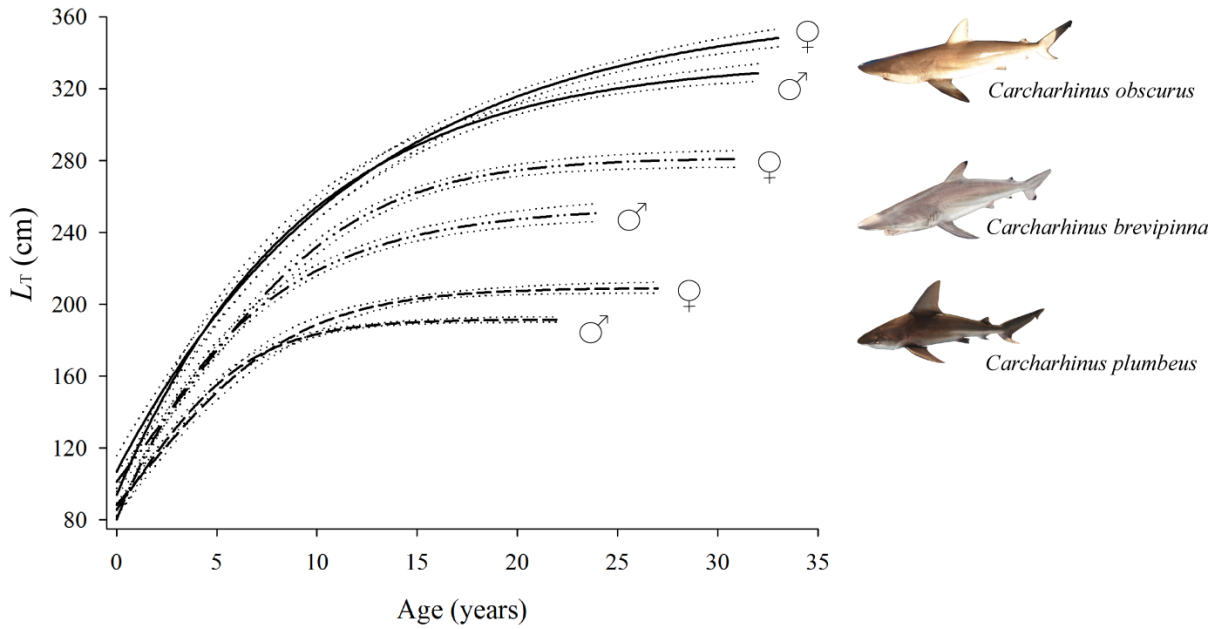

Figure 5.8 Comparative statistical 'best-fit' growth curves, as determined by Akaike's Information Criteria, for female and male *Carcharhinus obscurus*, *Carcharhinus brevipinna* and *Carcharhinus plumbeus* in New South Wales waters. Dotted lines indicate 95 % confidence intervals based on 10,000 bootstrap iterations.

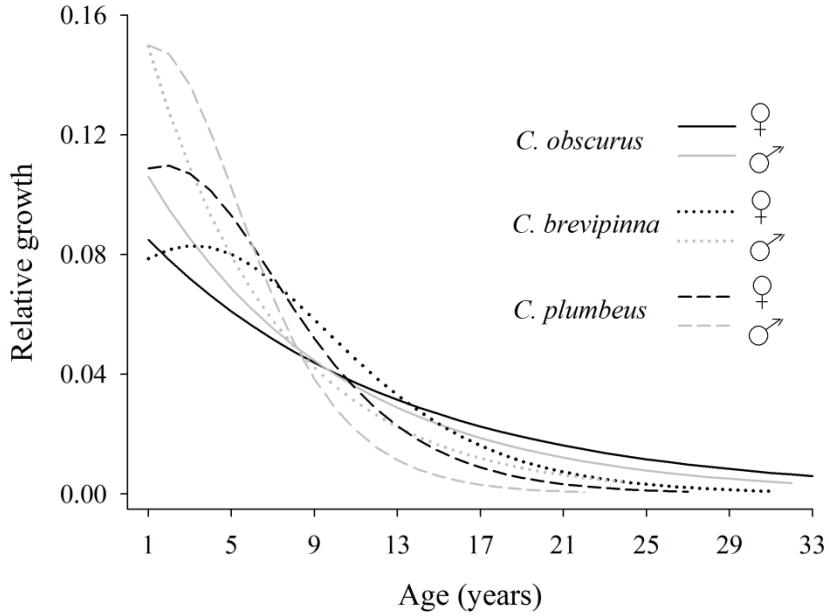

Figure 5.9 Relative growth (yearly growth increment/total growth) over observed life-spans of *Carcharhinus obscurus*, *Carcharhinus brevipinna* and *Carcharhinus plumbeus* in New South Wales waters. Total growth (L_∞ minus L_0) was calculated from values derived from statistical 'best-fit' growth models, as determined by Akaike's Information Criteria.

5.4.2 Carcharhinus brevipinna

Carcharhinus brevipinna was caught along the NSW coast between Tweed Heads and Crowdy Head (Figure 5.1). Vertebrae from 198 genetically-confirmed *C. brevipinna*, ranging in length from 81-300 cm L_T, were sectioned and read. Length-frequency distributions exhibited two modes for both sexes (Figure 5.3b).

Vertebral growth-band readability was high in individuals ≤ 230 cm L_T and lower in larger specimens (> 230 cm L_T) (Figure 5.4b). Overall mean (± S.E.) readability was high (3.1 ± 0.06), although three were deemed unreadable and excluded from further analyses. Growth, therefore, was investigated using observed length-at-age data from 195 individuals (110 females and 85 males), ranging in length from 86-300 cm L_T for females and 81-300 cm L_T for males.

No systematic bias in age counts was identified between Reader 1 and 2 ($\chi^2 = 69.7$, df = 55, $p > 0.05$) (Figure 5.5b). Inter-reader precision was acceptable (c.v. = 12.6) (Campana 2001) and overall PA was 36.4 % (Figure 5.5b, Supplementary material A). Agreement with final age count was 80 % for Reader 1 and 44.6 % for Reader 2.

Marginal increment analysis suggested band-pair deposition commencing in mid-winter. Marginal increment ratios were lowest in the summer months (December-February), increasing to a maximum value in early winter (June) (Figure 5.6b).

All growth models provided good fits of the observed length-at-age data for both sexes (Figure 5.7b). Statistically, the three-parameter logistic (LOGI-3) growth function was the best model for describing female *C. brevipinna* growth in NSW waters, with L_∞, k and L_0 estimated at 281.63 cm L_T, 0.212 and 101.43 cm L_T, respectively (Table 5.2b). The VB-2 model was considered the best for describing male growth, with L_0 fixed at 80.5 cm L_T and L_∞ and k estimated at 254.67 cm L_T and 0.158, respectively (Table 5.2b).

Observed mean, and predicted, length-at-age suggested similar sizes for both sexes over the first seven years of life (Figure 5.8, Supplementary material C). At all subsequent ages, females were considerably larger than males. Longevity varied between sexes, with the oldest observed *C. brevipinna* a 276 cm L_T female aged at 31 years and the oldest observed male a 300 cm L_T individual aged at 24 years (Figures 5.7b, 5.8).

Modelled yearly growth increments demonstrated males to grow at a faster rate than females for the first four years of life, with growth in the first three years being substantially greater in males than females (Figure 5.9, Supplementary material C). From the age of five onwards, females grow at a faster rate than males. Growth was greatest in the first and third year after birth in males and females, respectively (Figure 5.9, Supplementary material C).

5.4.3 Carcharhinus plumbeus

Carcharhinus plumbeus was caught along the NSW coast between Tweed Heads and Nambucca Heads (Figure 5.1). Vertebrae from 428 genetically-confirmed *C. plumbeus*, ranging in length from 76-251 cm L_T, were sectioned and read. Specimens were predominantly large (> 170 cm L_T) individuals for both sexes (Figure 5.3c).

Vertebral growth-band readability was moderately high in individuals ≤ 160 cm L_T, but generally poor in larger specimens (> 160 cm L_T) (Figure 5.4c). Overall mean (± S.E.) readability was quite low (2.4 ± 0.04). Following the exclusion of 35 vertebral sections deemed unreadable, growth was examined using observed length-at-age data from 393 individuals (156 females and 237 males), with lengths ranging from 81-251 cm L_T for females and 76-212 cm L_T for males.

Between-reader bias in age counts was identified for this species ($\chi^2 = 165.2$, df = 97, $p < 0.001$); Reader 2 systematically under-aged vertebrae relative to Reader 1 (Figure 5.5c). Consequently, overall inter-reader precision was low (c.v. = 19.8, PA = 15.3 %) (Campana

2001) (Figure 5.5c, Supplementary material A). Agreement with final age count was 63.4 % for Reader 1 and 25.2 % for Reader 2.

Marginal increment analysis provided limited information, but was suggestive of increasing ratios throughout the autumn months, peaking in May and remaining high in early winter (June) (Figure 5.6c). This supports, albeit tentatively, band-pair deposition from mid-winter onwards.

All candidate growth models provided good fits of the observed length-at-age data for both sexes (Figure 5.7c). Statistically, the LOGI-3 growth function was the best model for describing both female and male *C. plumbeus* growth in NSW waters (Table 5.2c). Asymptotic growth (L_∞) and L_0 estimates were larger for females (209.27 and 88.42 cm L_T) compared to males (191.74 and 85.75 cm L_T). In contrast, k was higher for males (0.332) than females (0.253) (Table 5.2c).

Observed mean length-at-age reported similar sizes for both sexes over the first five years of life; predicted length-at-age suggested similar sizes over the first nine years of life (Figure 5.8, Supplementary material D). At all subsequent ages, females were considerably larger than males. Longevity varied between sexes; the oldest observed *C. plumbeus* being a 216 cm L_T female aged at 27 years and the oldest observed males being two individuals measuring 183 and 193 cm L_T and aged at 22 years (Figures 5.7c, 5.8).

Analysis of modelled yearly growth increments indicated that males grow at a faster rate than females for the first four years of life, after which females grow faster than males (Figure 5.9, Supplementary material D). Rate of growth was greatest in the first and second years after birth for males and females, respectively (Figure 5.9, Supplementary material D).

5.5 Discussion

This study marks the first assessment of the age and growth of *C. brevipinna* in Australian waters, and of *C. obscurus* and *C. plumbeus* off Australia's east coast, where all three were demonstrated to be long-lived. The six candidate growth models fitted the observed length-at-age data well for all three species. Nevertheless, growth parameters varied markedly among models. Statistically, female growth was best described by the three-parameter von Bertalanffy function for *C. obscurus*, and by the three-parameter logistic model for *C. brevipinna* and *C. plumbeus*. Male growth was best modelled by the two-parameter von Bertalanffy function for *C. obscurus* and *C. brevipinna*, and by the three-parameter logistic curve for *C. plumbeus*. Based on criteria outlined by Branstetter (1987) and Musick (1999), our growth coefficients (*k* values) suggest that in south-eastern Australian waters *C. obscurus* is a slow-growing species, *C. brevipinna* has a slow to moderate rate of growth, and *C. plumbeus* is a moderate to rapidly-growing species.

Statistical 'best-fit' ranking, however, doesn't necessarily convey biological reality (Wang & Milton 2000, Romine et al. 2006, Bubley et al. 2012). Growth-model goodness-of-fit and resultant parameter estimates can be highly influenced by sampling biases, such as those imparted by gear selectivity or historic length-selective fishing mortality (Thorson & Simpfendorfer 2009, Harry et al. 2013). In the present study, a general under-representation of small-to-medium sized individuals resulted in three-parameter models overestimating length-at-birth (L_0) for all three species. In addition, the von Bertalanffy functions produced the most realistic estimates of theoretical asymptotic length (L_∞), while the logistic and Gompertz models underestimated L_∞ in all cases. Given that L_∞ and k are negatively correlated, an underestimate in the former causes an overestimate in the latter. Statistical output, therefore, must be considered in conjunction with observed biological data when determining the most suitable model (Cailliet et al. 2006).

With this in mind, we propose the two-parameter von Bertalanffy (VB-2) function to be the most appropriate for describing the growth of both sexes of all three species off the south-east coast of Australia. Despite a lack of statistical support in most cases (Table 5.2), the VB-2 model provided the most biologically-accurate fit to each data set given the incorporation of empirical lengths-at-birth and realistic L_∞ output, and are referred to henceforth. However, while models with fixed L_0 are highly applicable where small individuals are inadequately sampled, they are limited by a failure to account for variable length-at-birth or rapid early growth (Neer et al. 2005, Cailliet et al. 2006, Thorson & Simpfendorfer 2009), and are vulnerable to biased parameter estimates with slight variations in L_0 (Pardo et al. 2013).

Carcharhinus obscurus, *C. brevipinna* and *C. plumbeus* displayed both contrasts and consistencies in their growth characteristics in south-eastern Australian waters. With respect to attributes common to all three species: growth rates were greatest in the years immediately after birth and decreased progressively over time, males grew more rapidly than females in the juvenile phase (hence displaying greater *k* estimates) after which their growth rate slowed below that of females, and females were observed to grow larger, live longer and were generally larger at any given age. These growth patterns are typical of sharks (Cortés 2000) and corroborate the findings of previous work on these species from other parts of the world (refer to literature cited in Table 5.3). In addition, vertebral band-pair deposition appeared to occur annually in all three sharks commencing in mid-winter months, although our marginal increment analyses were severely limited in their sample size and monthly cover.

Longevity, however, varied among *C. obscurus*, *C. brevipinna* and *C. plumbeus* in the study area. In the case of the former two species, our longevity estimates are consistent with those reported from other oceanic regions where comparable methodologies were employed (Table 5.3). In contrast, our maximum age estimates for *C. brevipinna* are considerably higher than those previously reported for this species (Table 5.3) – such discrepancies between NSW

and other geographic regions, however, may be the result of a range of confounding factors, such as variations in technique of preparation and reading of vertebrae, reader accuracy and precision, as well as sample size and distribution (Cailliet et al. 1990, Carlson et al. 2006).

The parameters L_∞ and k, and hence rates of incremental and relative growth, also varied considerably among the study species in NSW waters. Yearly growth increments were largest in *C. obscurus* and smallest in *C. plumbeus* at any given age (Supplementary material B, C & D) – not an unexpected result given the difference in maximum size attained by these species (Figure 5.3, Last & Stevens 2009). Taking these differences into account, however, the reverse pattern was observed in the juvenile phase, where relative growth rates were highest in *C. plumbeus* and lowest in *C. obscurus* (Figure 5.9).

Our estimates of L_∞ and k did not necessarily agree with previous estimates for the same species in other areas – bearing in mind that direct comparisons of k are only appropriate among the same growth-model family. Similarly, rates of incremental growth were also observed to vary. Comparisons based solely on annual growth increments, however, are of limited value given that maximum attainable size within a species can vary among geographically-distinct locations (Last & Stevens 2009). We therefore recommend that measures of relative growth, as calculated in our study, be reported in conjunction with incremental growth so that more robust population (and species) comparisons can be drawn.

For *C. brevipinna*, our estimates of L_∞ and k are generally within the range of those reported for this species from other oceanic basins (Table 5.3). In contrast, our parameters for *C. obscurus* and *C. plumbeus* are markedly different from those reported by most other studies; our L_∞ and k estimates being comparatively low and high respectively (Table 5.3). However, rather than reflecting true conspecific differences, we propose that these discrepancies are driven by differences in sample size and length-distribution – in most cases highlighting the shortcomings of previous studies.

Table 5.3 Comparative growth-model parameters based on vertebral analysis. L_∞ = theoretical asymptotic length; k = growth coefficient; L_0 = length-at-birth. VB = von Bertalanffy; GOM = Gompertz; LOGI = logistic; number of model parameters in parentheses. All length measurements expressed as total length (L_T, cm) unless otherwise stated, and converted where appropriate using publication-specific morphometric equations (if provided). NB: the parameter L_∞ is common to all models, however k and fitted L_0 are not directly comparable between growth-model families.

Species	Oceanic region	Reference	*n*	Size range	Max. ages (sex)	Female			Male		
						Model	L_∞	k	L_0	L_∞	k	L_0
C. obscurus	SE Indian	Simpfendorfer et al. (2002)	305	77.7 – 333.9	32 (F), 25 (M)	VB (2)	418.6	0.043	92.1 [f]	397.7	0.045	92.1 [f]
	NW Atlantic	Natanson et al. (1995)	120	89.7 – 356.7	33 (F), 25 (M)	VB (3)	420.2	0.039	102.9	448.9	0.038	95.7
	SW Indian	Natanson & Kohler (1996)	42	99.1 – 353.6	34 (F)	VB (3)	395.7 [c]	0.047 [c]	–			
	SW Pacific	**Present study**	**257**	**92.0 – 386.0**	**33 (F), 32 (M)**	**VB (2)**	**357.2**	**0.095**	**94.0 [f]**	**336.3**	**0.108**	**94.0 [f]**
C. brevipinna	SW Indian	Allen & Wintner (2002)	67	78.4 – 282.5	17 (F), 19 (M)	VB (3)	307.9	0.100	–	261.1	0.146	–
	NW Atlantic	Branstetter (1987)	15	67.0 – 208.0	11.3 (F), 8 (M)	VB (3)	214.0 [c]	0.212 [c]	72.2			
	NW Atlantic	Carlson & Baremore (2005)	259	57.8 – 233.7	17.5 (F), 13.5 (M)	VB (3)	270.6	0.080	–	500.5	0.030	–
						VB (2)	242.8	0.110	64.9 [f]	333.0	0.070	64.9 [f]
						GOM (3)	263.2	0.160	75.2	239.6	0.140	74.9
	W Pacific	Joung et al. (2005)	208	125.0 – 304.0	21 (F), 17 (M)	VB (3)	288.2	0.151	75.0	257.4	0.203	75.0
	SW Pacific	**Present study**	**195**	**81.0 – 300.0**	**31 (F), 24 (M)**	**VB (2)**	**291.7**	**0.124**	**80.5 [f]**	**254.7**	**0.158**	**80.5 [f]**
C. plumbeus	Central Pacific	Romine et al. (2006)	187	46.0 – 147.0 [P]	23 (F), 19 (M)	VB (3)	164.9 [P]	0.080	–	151.1 [P]	0.090	–
						VB (2)	152.8 [P]	0.100	47.0 [f, P]	138.5 [P]	0.120	47.0 [f, P]
						GOM (2)	143.5 [P]	0.170	47.0 [f, P]	130.4 [P]	0.190	47.0 [f, P]
						LOGI (3)	146.4 [P]	0.170	–	134.3 [P]	0.190	–
	NW Atlantic	Hale & Baremore (2010)	1194	39.0 – 202.0 [F]	27 (F), 22 (M)	VB (3)	181.2 [F]	0.120	–	173.0 [F]	0.150	–
						VB (2)	178.3 [F]	0.140	46.0 [f, F]	172.1 [F]	0.150	46.0 [f, F]
	NW Atlantic	Casey et al. (1985)	475	~51.9 – 241.0	21 (F), 15 (M)	VB (3)	360.4	0.040	–	309.6	0.050	–
	W Pacific	Joung et al. (2004)	362	82.0 – 219.0	20.8 (F), 19.8 (M)	VB (3)	210.0 [c]	0.170 [c]	–			
	SE Indian	McAuley et al. (2006)	235	58.7 – 178.8	25 (F), 19 (M)	VB (2)	279.4	0.039	53.7 [f]	259.3	0.044	53.7 [f]
	NW Atlantic	Sminkey & Musick (1995) [a]	188	67.7 – 229.8	24 (F), 20 (M)	VB (3)	263.3	0.059	–	245.9	0.059	–
		Sminkey & Musick (1995) [b]	412	57.0 – 215.1	22 (F), 18 (M)	VB (3)	220.5	0.086	–	221.8	0.087	–
	SW Pacific	**Present study**	**393**	**76.0 – 251.0**	**27 (F), 22 (M)**	**VB (2)**	**211.8**	**0.182**	**71.0 [f]**	**193.5**	**0.244**	**71.0 [f]**

[a] 1980 – 1981; [b] 1991 – 1992; [c] combined sexes; [f] fixed parameter; [P] Pre-caudal length (L_{PC}); [F] Fork length (L_F).

All published works describing the growth of *C. obscurus* have grossly overestimated L_∞ (and hence underestimated *k*) relative to biological reality; the same can be said for *C. plumbeus*, but with notable exceptions (Table 5.3). These inaccuracies stem from either small sample sizes (Natanson et al. 1995, Natanson & Kohler 1996) or a comparative over-representation of small individuals, resulting in poorly-defined growth curve asymptotes (e.g. Casey et al. 1985, Sminkey & Musick 1995, Simpfendorfer et al. 2002, McAuley et al. 2006). While the present study also displayed generally poor balance among size classes, the contrasting bias towards large individuals of *C. obscurus* and *C. plumbeus* translated to pronounced growth asymptotes and hence lower (more realistic) L_∞ and higher (more accurate) *k* values. The influence of sample length-distribution on growth parameters is further emphasised by far less variation being observed between NSW waters and other geographic regions where species-specific length-distributions more closely resembled those of the present study (e.g. Allen & Wintner 2002, Joung et al. 2004, 2005, Hale & Baremore 2010). We propose, therefore, that our growth parameters are among the most accurate and robust for all three study taxa to date.

Notwithstanding the abovementioned limitations, differences in growth characteristics between south-eastern and western Australian waters should not be ruled out entirely for *C. obscurus*, possibly warranting further investigation. Our predicted annual growth increments for juveniles of this species were markedly larger than those reported by Simpfendorfer (2000) based on tag-recapture data, and a study by Geraghty et al. (*Chapter 2*) demonstrated evidence for genetic differentiation in this species, albeit weak, between the two abovementioned regions.

On the bases of genetic validation and sample size and distribution, we propose the growth-model parameters presented herein to be among the more robust currently available for all three taxa. That said, however, due consideration must be given to the lack of age-validated longevity in the present study. Tag-recapture and bomb radiocarbon data have

provided compelling evidence for vertebral-band analysis underestimating age in large adult sharks, including in our study species (Casey & Natanson 1992, Natanson et al. 1995, Francis et al. 2007, Andrews et al. 2011) - purportedly a result of discontinued band-pair deposition coinciding with a cessation of somatic growth, and/or problems with the interpretation of growth bands on the centrum outer edge. This is particularly relevant to the present study in which most sharks aged were large adult individuals. It is worth noting too that various studies have computed maximum theoretical ages based on reported maximum sizes and modelled growth parameters, yielding greatly elevated longevity estimates (e.g. Natanson & Kohler 1996, McAuley et al. 2006). However, such calculations are highly speculative and likely of limited value. Nevertheless, by compromising longevity estimates and hence growth model parameters, age underestimation has far-reaching implications for shark population modelling and assessment - highlighting the need for age validation of older age classes.

Similarly, the influence of section readability on our results also warrants some consideration. In all three study species, readability demonstrated a generally decreasing trend as shark size increased. This emphasises a potential source of inaccuracy in our age counts given that the majority of sharks aged in the present study were large adults.

The results of the present study indicate that *C. obscurus*, *C. brevipinna* and *C. plumbeus* are all long-lived species displaying both contrasts and consistencies in their growth dynamics in temperate eastern Australian waters. While our results appear to challenge findings emanating from other parts of the world, confounding factors render definitive inter-region conclusions potentially misleading. Nevertheless, we report the least conservative *k* estimates for *C. obscurus* and *C. plumbeus* of the published literature to date, which has profound implications relating to assessments of natural mortality and survival. Using *k* as an index of potential stock vulnerability to excessive mortality (Musick 1999), our results suggest that these two species may in fact be somewhat more resilient to overexploitation (at least in NSW

waters) than current population models would assert (Sminkey & Musick 1996, McAuley et al. 2007a, Romine et al. 2009). This study also extends current estimates of maximum age in *C. brevipinna* – suggestive of greater reproductive potential. While the intrinsic susceptibilities of the three study species to overfishing are well established (particularly for *C. obscurus* and *C. plumbeus*), our results potentially warrant some level of optimism when considering the resilience of these species to fishing pressure, at least in NSW waters. That said, however, the true implications of our findings remain purely speculative in the absence of reproductive parameters, and hence demographic analyses, defined from the study region.

5.6 Acknowledgements

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5.7 Supplementary material A – Percentage agreement (PA) between Reader 1 and Reader 2 age counts.

Size range (L_T, cm)	*C. obscurus*					*C. brevipinna*					*C. plumbeus*				
	Total read	Percentage agreement				Total read	Percentage agreement				Total read	Percentage agreement			
		± 0	± 1	± 2	± 3		± 0	± 1	± 2	± 3		± 0	± 1	± 2	± 3
71 – 80											1	100			
81 – 90						3	100				4	100			
91 – 100	5	100				1	0	100			5	20.0	100		
101 – 110	6	100				7	85.7	100			16	18.8	87.5	100	
111 – 120	2	100				8	50.0	100			6	66.6	100		
121 – 130	2	50.0	100			9	66.6	100			8	37.5	75.0	100	
131 – 140	8	62.5	100			13	53.8	84.6	100		12	33.3	83.3	100	
141 – 150	5	60.0	100			18	55.5	83.3	88.9	100	7	28.6	71.4	85.7	100
151 – 160	4	100				11	54.5	90.9	100		1	0	100		
161 – 170	2	50.0	100			12	50.0	75.0	100		6	16.7	16.7	50.0	83.3
171 – 180	2	0	100			11	36.4	72.7	100		27	14.8	40.7	59.3	70.4
181 – 190	7	28.6	71.4	100		5	40.0	80.0	100		99	9.1	35.4	60.6	73.7
191 – 200	4	25.0	100			5	20.0	100			113	9.7	35.4	55.8	71.7
201 – 210	3	33.3	66.6	100		1	100				51	11.8	35.3	58.8	76.5
211 – 220	6	16.7	50.0	100		2	0	100			33	18.2	33.3	51.5	57.6
221 – 230	5	40.0	60.0	80.0	80.0	7	28.6	71.4	100		3	33.3	33.3	33.3	33.3
231 – 240	3	33.3	100			13	23.1	61.5	84.6	84.6	0				
241 – 250	3	66.6	66.6	100		13	23.1	61.5	84.6	92.3	0				
251 – 260	0					21	9.5	52.4	76.2	81.0	1	0	0	0	100
261 – 270	2	0	100			9	11.1	44.4	44.4	55.6					
271 – 280	5	0	20.0	60.0	100	11	18.2	45.5	45.5	45.5					
281 – 290	10	20.0	60.0	80.0	90.0	10	20.0	40.0	50.0	50.0					
291 – 300	12	33.3	66.6	83.3	100	5	0	20.0	60.0	60.0					
301 – 310	28	14.3	53.6	78.6	89.3										
311 – 320	38	23.7	55.3	76.3	84.2										
321 – 330	35	20.0	51.4	71.4	91.4										
331 – 340	31	16.1	54.8	77.4	87.1										
341 – 350	19	26.3	42.1	63.2	78.9										
351 – 360	8	0	12.5	25.0	50.0										
361 – 370	0														
371 – 380	1	0	100												
381 – 390	1	0	100												
Overall	257	28.4	61.1	79.8	89.9	195	36.4	71.3	85.1	87.7	393	15.3	43.0	63.4	75.8

5.8 Supplementary material B – Mean ($\bar{x}$) and predicted (P) length-at-age (total length, L_T, cm) and growth rate (yearly growth increment, G, cm·yr^{-1}) for female and male *Carcharhinus obscurus* in New South Wales waters. Mean length-at-age calculated from observed vertebral analysis data; predicted length-at-age and growth rates derived from sex-specific 'best-fit' growth model as determined by Akaike's Information Criteria. S.E. = standard error.

Female (n = 126)	Age (years)																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$\bar{x}$	105.4	134.5	142.7	168.7	178.3	207.3	205.5	216.6	232.0	245.0	220.0	274.5	286.0	278.5		305.0	304.0	291.0	288.3	330.0	309.0
S.E.	2.2	0.5	3.1	6.9	5.4	12.7	5.5	9.1	4.0			5.5		2.5				5.0	6.9		
n	5	2	6	3	7	3	4	5	5	1	1	2	1	2	0	1	1	2	3	1	1
P	107.0	127.5	146.4	163.7	179.7	194.4	208.0	220.4	231.9	242.5	252.2	261.2	269.4	277.0	284.0	290.4	296.3	301.8	306.8	311.4	315.7
G		20.5	18.9	17.4	16.0	14.7	13.5	12.5	11.5	10.6	9.7	9.0	8.2	7.6	7.0	6.4	5.9	5.5	5.0	4.6	4.3

	21	22	23	24	25	26	27	28	29	30	31	32	33
$\bar{x}$	320.5	336.8	321.6	324.5	334.0	335.1	341.8	338.2	340.0	343.3	335.7	360.0	359.0
S.E.	2.5	4.9	3.1	5.4	4.9	4.9	5.2	1.0	10.0	3.3	5.8		
n	2	5	5	10	5	18	9	5	3	3	3	1	1
P	319.6	323.2	326.5	329.6	332.4	335.0	337.4	339.6	341.6	343.4	345.2	346.7	348.2
G	3.9	3.6	3.3	3.1	2.8	2.6	2.4	2.2	2.0	1.9	1.7	1.6	1.5

Male (n = 131)	Age (years)																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$\bar{x}$	99.9	125.5	139.0	153.5		193.0	220.5	219.0			219.0	274.0						297.5	301.0	313.2	314.3
S.E.	2.5	4.3	3.1	3.5		6.5	4.5	20.0										11.1	4.1	8.2	7.1
n	7	4	4	2	0	3	2	2	0	0	1	1	0	0	0	0	0	4	5	6	6
P	94.0	118.9	141.2	161.2	179.2	195.3	209.8	222.8	234.4	244.9	254.2	262.7	270.2	277.0	283.1	288.5	293.4	297.8	301.8	305.3	308.5
G		24.9	22.3	20.0	18.0	16.1	14.5	13.0	11.7	10.5	9.4	8.4	7.6	6.8	6.1	5.5	4.9	4.4	3.9	3.5	3.2

	21	22	23	24	25	26	27	28	29	30	31	32
$\bar{x}$	310.5	308.5	319.3	320.3	320.6	317.5	321.0	327.3	305.5			347.0
S.E.	3.3	3.4	5.1	3.7	3.9	5.4	4.4	3.7	0.5			
n	11	11	12	18	12	11	3	3	2	0	0	1
P	311.4	313.9	316.2	318.3	320.1	321.8	323.3	324.6	325.8	326.9	327.8	328.7
G	2.9	2.6	2.3	2.1	1.8	1.7	1.5	1.3	1.2	1.1	1.0	0.9

5.9 Supplementary material C – Mean ($\bar{x}$) and predicted (P) length-at-age (total length, L_T, cm) and growth rate (yearly growth increment, G, cm·yr^{-1}) for female and male *Carcharhinus brevipinna* in New South Wales waters. Mean length-at-age calculated from observed vertebral analysis data; predicted length-at-age and growth rate derived from sex-specific 'best-fit' growth model as determined by Akaike's Information Criteria. S.E. = standard error.

Female (n = 110)	Age (years)																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$\bar{x}$	92.0	118.5	133.5	145.3	157.9	179.7	178.3	194.2		221.5		251.5	242.0	259.5	263.8	262.3	255.0	266.3	259.0	266.0	264.7
S.E.	5.5	1.9	3.9	2.9	2.3	1.6	5.1	8.2		2.5		1.6	6.0	2.5	4.7	9.3		9.0	5.2	6.0	11.1
n	3	4	8	10	7	9	4	5	0	2	0	4	6	2	6	3	1	4	3	2	3
P	101.4	115.5	130.2	145.1	159.9	174.3	187.9	200.7	212.3	222.8	232.0	240.1	247.1	253.0	258.0	262.2	265.7	268.6	271.0	273.0	274.6
G		14.1	14.7	14.9	14.8	14.4	13.7	12.7	11.6	10.5	9.3	8.1	7.0	5.9	5.0	4.2	3.5	2.9	2.4	2.0	1.6

	21	22	23	24	25	26	27	28	29	30	31
$\bar{x}$	281.4	278.2	276.0	275.0	300.0		287.0	275.5			276.0
S.E.	2.6	3.6	5.7					8.5			
n	9	6	3	1	1	0	1	2	0	0	1
P	275.9	277.0	277.8	278.6	279.1	279.6	280.0	280.3	280.6	280.8	280.9
G	1.3	1.1	0.9	0.7	0.6	0.5	0.4	0.3	0.3	0.2	0.2

Male (n = 85)	Age (years)																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$\bar{x}$	89.0	110.1	125.5	147.2	165.6	169.4	184.5	196.0			225.0	222.0		248.0	240.3	246.0	235.0	237.6	238.3	249.8	241.7
S.E.	8.0	2.0	2.6	1.7	5.5	0.9	7.5								6.2		5.0	4.1	5.4	3.2	4.4
n	2	8	13	16	8	5	2	1	0	0	1	1	0	1	3	1	2	5	3	5	3
P	80.5	106.0	127.7	146.2	162.1	175.6	187.2	197.0	205.5	212.7	218.8	224.0	228.5	232.3	235.6	238.4	240.8	242.8	244.5	246.0	247.3
G		25.5	21.7	18.6	15.8	13.5	11.6	9.9	8.4	7.2	6.1	5.2	4.5	3.8	3.3	2.8	2.4	2.0	1.7	1.5	1.3

	21	22	23	24
$\bar{x}$	240.8			300.0
S.E.	6.9			
n	4	0	0	1
P	248.4	249.3	250.1	250.7
G	1.1	0.9	0.8	0.7

5.10 Supplementary material D – Mean ($\bar{x}$) and predicted (P) length-at-age (total length, L_T, cm) and growth rate (yearly growth increment, G, cm·yr^{-1}) for female and male *Carcharhinus plumbeus* in New South Wales waters. Mean length-at-age calculated from observed vertebral analysis data; predicted length-at-age and growth rate derived from sex-specific 'best-fit' growth model as determined by Akaike's Information Criteria. S.E. = standard error.

Female (n = 156)	Age (years)																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$\bar{x}$	81.0	102.4	113.0	131.4	142.0	145.5	147.0		163.0		197.0	203.5	201.0	198.6	207.3	199.6	203.6	203.3	212.2	203.0	206.6
S.E.		3.5	4.0	1.8	3.6	2.5						8.5		2.2	2.4	1.6	1.9	2.1	3.4	3.3	2.3
n	1	7	7	7	3	2	1	0	1	0	1	2	1	8	11	23	20	18	16	9	9
P	88.4	101.5	114.8	127.7	139.9	151.1	161.1	169.8	177.3	183.6	188.8	193.0	196.4	199.2	201.3	203.1	204.4	205.5	206.3	207.0	207.5
G		13.1	13.2	12.9	12.2	11.2	10.0	8.7	7.5	6.3	5.2	4.2	3.4	2.7	2.2	1.7	1.4	1.1	0.8	0.7	0.5

	21	22	23	24	25	26	27
$\bar{x}$	212.5	202.0			213.0		216.0
S.E.	3.7	4.6					
n	4	3	0	0	1	0	1
P	207.9	208.2	208.4	208.6	208.8	208.9	209.0
G	0.4	0.3	0.2	0.2	0.1	0.1	0.1

Male (n = 237)	Age (years)																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
$\bar{x}$	84.2	102.6	116.3	133.3	145.0	145.0	161.0		176.5	180.8	186.4	185.0	188.0	187.0	190.4	189.4	189.6	194.3	201.0	188.5	197.0
S.E.	3.3	0.9	3.1	2.7	4.0				1.5	1.5	2.5	1.2	1.4	1.1	1.0	1.2	2.0	3.4	7.0	11.5	6.7
n	5	9	7	7	3	1	1	0	2	9	12	24	20	38	30	32	18	6	2	2	5
P	85.8	101.6	117.2	131.6	144.4	155.2	164.1	171.0	176.4	180.5	183.5	185.8	187.4	188.6	189.5	190.1	190.6	190.9	191.1	191.3	191.4
G		15.9	15.6	14.5	12.8	10.8	8.8	7.0	5.4	4.1	3.0	2.2	1.6	1.2	0.9	0.6	0.5	0.3	0.2	0.2	0.1

	21	22
$\bar{x}$	189.0	188.0
S.E.	3.0	5.0
n	2	2
P	191.5	191.6
G	0.1	0.1

CHAPTER 6. Reproductive Parameters for Dusky, Spinner and Sandbar Sharks (Family Carcharhinidae) in the South-Western Pacific Ocean

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Plate 7. An adult dusky shark (*Carcharhinus obscurus*) prior to examination by a scientific observer.

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6.1 Abstract

Increased harvest pressure exerted on sharks worldwide has created a necessity for more information concerning the basic biology of targeted species. This study marks the first attempt at a detailed assessment of the reproductive biology of *Carcharhinus obscurus*, *C. brevipinna* and *C. plumbeus* in eastern Australian waters, where these species support a demersal longline fishery. Although generally limited in sample size and temporal distribution, we demonstrate all three species to be relatively late-maturing species exhibiting similar reproductive characteristics (i.e. fecundity, embryo sex ratio, gestation period and parturition season) marked by low productivity – highlighting their susceptibilities to stock depletion off the New South Wales coast. Length- (L_{50}, cm L_T) and age-at-maturity (A_{50}, years), for females and males respectively, were 281.1 and 15.5, and 271.9 and 14.6 for *C. obscurus*; 224.9 and 10.1, and 208.9 and 8.5 for *C. brevipinna*; and, 174.8 and 9.5, and 164.5 and 7.0 for *C. plumbeus*. Pregnancy was observed at a mean length (cm L_T) and age (years) of 329.4 and 23.8, 276.9 and 18, and 204.8 and 16.1 for *C. obscurus*, *C. brevipinna* and *C. plumbeus*, respectively. Uterine fecundity measured 5-12 ($\bar{x}$= 9.6), 5-14 ($\bar{x}$= 10.6) and 3-12 ($\bar{x}$= 7.8) for the same three species, and litter size increased significantly with maternal length in *C. brevipinna*. Length-at-birth (L_0) (cm L_T) ranged from 92-96 in *C. obscurus*, 79-82 in *C. brevipinna*, and 66-76 in *C. plumbeus*. All three species exhibited lengthy gestation periods, overall embryonic sex ratios of 1:1 and parturition in autumn. While appearing to challenge a range of findings emanating from other parts of the world, we urge that our findings be considered preliminary rather than definitive. Nevertheless, our study raises pertinent questions relating to the comparative resilience of these species to targeted fishing activities in NSW waters, and again highlights the importance of locally-derived demographic parameters for accurate population modelling, demographic analyses and stock assessment.

6.2 Introduction

Anthropogenic activities are responsible for accelerating rates of mortality beyond sustainable thresholds in many species, resulting in population declines and even extinctions (e.g. Miller et al. 1999, Fuller 2003, Burney & Flannery 2005). Intense targeted-fishing pressure, for example, has led to precipitous declines in the biomass of predatory fish (e.g. Safina 1993, Hutchings & Myers 1994, Myers & Worm 2003), including sharks (e.g. Baum et al. 2003; Otway et al. 2004, Ferretti et al. 2008). Species' resilience under harvest pressure is determined largely by reproductive potential and mortality, both being crucial for demographic and fishery stock assessment modelling as well as for estimates of sustainable rates of harvest (Walker 2005b, McAuley et al. 2005, 2007a, Cortés et al. 2006, Romine et al. 2009). Knowledge of the reproductive characteristics of impacted species is therefore essential for their effective conservation and management.

Dusky (*Carcharhinus obscurus*), spinner (*Carcharhinus brevipinna*) and sandbar (*Carcharhinus plumbeus*) sharks are three large-medium sized carcharhinids inhabiting much of the world's tropical and warm-temperate coastal and continental-shelf waters (Last & Stevens 2009). All three are landed as either target or by-catch species in commercial and artisanal fisheries throughout much of their distributional ranges (e.g. Amorim et al. 1998, Castillo-Géniz et al. 1998, McVean et al. 2006, Henderson et al. 2007, White 2007, Hale et al. 2011, Manojkumar et al. 2012). Rates of decline have seen *C. obscurus* and *C. plumbeus* globally IUCN listed as 'vulnerable' (Musick et al. 2009a, 2009b) and *C. brevipinna* as 'near threatened' (Burgess 2009).

In Australian waters all three species are actively targeted by commercial fisheries along the eastern, northern and western coastlines, as well as the southern coastline for *C. obscurus* (Simpfendorfer & Donohue 1998, Macbeth et al. 2009, Harry et al. 2011a, Tillett et al. 2012a, Rogers et al. 2013). Off Australia's south-east coast, a fishery-observer study revealed *C.*

plumbeus, C. obscurus and *C. brevipinna* to be the three most abundant large sharks caught in the New South Wales Ocean Trap and Line Fishery (NSW OTLF), respectively (Macbeth et al. 2009).

Cosmopolitan distributions, commercial importance, known susceptibility to overfishing and slow rates of recovery (Smith et al. 1998, Stevens et al. 2000, Field et al. 2009) have led to numerous studies on the reproductive biology of *C. obscurus*, *C. brevipinna* and *C. plumbeus*. Reproductive parameters are available for all three species from the waters of the NW Atlantic (e.g. Branstetter 1981, 1987, Natanson et al. 1995, Romine et al. 2009, Baremore & Hale 2012), South Africa (e.g. Bass et al. 1973, Cliff et al. 1988, Allen & Cliff 2000, Dudley et al. 2005), Brazil (Sadowsky 1967, Amorim et al. 1998, Hazin et al. 2007), Indonesia (White 2007) and Australia (e.g. Stevens 1984, Simpfendorfer et al. 2002, McAuley et al. 2005, 2007b). Reproductive parameters are also available for *C. brevipinna* and *C. plumbeus* from the Mediterranean (Capapé et al. 2003, Saïdi et al. 2005), the Middle East (Baranes & Ben-Tuvia 1978, Moore et al. 2012) and Taiwan (Joung and Chen 1995, Joung et al. 2005), as well as from Hawaii (Wass 1973), Senegal (Diatta et al. 2008), Mauritius (Wheeler 1962) and the East China Sea (Taniuchi 1971) for *C. plumbeus*.

These studies revealed highly conservative life-history traits for all three species. *Carcharhinus obscurus* was demonstrated to be late-maturing (260-300 cm L_T, 17-32 years), of low fecundity (3-16 pups every 3 years), lengthy gestation period (20-24 months) and born at large size (85-100 cm L_T). *Carcharhinus brevipinna* was shown to mature at lengths between 160-220 cm L_T and ages 6-10 years, give birth to 3-17 pups every two years following a gestation period of 10-18 months, with pups born at 60-80 cm L_T. *Carcharhinus plumbeus* was mature at 150-190 cm L_T and at ages 8-16, produced litters of 1-14 pups every 2 years following a maximum gestation period of 12 months, and was born at 45-75 cm L_T. For all three species males matured at smaller lengths and similar or younger ages compared

to females, fecundity typically increased with maternal length, and *in utero* embryos generally exhibited a 1:1 sex ratio overall.

Reproductive characteristics, however, can vary among conspecific shark populations (Yamaguchi et al. 2000), even on relatively small spatial scales (Lombardi-Carlson et al. 2003, Walker 2007). Considerable inaccuracy, therefore, may be associated with population models and management strategies based on reproductive parameters defined elsewhere, emphasising the need for locally-derived data.

Given the paucity of information pertaining to eastern Australian waters, the objective of this study was to provide parameters of reproduction for *C. obscurus*, *C. brevipinna* and *C. plumbeus* off the New South Wales (NSW) coast to be available for use in demographic modelling and stock assessment. Parameters include length- and age-at-maturity, parturition periods, duration of embryonic development, litter size, embryonic sex ratio and length-at-birth.

6.3 Materials and methods

6.3.1 Sampling location and methodology

Carcharhinus obscurus, *C. brevipinna* and *C. plumbeus* were sampled via fishery-dependent methods off Australia's NSW coast between November 2007 and September 2010 (Figure 6.1). Data and samples were collected as part of a fishery-observer program monitoring the catch of sharks in the NSW OTLF (Macbeth et al. 2009). Sharks were caught using demersal longlines set at depths between 10-130 m, with a small number also caught using handlines. Landed sharks were examined by on-board scientific observers.

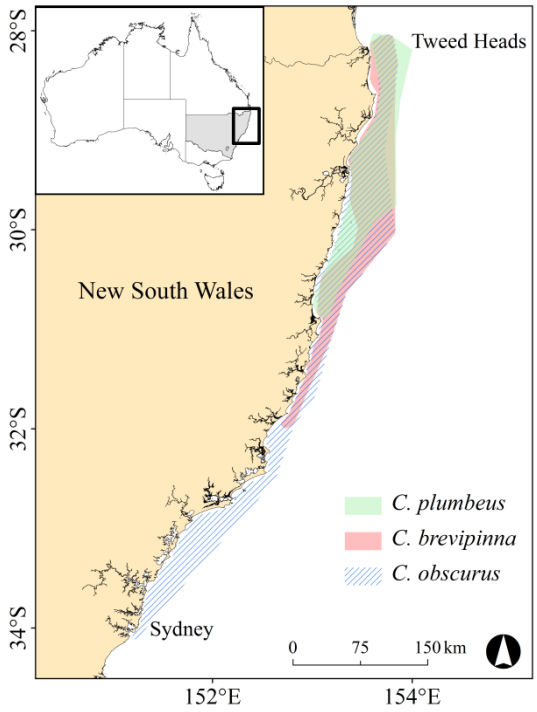

Figure 6.1 Reproductive data collection regions for the three study species.

6.3.2 Data collection and analyses

Sharks were sexed, and pre-caudal (L_{PC}), fork (L_F) and total (L_T) lengths measured to the nearest centimetre (cm). Measurements were defined as the distance from the tip of the snout to a point on the horizontal axis intersecting a perpendicular line extending downward from the pre-caudal notch (pre-caudal), the fork of the caudal fin (fork) and the tip of the upper caudal lobe (total).

Total lengths (L_T) are reported throughout this study, and can be converted to L_{PC} and L_F according to the following morphometric equations (sexes combined) defined from the study area (Geraghty et al. 2013a – *Chapter 5*):

C. obscurus, $L_T = 1.305 \cdot (L_{PC}) + 8.021$ ($n = 255$, $r^2 = 0.99$); $L_T = 1.203 \cdot (L_F) + 4.226$ ($n = 236$, $r^2 = 0.99$),

C. brevipinna, $L_T = 1.286 \cdot (L_{PC}) + 6.208$ ($n = 183$, $r^2 = 0.99$); $L_T = 1.188 \cdot (L_F) + 3.519$ ($n = 191$, $r^2 = 0.99$),

C. plumbeus, $L_T = 1.316 \cdot (L_{PC}) + 4.566$ ($n = 424$, $r^2 = 0.98$); $L_T = 1.206 \cdot (L_F) + 2.747$ ($n = 427$, $r^2 = 0.98$).

From each shark, a vertebrae sample was collected from the cervical region; age estimates were subsequently determined via vertebral band analysis (Geraghty et al. 2013a – *Chapter 5*). A small quantity (< 2 g) of white muscle tissue was also collected from each individual and tested to validate species identity using mitochondrial DNA (*Chapter 2*, Geraghty et al. 2013b – *Chapter 3*). Data associated with misidentified individuals, and those for which a genetic species identity could not be determined, were excluded from analyses.

Reproductive condition was determined for each individual according to macroscopic criteria (Table 6.1). Males were examined for clasper length and degree of clasper calcification, the former being measured to the nearest millimetre (mm) and defined as the distance from the distal tip to the junction with the pelvic fin (i.e. outer-clasper length). Females were assessed for uterus width (mm) and degree of uterine development. Where statistical analyses were performed, maturity-stage data were converted into binary form (immature = 0, mature = 1), whereby individuals of both sexes classified as stage 3 or above (i.e. C3 and U3-6) were considered mature, while those classified as stage 1 and 2 (i.e. C1-2 and U1-2) were deemed immature (Table 6.1).

Table 6.1 Sexual maturity stages assigned to sharks sampled in the present study. Adapted from Harry et al. (2013).

Organ	Stage	Description	Reproductive condition
Male			
Clasper	C1	Small, lacking calcification and flexible	Immature
	C2	Elongated, partially calcified and flexible	Maturing
	C3	Elongated, fully calcified and rigid	Mature
Female			
Uterus	U1	Uniformly thin and empty	Immature
	U2	Thin, enlarged posteriorly and empty	Maturing
	U3	Uniformly enlarged and empty	Mature
	U4	*In utero* yolky eggs present but no visible embryos	Ovulatory
	U5	*In utero* embryos visible	Pregnant
	U6	Enlarged and distended	Post-birth

Logistic regression analysis was employed to establish population estimates of length- (cm L_T) and age (years) -at-maturity where 50 % ($L_{50,}$ A_{50}) and 95 % ($L_{95,}$ A_{95}) were mature. Length and age were modelled separately as a function of binomial maturity stage (logit transformed) using generalised linear models (GLM), and computed in the statistical package R (R Development Core Team 2010). The 95 % confidence intervals (C.I.) of the abovementioned maturity parameters were derived from 10,000 bootstrap re-sampled data sets. Scatter-plots of clasper length and uterus width versus L_T and age were used in conjunction with raw maturity stage to validate the range of lengths and ages over which maturity occured in each species.

For gravid females, the total number of *in utero* embryos was recorded. All embryos were sexed and their L_T measured. Linear regression analysis was applied to establish relationships between uterine fecundity (i.e. litter size) and maternal L_T and age. The sex ratio of total *in utero* embryos was tested for significant deviation from a 1:1 ratio with chi-square tests. All sex ratios are expressed as female:male. A scatter-plot of embryo length by month was used to evaluate length of gestation and time of parturition. Length-at-birth (L_0, cm L_T) was inferred from observed embryo and free-swimming neonate length-frequency distributions, the latter defined as individuals belonging to the 0+ age class as determined from vertebral ageing analysis.

6.4 Results

6.4.1 Carcharhinus obscurus

A total of 268 genetically-validated *C. obscurus* (females, n = 127; males, n = 141), ranging in size from 92-386 cm L_T, were examined for this study (Table 6.2). The majority of individuals sampled were mature (Figure 6.2a).

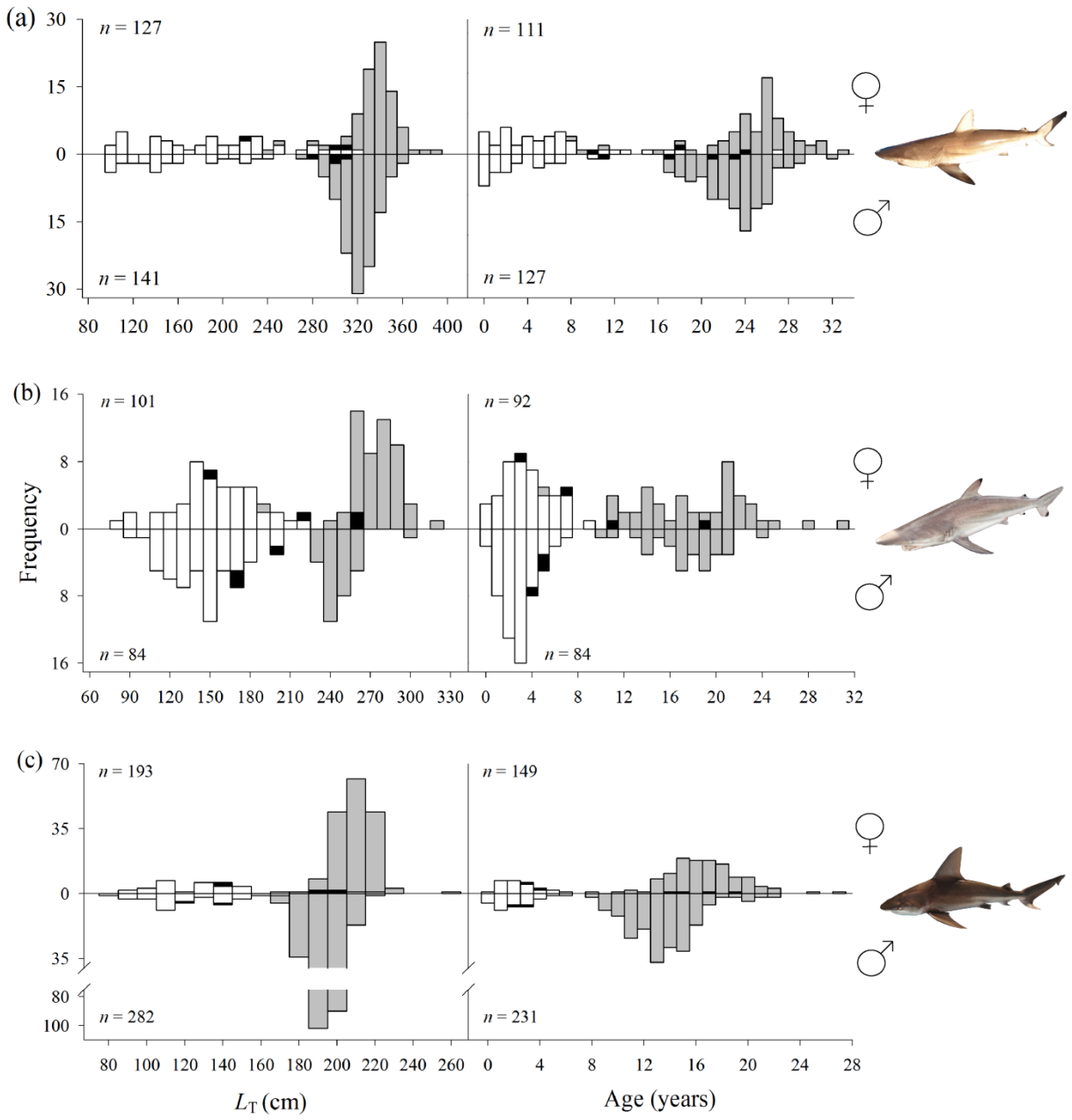

Figure 6.2 Length- (L_T) and age-frequency distributions, incorporating raw maturity-stage data, for (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus* specimens examined in the present study. White, black and grey columns denote immature, maturing and mature individuals, respectively.

A length-frequency distribution of *in utero* embryos (n = 66) and free-swimming neonates (n = 14) indicated that these two life stages overlap in the range 92-105 cm L_T (Figure 6.3a). The measurement of the 105 cm L_T embryo, however, is questionable in light of estimates of length-at-birth from other populations (Table 6.3) and given the next largest embryo in the same litter (n = 8) measured only 96 cm L_T. A more robust estimate of L_0 for *C. obscurus*, therefore, is within the range 92-96 cm L_T (Figure 6.3a).

Table 6.2 Summary of samples and raw reproductive parameters observed from New South Wales waters. Lengths expressed as total length (L_T, cm); ages expressed in years; $\bar{X}$ = mean (± S.E.).

Parameter	***C. obscurus***		***C. brevipinna***		***C. plumbeus***	
	♂	♀	♂	♀	♂	♀
n	141	127	84	101	282	193
Length range	92 - 356	92 - 386	81 - 300	79 - 317	76 - 212	81 - 251
Largest immature	303	320	196	260	161	215
Smallest mature	268	231	222	185	168	175
Oldest immature	23	27	7	19	6	19
Youngest mature	17	8	10	5	8	10
Smallest pregnant	—	315	—	247	—	175
Youngest pregnant	—	21	—	11	—	13
$\bar{X}$ length at pregnancy	—	329.4 (± 4.7)	—	276.9 (± 4.7)	—	204.8 (± 2.3)
$\bar{X}$ age at pregnancy	—	23.8 (± 1.5)	—	18 (± 1.6)	—	16.1 (± 0.5)

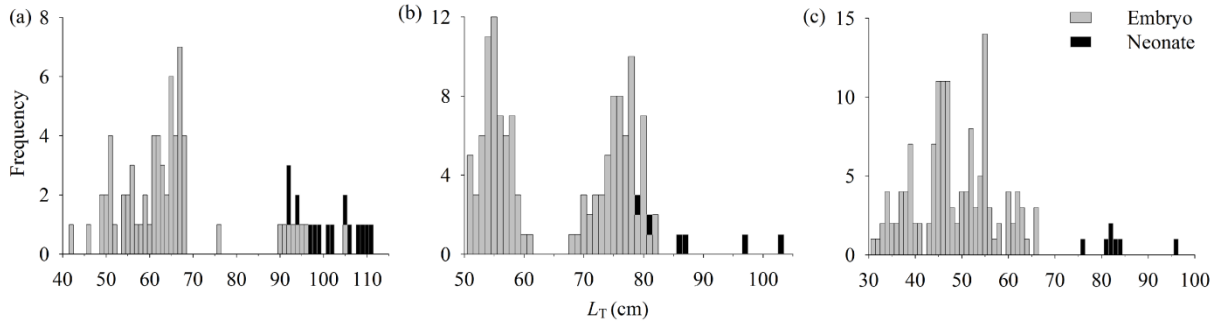

Figure 6.3 Length-frequency distribution of embryo and neonate (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus* observed during this study.

For males, length- and age-frequency distributions and clasper lengths suggested onset of maturity occurred within the ranges 240-280 cm L_T and 11-17 years (Figures 6.2a, 6.4a). Maturity ogives estimated L_{50} and L_{95} (95 % C.I.) respectively at 271.9 (255.1, 286.9) and 301.1 (279.3, 309.7) cm L_T, and A_{50} and A_{95} (95 % C.I.) at 14.6 (13.0, 17.0) and 20.2 (14.4, 22.4) years (Figure 6.5a). The largest immature and smallest mature males observed were 303 and 268 cm L_T, respectively; the oldest immature and youngest mature males were 23 and 17 years old, respectively (Table 6.2).

Onset of maturity in females occurred within the ranges 280-310 cm L_T and 15-18 years, according to length- and age-frequency distributions and uterus widths (Figures 6.2a, 6.4a). Maturity ogives returned L_{50} and L_{95} (95 % C.I.) values respectively of 281.1 (261.1, 302.6) and 328.4 (310.6, 339.4) cm L_T, and A_{50} and A_{95} (95 % C.I.) values of 15.5 (12.9, 18.4) and 24.7 (20.6, 27.8) years (Figure 6.5a). The largest immature and smallest mature females observed were 320 and 231 cm L_T, respectively; the oldest immature and youngest mature females were 27 and 8 years old, respectively (Table 6.2).

Pregnancy was observed at a mean length of 329.4 cm L_T and age of 23.8 years (Table 6.2). The smallest and youngest pregnant individuals measured were 315 cm L_T and 21 years, respectively (Table 6.2). Uterine fecundity was estimated from 8 pregnant females and revealed litter sizes varying from 5 to12 pups, with a mean of 9.6 (± S.E. 0.8). Fecundity did not increase significantly with maternal length or age. The embryo sex ratio from 7 litters varied from 0.6:1 to 4.5:1, but was not significantly different from a 1:1 ratio overall (χ^2 = 0.1385, df = 1, p = 0.71). *In utero* embryo lengths plotted by month suggested parturition in autumn and provided some, albeit weak, evidence that 11 months were necessary for development from approximately $L_0/2$ to full-term length (L_0) (Figure 6.6a). Assuming a constant rate of growth throughout *in utero* development, this tentatively equates to a ~22 month gestation period (Figure 6.6a).

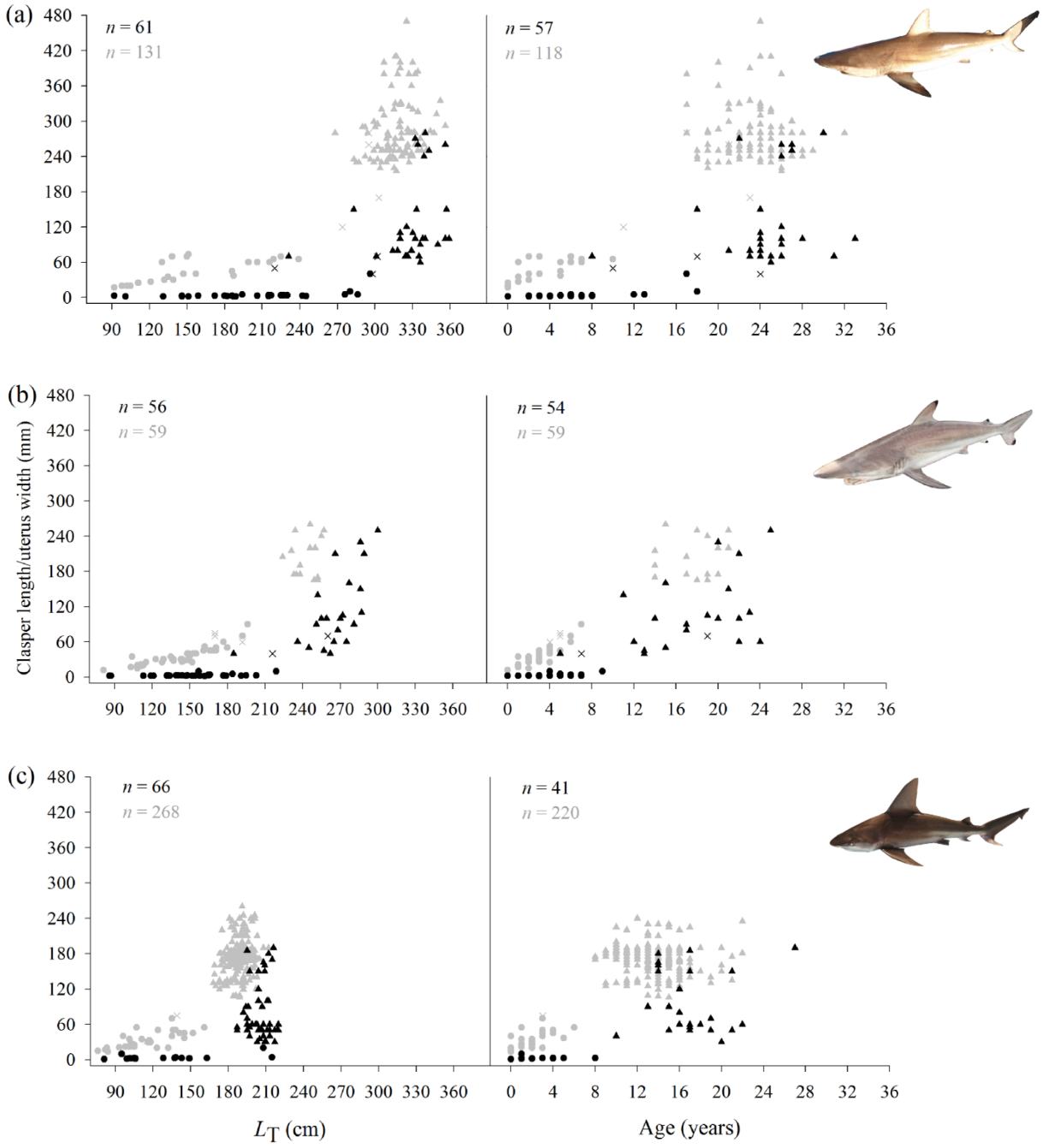

Figure 6.4 Comparative relationships between clasper length/uterus width and total length (L_T) and age for (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus*. Immature (○), maturing (×) and mature (Δ). Black symbols = females; grey symbols = males.

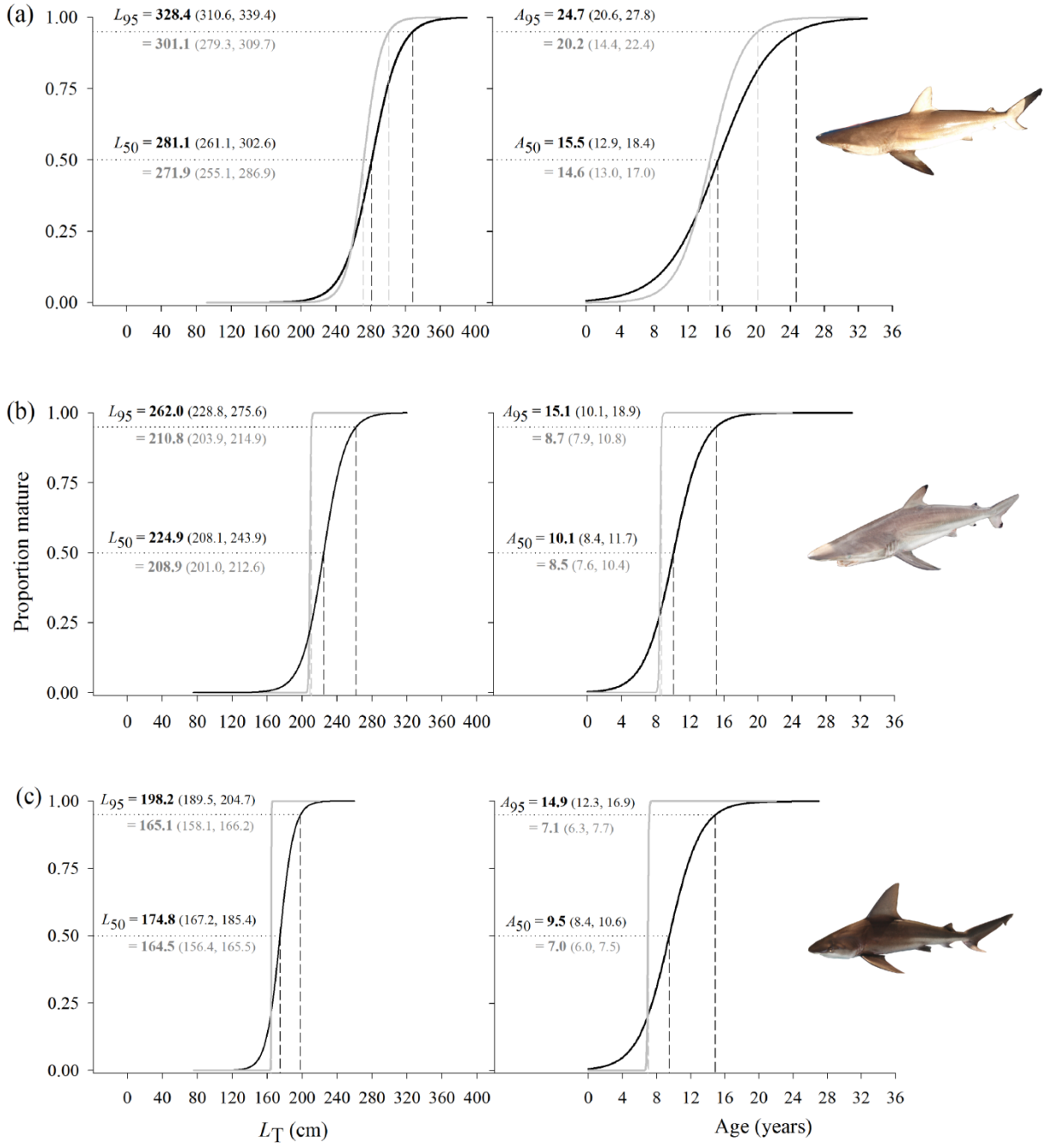

Figure 6.5 Comparative length- (L_T) and age-at-maturity ogives for (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus*. Sex-specific $L_{50\ \&\ 95}$ and $A_{50\ \&\ 95}$ parameters are displayed (with 95 % C.I.). Black ogives/parameters = females; grey ogives/parameters = males.

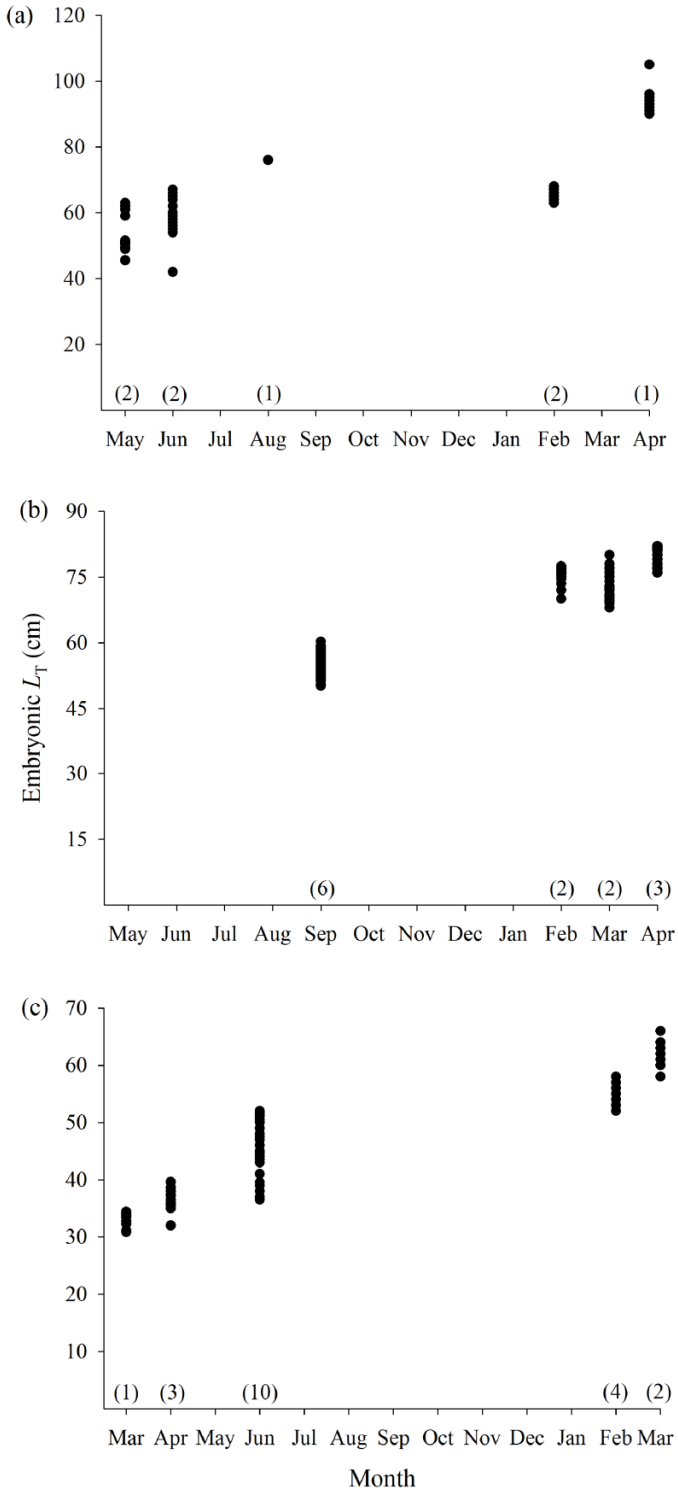

Figure 6.6 Embryonic total lengths (L_T) by month for (a) *Carcharhinus obscurus*, (b) *Carcharhinus brevipinna* and (c) *Carcharhinus plumbeus*. Number of litters in parentheses. Note the different x-axis in (c).

6.4.2 Carcharhinus brevipinna

We examined a total of 185 genetically-validated *C. brevipinna* (females, n = 101; males, n = 84), ranging in size from 79-317 cm L_T (Table 6.2). The length-frequencies for both sexes were bi-modally distributed (Figure 6.2b). Overlap of *in utero* embryo (n = 124) and free-swimming neonate (n = 6) life stages indicated that L_0 occured within the range 79-82 cm L_T (Figure 6.3b).

Clasper lengths suggested onset of maturity in males to occur between 190-220 cm L_T and 7-14 years (Figure 6.4b), while greater sample numbers indicated maturity between 200-220 cm L_T and 7-10 years inferred from length- and age-frequency distributions (Figure 6.2b). Male maturity ogives estimated L_{50} and L_{95} (95 % C.I.) respectively at 208.9 (201.0, 212.6) and 210.8 (203.9, 214.9) cm L_T, and A_{50} and A_{95} (95 % C.I.) at 8.5 (7.6, 10.4) and 8.7 (7.9, 10.8) years (Figure 6.5b). The largest immature and smallest mature males observed were 196 and 222 cm L_T, respectively; the oldest immature and youngest mature males were 7 and 10 years, respectively (Table 6.2).

For females, length- and age-frequency distributions and uterus widths suggested maturity to occur within the ranges 220-240 cm L_T and 7-11 years (Figures 6.2b, 6.4b). From maturity ogives, L_{50} and L_{95} (95 % C.I.) respectively were 224.9 (208.1, 243.9) and 262.0 (228.8, 275.6) cm L_T, and A_{50} and A_{95} (95 % C.I.) were 10.1 (8.4, 11.7) and 15.1 (10.1, 18.9) years (Figure 6.5b). The largest immature and smallest mature females observed were 260 and 185 cm L_T, respectively; the oldest immature and youngest mature females were 19 and 5 years, respectively (Table 6.2).

Pregnancy was observed at a mean length of 276.9 cm L_T and age of 18 years, with the smallest and youngest pregnant individuals being measured at 247 cm L_T and 11 years, respectively (Table 6.2). Uterine fecundity was estimated from 14 pregnant females and revealed litter sizes varying between 5 and 14 pups, with a mean of 10.6 (± S.E. 0.7). A weak

but statistically significant increase in litter size with maternal length was observed (Figure 6.7); the relationship between litter size and maternal age, however, was not significant.

The embryo sex ratio from 12 litters varied from 0.3:1 to 2.3:1, but was not significantly different from a 1:1 ratio overall ($\chi^2 = 0.0308$, df = 1, $p = 0.86$). *In utero* embryo lengths plotted by month suggested parturition occurs in autumn months and provided weak evidence that 7 months were necessary for development from approximately $2/3 \cdot L_0$ to full-term length (L_0) – suggestive of a ~21 month gestation period assuming, once again, a constant *in utero* growth rate (Figure 6.6b).

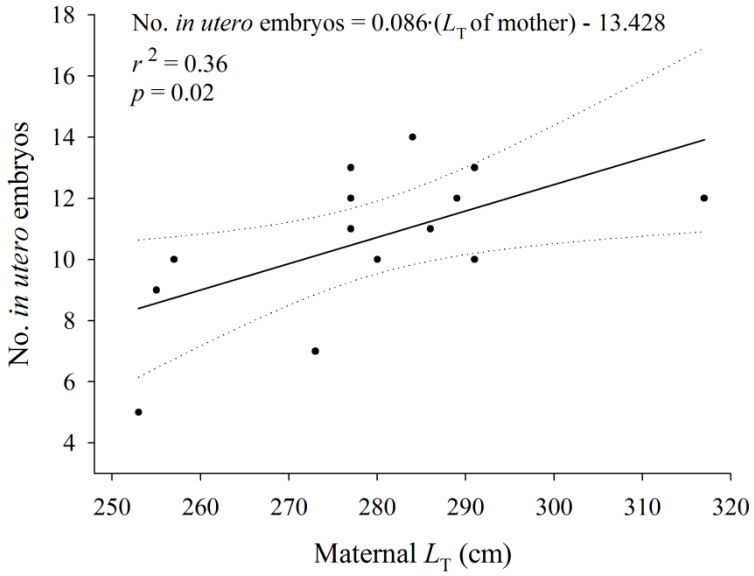

Figure 6.7 Relationship between uterine fecundity and maternal total length (L_T) observed for *Carcharhinus brevipinna* during the present study. Dotted lines denote 95 % C.I.

6.4.3 Carcharhinus plumbeus

A total of 475 genetically-validated *C. plumbeus* (females, $n = 193$; males, $n = 282$), ranging in size from 76-251 cm L_T, were examined here (Table 6.2); the vast majority were mature individuals (Figure 6.2c). *In utero* embryo ($n = 139$) and neonate ($n = 7$) lengths suggested L_0 to occur between 66-76 cm L_T, however no overlap was observed between the two life stages (Figure 6.3c).

For males, length- and age-frequency distributions and clasper lengths supported onset of maturity within the ranges of 160 to 170 cm L_T and 6 to 8 years (Figures 6.2c, 6.4c). Maturity ogives returned L_{50} and L_{95} (95 % C.I.) parameters of 164.5 (156.4, 165.5) and 165.1 (158.1, 166.2) cm L_T respectively, and A_{50} and A_{95} (95 % C.I.) values of 7.0 (6.0, 7.5) and 7.1 (6.3, 7.7) years (Figure 6.5c). The largest immature and smallest mature males observed were 161 and 168 cm L_T, respectively; while the oldest immature and youngest mature males were 6 and 8 years, respectively (Table 6.2).

Length, age and uterus width data proposed onset of maturity in females to be within the ranges 170-190 cm L_T and 8-10 years (Figures 6.2c, 6.4c). From maturity ogives, L_{50} and L_{95} (95 % C.I.) were 174.8 (167.2, 185.4) and 198.2 (189.5, 204.7) cm L_T respectively, and A_{50} and A_{95} (95 % C.I.) were 9.5 (8.4, 10.6) and 14.9 (12.3, 16.9) years (Figure 6.5c). The largest immature and smallest mature females observed were 215 and 175 cm L_T; the oldest immature and youngest mature females were 19 and 10 years, respectively (Table 6.2).

Pregnancy occurred at a mean length of 204.8 cm L_T and age of 16.1 years, with the smallest and youngest pregnant individuals being measured at 175 cm L_T and 13 years respectively (Table 6.2). Uterine fecundity was estimated from 23 pregnant females and revealed litter sizes of 3-12 pups, with a mean of 7.8 (± S.E. 0.5). Litter size did not increase significantly with maternal length or age. The embryo sex ratio from 19 litters varied from exclusively females to exclusively males, but was not significantly different from a 1:1 ratio overall ($\chi^2 = 0.3475$, df = 1, $p = 0.56$). *In utero* embryo lengths plotted by month suggested parturition occurs in autumn. Embryonic development was bi-modal in the month of March, suggestive of development from approximately $L_0/2$ to full-term length (L_0) having taken 12 months (Figure 6.6c). Assuming a constant rate of growth *in* utero, therefore, these data tentatively suggest a ~24 month gestation period.

6.5 Discussion

This study provides the first attempt at a detailed assessment of the reproductive biology of *C. obscurus*, *C. brevipinna* and *C. plumbeus* in eastern Australian waters. Although generally limited by modest sample sizes and restricted temporal distributions, all three were demonstrated to be relatively late-maturing species of low fecundity and lengthy gestation – highlighting their susceptibilities to stock depletion (Musick 1999). Comparatively, *C. obscurus* matured at the greatest lengths and oldest ages; *C. plumbeus* was the smallest and youngest at maturity. Accounting for differences in attainable size and longevity in the region, both sexes of all three species matured at similar sizes (i.e. 77-85 %) relative to their theoretical asymptotic lengths (L_∞), as modelled by two-parameter von Bertalanffy growth functions (Geraghty et al. 2013a – *Chapter 5*). With respect to age, male and female *C. brevipinna* and *C. plumbeus* matured at similar life-stages (i.e. at ages 32-35 % of their maximum longevities observed in NSW waters); both *C. obscurus* sexes, however, matured comparatively later in life – at ages 46-47 % of their maximum observed life spans in the sampling area (Geraghty et al. 2013a – *Chapter 5*). These results suggest that, of the three study species, *C. obscurus* is the most susceptible to population decline off Australia's south-east coast.

Our maturity and L_0 parameters, as well as our observations regarding pregnancy and fecundity, generally challenge the findings reported from other parts of the world for all three species, with notable exceptions. While the intrinsic vulnerabilities of the study species (but particularly *C. obscurus* and *C. plumbeus*) to overfishing are well established (Sminkey & Musick 1996, McAuley et al. 2007a, Romine et al. 2009), these data raise important implications relating to the comparative abilities of these species to withstand targeted-fishing pressure in the SW Pacific Ocean (Table 6.3). For example, *C. obscurus* and *C. plumbeus* appear to mature at younger ages in south-east Australian waters compared to other regions

(Table 6.3); earlier onset of maturity implies greater reproductive potential and, in turn, a comparatively greater resilience to population decline in NSW waters.

Similarly, our estimates of fecundity for *C. obscurus* and *C. brevipinna* support greater productivity in temperate eastern Australian waters compared to some other regions. In this study, *C. brevipinna* exhibited the greatest uterine fecundity and *C. plumbeus* the lowest. Our estimate for the latter species was largely consistent with those reported from other populations around the world (Table 6.3). In contrast, mean litter sizes estimated here for *C. obscurus* and *C. brevipinna* were consistent with most southern hemisphere regions but were markedly higher than those reported from all sampled northern hemisphere populations (Table 6.3). Higher fecundity not only suggests a greater tolerance to harvest pressure, but also a greater potential for stock recovery following excessive mortality.

Litter sex ratios proved highly consistent among studies. In NSW waters, we observed overall sex ratios of 1:1 for all three species. These findings support those from all previously sampled populations of our study species, with the exception of *C. plumbeus* from Western Australia (McAuley et al. 2007b) and the Mediterranean (Saïdi et al. 2005) (Table 6.3). Sex ratios, however, did vary considerably among individual litters in all studies. The most extreme case of which is demonstrated by *C. plumbeus*, which exhibits broods varying from exclusively females to exclusively males; a phenomenon observed in this study and also by Taniuchi (1971).

In considering uterine fecundity and pup sex ratios, however, it is important to acknowledge the tendency for viviparous sharks (including our study species) to abort part, or all, of their brood upon capture (Dudley et al. 2005, McAuley et al. 2007b). Quantitative observations of *in utero* embryos, therefore, may underestimate fecundity and bias sex ratios in some instances, thereby confounding population comparisons and relationships between litter size and maternal length and age.

Table 6.3 Comparative reproduction parameters described for *Carcharhinus obscurus*, *Carcharhinus brevipinna* and *Carcharhinus plumbeus* from around the world. Populations arranged latitudinally. All length measurements expressed as rounded total length (L_T, cm), unless otherwise stated, and converted where appropriate using publication-specific morphometric equations. Age and reproductive cycle expressed in years; gestation period in months.

Carcharhinus obscurus

Population	*n*	Female		Male		Mating	*G* [c]	*P* [d]	*R* [e]	Uterine fecundity		L_0 [g]	Reference
		L_M [a]	A_M [b]	L_M	A_M					Litter size	Sex [f]		
NW Atlantic	120	284	21	279	19	—	—	—	—	—	—	85-100	Natanson et al. (1995) ; Compagno (1984)
	—	226 [FL]	20	—	—	—	> 20	spr-sum	3	3-12; 7.1 ($\bar{X}$)	—	—	Romine et al. (2009)
	49	—	—	—	—	—	—	—	—	6-10; 7.7 ($\bar{X}$)	1:1	85-97	Bigelow & Schroeder (1948); Clarke & von Schmidt (1965)
Brazil	2	—	—	—	—	—	—	—	—	6-12	1:1	—	Amorim et al. (1998)
Indonesia	86	280	—	280-300	—	—	—	—	—	—	—	—	White (2007)
South Africa	871	285	20	280	19	—	≤ 24	win	3	≤ 16; 10 ($\bar{X}$)	1:1	85-96	Dudley et al. (2005); Natanson & Kohler (1996)
	42	260-300	17-24	280	20.5	—	—	—	—	9.9 ($\bar{X}$)	—	80-100	Bass et al. (1973); Natanson & Kohler (1996)
W Australia	305	261-297	17-22	273-288	20-23	—	—	—	2-3	3-14	—	92	Simpfendorfer (2000, 1999); Simpfendorfer et al. (2002)
	460	298	27-32	—	—	—	—	—	—	6-13; 9.9 ($\bar{X}$)	—	—	McAuley et al. (2005)
SE Australia	**268**	**281**	**15.5**	**272**	**14.6**	**—**	**~22**	**aut**	**—**	**5-12; 9.6 ($\bar{X}$)**	**1:1**	**92-96**	**Present study**

Table 6.3 cont.

Carcharhinus brevipinna

Population	*n*	Female		Male		Mating	*G*	*P*	*R*	Uterine fecundity		L_0	Reference
		L_M	A_M	L_M	A_M					Litter size	Sex		
NW Atlantic	49	180	7-8	170	6-7	sum	11-12	spr-sum	2	7 ($\bar{X}$)	—	60-70	Branstetter (1981, 1987)
Mediterranean	166	158-196	—	155-172	—	spr-sum	≥ 13-14	sum-aut	—	6-10; 7.5 ($\bar{X}$)	1:1	61-69	Capapé et al. (2003)
Persian Gulf	29	—	—	204	—	—	—	—	—	—	—	—	Moore et al (2012)
Taiwan	383	223	8	221	8	aut-win	10-12	spr	2	3-14; 8.5 ($\bar{X}$)	1:1	65-70	Joung et al. (2005)
Brazil	—	170	—	160		spr-sum	12	spr-sum	—	6 ($\bar{X}$)	—	—	Sadowsky (1967)
Indonesia	135	—	—	189-196	—	—	—	—	—	6-15	—	68-81	White (2007)
N Australia	—	—	—	165-195	—	—	—	—	1	12	—	—	Stevens & McLoughlin (1991)
South Africa	760	207	—	202	—	sum-aut	13-18	aut-win	≥ 2	≤ 17; 9 ($\bar{X}$)	1:1	73-86	Allen & Cliff (2000)
	67	215	8-10	202	8-10	—	—	—	—	—	—	80	Allen & Wintner (2002)
	—	200-210	—	180-200	—	sum-aut	12-15	aut	—	≤ 15; 10.7 ($\bar{X}$)	—	60-80	Bass et al. (1973)
SE Australia	33	—	—	215	—	—	12	aut	—	8-13	—	70-80	Stevens (1984)
	185	**225**	**10.1**	**209**	**8.5**	**—**	**~21**	**aut**	**—**	**5-14; 10.6 ($\bar{X}$)**	**1:1**	**79-82**	**Present study**

Table 6.3 cont.

Carcharhinus plumbeus

Population	*n*	Female		Male		Mating	*G*	*P*	*R*	Uterine fecundity		L_0	Reference
		L_M	A_M	L_M	A_M					Litter size	Sex		
NW Atlantic	1194	155 [FL]	13	152 [FL]	12	spr-sum	12	spr-sum	≥ 2	3-12; 8 ($\bar{X}$)	1:1	48-64	Baremore & Hale (2012)
	—	175	—	180-190	—	spr-sum	8-12	spr-sum	2	1-14; 9 ($\bar{X}$)	1:1	43-62	Springer (1960)
	—	—	—	—	—	—	—	—	—	4-12; 9 ($\bar{X}$)	1:1	—	Clark & von Schmidt (1965)
	20	185-190	—	180-184	—	—	—	—	2	6-11	—	—	Branstetter (1981)
	354	178	15-16	177	15-16	—	—	—	—	—	—	—	Sminkey & Musick (1995)
Mediterranean	932	166-172	—	155-160	—	—	12	spr-sum	2	4-10; 6.9 ($\bar{X}$)	1.2:1	45-65	Saïdi et al. (2005)
	—	170	—	166	—	—	12	—	1	3-14	—	58-65	Capapé (1984)
East China Sea	549	—	—	—	—	sum	10-12	sum	—	2-10; 6 ($\bar{X}$)	1:1	65-75	Taniuchi (1971)
Taiwan	885	170-175	7.5-8	175	8	spr	10-12	win-spr	2	4-12; 7.5 ($\bar{X}$)	1:1	60-65	Joung & Chen (1995); Joung et al. (2004)
Hawaii	320	110-120 [PCL]	10	100-110 [PCL]	8	—	—	—	—	—	—	47 [PCL]	Romine et al. (2006)
	789	150	—	143	—	sum	12	sum-aut	≥ 2	1-8; 5.5 ($\bar{X}$)	1:1	60-68	Wass (1973)
Red Sea	—	180	—	170	—	sum	—	—	—	6	1:1	60-65	Baranes & Ben-Tuvia (1978); Baranes & Wendling (1981)
Senegal	136	179-185		165			11-12	spr-sum	2	4-12; 7.7 ($\bar{X}$)	1:1	58-65	Diatta et al. (2008)
	—	185	—	180	—	—	—	—	—	5-12	—	55-61	Cadenat & Blache (1981)
Brazil	28	—	—	—	—	—	12	sum	2-3	7-10; 8.6 ($\bar{X}$)	1:1	> 57	Hazin et al. (2007)
	7	—	—	—	—	—	—	—	—	7-10	1:1	> 60	Amorim et al. (1998)
Indonesia	6	—	—	183	—	—	—	—	—	—	—	—	White (2007)
N Australia	—	155	—	156	—	spr-aut	12	sum-aut	2	3-8; 6 ($\bar{X}$)	1:1	60	Stevens & McLoughlin (1991)
Mauritius	—	177	—	180	—	—	—	—	—	6-11; 8.3 ($\bar{X}$)	—	—	Wheeler (1962)
South Africa	—	190	—	163	—	—	11-12	sum-aut	2	4-12	1:1	60-75	Bass et al. (1973)
	—	130 [PCL]	12	125-129 [PCL]	12	spr-sum	12	spr-sum	2	4-10; 7.2 ($\bar{X}$)	1:1	54-62	Cliff et al. (1988); Dudley & Simpfendorfer (2006)
W Australia	2163	157	16	148	14	sum-aut	12	sum-aut	2	4-10; 6.5 ($\bar{X}$)	0.6:1	51-57	McAuley et al. (2006, 2007b)
SE Australia	**475**	**175**	**9.5**	**165**	**7.0**		**~24**	**aut**		**3-12; 7.8 ($\bar{X}$)**	**1:1**	**66-76**	**Present study**

[a] Length (L_M) and [b] age (A_M) at maturity - expressed as length (L_{50}) and age (A_{50}) at which 50 % are mature where possible; [c] gestation period (G); [d] peak parturition season (P);

[e] reproductive cycle (R); [f] sex ratio (Sex) following significance test; [g] length-at-birth (L_0); [FL] fork length (L_F); [PCL] pre-caudal length (L_{PC})

While not conducted in the present study due to practical limitations, a more robust measure of uterine fecundity involves the quantification of placental scars associated with uterine compartments (Baremore & Hale 2012).

Empirical lengths-at-birth (L_0) for *C. plumbeus* and *C. brevipinna* suggest further differences between south-east Australia and other regions. *Carcharhinus plumbeus* appears to be born at considerably larger size in NSW waters, while our results for *C. brevipinna* support previous findings hinting at larger size-at-birth in the southern compared to the northern hemisphere (Table 6.3). Larger L_0 is thought to have positive implications for neonate survivorship and hence population growth via reduced predation risk and natural mortality (Cortés & Parsons 1996, Cortés 1998). Off Australia's east coast, *C. plumbeus* are not known to use discrete nursery grounds – a trait in direct contrast with NW Atlantic populations (Conrath & Musick 2007, Grubbs et al. 2007). Comparatively high L_0, therefore, may be a local adaption to offset increased predation risk. Lack of coastal nursery use by *C. plumbeus* in Australian waters is supported by McAuley et al. (2007b), who reported juvenile distribution in predominantly offshore continental-shelf regions off Australia's west coast. Interestingly, however, L_0 in the latter region appears to have remained small (Table 6.3).

Not all comparisons auger well, however, for the comparative resilience of south-east Australian populations of *C. obscurus*, *C. brevipinna* and *C. plumbeus* to overexploitation. Observations of gravid females provide some, albeit weak, evidence that pregnancy occurs at larger mean lengths in NSW waters, particularly in the former two species. Our estimates of mean length-at-pregnancy (Table 6.2) were larger than those reported from the NW Atlantic (317.2 ± 1.3; Clarke & von Schmidt 1965) for *C. obscurus* and from the Mediterranean (239.6 ± 5.1; Capapé et al. 2003) for *C. brevipinna*. For *C. plumbeus*, our estimate was somewhat consistent with studies from the NW Atlantic (208.1 ± 1.3; Clarke & von Schmidt, 1965) and Brazil (193-208; Hazin et al. 2007), but was slightly larger than that reported from the coast

of Senegal (195.4 ± 4.0; Diatta et al. 2008). In contrast to some of the previous results discussed above, the larger mean lengths- and ages-at-pregnancy observed in this study would suggest lower reproductive output and hence elevated susceptibilities to population decline in NSW waters. This assertion is further emphasised for all three species by the apparent lag between length and age-at-maturity (L_{50}, A_{50}) and mean length and age-at-pregnancy (Table 6.2, Figure 6.5). Although likely a direct consequence of low sample numbers and data gaps, this lag is, however, negligible or non-existent when considering L_{95} and A_{95}.

Observations of *in utero* embryos supported lengthy gestation cycles in all three species, and parturition in autumn months. While gestation in *C. obscurus* is widely recognised to be > 20 months in length (Dudley et al. 2005, Romine et al. 2009), our data suggest that gestation may be longer in NSW waters than in other regions for *C. brevipinna* and *C. plumbeus* (Table 6.3). This is emphasised for the latter species in particular, for which previous studies unanimously propose a maximum gestation period of 12 months compared with our tentative estimate of up to 24 months, the latter being based primarily on the observation of bi-model embryonic development in the month of March (Table 6.3). Such low apparent reproductive outputs in these species support considerable vulnerabilities to the detrimental effects of fishing pressure in the study region. As noted previously, however, our gestation periods have been estimated based on assumptions of constant *in utero* growth rate, which we concede is unlikely. In addition, bi-modal embryonic development as observed in *C. plumbeus* may be indicative of a prolonged mating season rather than prolonged gestation. Nevertheless, our data still support lengthy gestation periods in excess of 12 months for each species.

Of those populations for which reproductive parameters are available, direct comparisons suggest the NSW population of *C. obscurus* is most similar to those of Western Australia and South Africa; the NSW population of *C. brevipinna* appears to also closely resemble that of South Africa (Table 6.3). Our reproductive parameters for *C. plumbeus* are generally

consistent with a range of other locations, including Taiwan, Hawaii, Senegal and South Africa (Table 6.3). These results are interesting in light of recent studies by Geraghty et al. that report evidence of genetic differentiation in *C. obscurus* between eastern and western Australian waters (*Chapter 2*), as well as between Australian and South African waters in *C. brevipinna* (Geraghty et al. 2013b – *Chapter 3*).

Comparisons of reproductive characteristics between conspecific populations, such as those discussed above, however, are subject to a range of confounding factors and should be treated with considerable caution. Perceived geographic differences may be the result of sampling biases, variations in species' maximum attainable sizes, differing maturity-stage criteria and methodology, or combinations thereof. Comparisons involving estimates of age (e.g. age-at-maturity) can be compromised by variations in technique of preparation and reading of vertebrae, or reader accuracy and precision (Cailliet et al. 1990, Carlson et al. 2006). Furthermore, studies determining age-at-maturity via substitution of length-at-maturity into published growth curves are subject to the biases inherent in the respective growth parameters. For example, underestimated growth coefficients (k) resulting from overestimated asymptotic lengths (L_∞) will invariably translate to overestimates of age-at-maturity.

Admittedly this study was subject to a range of limitations impacting on the strength of our conclusions. Most notably, a distinct lack of data pertaining to neonate and maturing individuals resulted in high resolution estimates of length- and age-at-maturity and L_0 being difficult to achieve. In addition, the range of lengths (and hence ages) over which all maturity stages (but especially immaturity and intermediate maturity) were allocated, particularly for females, is suggestive of some observer inaccuracy (Figures 6.2, 6.4). Furthermore, our estimates of fecundity, mean length- and age-at-pregnancy and gestation period were all based on conspicuously low sample numbers and restricted temporal distributions of gravid females. As such we urge that our results be viewed as preliminary rather than definitive. We therefore

encourage future studies in this region to strive for greater resolution with resect to size- and age-at-maturity and timing and duration of development through more robust sample numbers and assessments of placental scars and number and size of ova in females, as well as the presence/absence of spermatozoa in the epididymis for males.

Nevertheless, the present study indicates that *C. obscurus*, *C. brevipinna* and *C. plumbeus* are all relatively late-maturing species in south-eastern Australian waters where they exhibit similar reproductive characteristics (i.e. fecundity, embryo sex ratio, gestation period and parturition season) marked by low productivity. When compared to populations inhabiting other parts of the world, our study also raises pertinent questions relating to the comparative resilience of these species to targeted-fishing activities in NSW waters. In doing so this study highlights the importance of measuring local life-history parameters for population modelling, demographic analyses and stock assessments.

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CHAPTER 7. General Discussion & Conclusions

Plate 8. A shark-fishing day draws to a close off the east coast of Australia. Photos by P. Geraghty.

7.1 Project summary & context

This study marks an investigation into the genetic diversity and population structure, growth dynamics and reproductive characteristics of *Carcharhinus obscurus*, *Carcharhinus brevipinna* and *Carcharhinus plumbeus* in temperate eastern Australian waters; where they support a demersal longline shark fishery. We also established basic estimates of scientific-observer accuracy in the identification of these species within the fishery. The resultant findings were used to explore implications for the management and conservation of these species in the study region.

Notably, this work represents: the first assessment of genetic diversity and structure in *C. brevipinna* from any part of its distributional range; a re-assessment of the genetic structure of *C. obscurus* from Indo-Australian waters; the first evaluation of age and growth for *C. brevipinna* in Australian waters and for *C. obscurus* and *C. plumbeus* in eastern Australian waters; and, the first attempt at a detailed assessment of the reproductive biology of all three species off Australia's east coast.

Carcharhinus obscurus, *C. brevipinna* and *C. plumbeus* exhibited both remarkable similarities and stark contrasts in aspects of their genetic structures and diversities, longevities, growth characteristics and reproductive biologies off Australia's south-east coast. These results highlighted a range of both positive and negative implications for their management in the New South Wales Ocean Trap and Line Fishery (NSW OTLF) when compared to other oceanic regions.

7.2 Significant findings & management implications

7.2.1 Fishery-observer accuracy

Fishery observers were demonstrated, via genetic validation, to be highly accurate in the identification of *C. obscurus*, *C. brevipinna* and *C. plumbeus* in the NSW OTLF; where they

are the three most harvested shark species (by number). This is most likely a function of large modal size-at-capture coupled with morphologic distinction at those sizes; the vast majority of landed sharks in the fishery being mature, adult individuals (Macbeth et al. 2009). These factors contribute to the NSW OTLF being less susceptible to observer-generated catch-composition inaccuracies than related shark fisheries in other parts of Australia (Tillett et al. 2012a).

Sound species identification, lending to robust catch-composition data, is of fundamental importance for effective fishery and species management. It provides a valuable means of recognising critical fishing-induced ecosystem consequences such as species-specific shifts in abundance, size-at-capture and/or catch per unit effort (Burgess et al. 2005, Field et al. 2009, Tillett et al. 2012a). The high level of accuracy observed here in south-eastern Australian waters, therefore, confirms the value of observer data in the management of the large-shark fishery under study.

7.2.2 Contemporary gene flow & genetic structure

In the case of *C. obscurus*, we discovered evidence for restricted contemporary gene flow between eastern and western Australian waters. Gene flow, however, appeared to be unencumbered between northern Australia and more southern regions, but was possibly constrained between Australia and central Indonesia by the Timor Trench. While genetic differentiation has been documented over directly comparable spatial scales in a range of similar shark species (Keeney et al. 2005, Duncan et al. 2006, Ahonen et al. 2009, Ovenden et al. 2009, 2011, Portnoy et al. 2010, Karl et al. 2011, Blower et al. 2012, Tillett et al. 2012b), our results for *C. obscurus* both challenge and reinforce the various findings of previous studies (Benavides et al. 2011b, Ovenden et al. 2009).

From a fishery management perspective, our findings support the allocation of two management units for *C. obscurus* in Australian waters – eastern and western regions. This implies that, in the event of a population collapse in the east, stock recovery would rely on reproduction by surviving local individuals and replenishment by immigrants from northern Australia. Replenishment via immigration from the west would appear to be limited, and levels of gene flow between Indonesia and Australia were essentially inconclusive. In spite of this, the most suitable boundary between the two purported management units remains uncertain, highlighting the need for greater resolution achieved via more extensive sampling. Nevertheless, our study would suggest a more integrated approach between adjacent states is appropriate for the management of *C. obscurus* in Australia.

Carcharhinus plumbeus and *C. obscurus* displayed very similar genetic diversities in NSW waters. Moreover, haplotype-network topologies suggested that populations of both species had been shaped by closely related evolutionary histories in this region. In light of this we propose that *C. obscurus* may represent a suitable proxy for patterns of gene flow in *C. plumbeus*, and vice versa, around Australia (excluding southern waters where the latter species does not occur). This hypothesis is given more weight by the finding of genetic differentiation between the east and west coasts of Australia in *C. plumbeus* by Portnoy et al. (2010) as is presented for *C. obscurus* in the present study.

Similar patterns of genetic diversity shaped by closely related evolutionary histories raise important implications for the management and conservation of these two species in the study area. Given that *C. obscurus* and *C. plumbeus* populations appear to have responded in the same way to evolutionary influences over time, and given the general similarities in their biological traits in Australian waters (Simpfendorfer et al. 2002, McAuley et al. 2006, 2007b, Geraghty et al. 2013a – *Chapter 5*, *Chapter 6*), it is likely that contemporary environmental and/or anthropogenic pressures will impact the two species' populations off the east coast in a

similar manner. Of concern, therefore, is that the majority of the genetic diversities displayed by both species in NSW waters is present as low-frequency haplotypes, implying a certain vulnerability to loss of genetic diversity under intense fishing pressure in this region. We therefore recommend that both species be considered simultaneously in any future management interventions.

In contrast, *C. brevipinna* exhibited greater genetic diversity off Australia's south-east coast stemming from a vastly different evolutionary history. The haplotype network for this species was distinctly star-shaped – indicative of a rapid demographic expansion event having occurred throughout the study region. The comparatively high diversity observed in this species implies that *C. brevipinna* may be more resilient to a fishing-induced loss of genetic diversity than both *C. plumbeus* and *C. obscurus*. This is cause for some optimism when considering the conservation of this commercially-targeted species in Australian waters.

With respect to gene flow in *C. brevipinna*, we detected subdivision over a broad spatial scale between Australia and South Africa – suggestive that the Indian Ocean is a robust barrier to contemporary gene flow in this species. This result is consistent with a range of other shark species (Pardini et al. 2001, Duncan et al. 2006, Ahonen et al. 2009, Chabot & Allen 2009, Portnoy et al. 2010, Benavides et al. 2011a, 2011b, Daly-Engel et al. 2012) and reiterates that large oceanic expanses represent major barriers to mitochondrial gene flow in coastal shark taxa. We also detected evidence for genetic differentiation between south-eastern and more northern regions of Australia's continuous continental margin. Although a surprising result, genetic subdivision has been reported over comparable geographic scales in the waters of Australia and abroad for a range of similar, highly-vagile shark species (Keeney et al. 2005, Karl et al. 2011, Morgan et al. 2011, Blower et al. 2012, Tillett et al. 2012b, 2012c).

From a management viewpoint, these results support the delineation of two management units in the southern Indo-Pacific for *C. brevipinna* – Australia and South Africa. The most appropriate boundary between them, however, is unknown and would require extensive sampling throughout the Indian Ocean basin. Our results also suggest two management units within Australian waters for *C. brevipinna* – i.e. south-eastern (NSW) and northern (QLD and NT) Australia, the implication being that in the event of a population collapse in south-eastern Australia recovery of genetic diversity would rely predominantly on reproduction by surviving local individuals in NSW waters. Despite statistical significance, however, this proposal and that concerning *C. obscurus* between east and west Australia are largely tentative given the weakness of the original findings of fine-scale genetic differentiation coupled with analyses indicating that biased sample numbers may have exerted considerable influence upon these results.

7.2.3 Shark ageing via micro-CT

This study identified micoCT as a valid alternative method for visualising growth bands in shark vertebrae for the purposes of ageing. This novel technique offered several distinct advantages over the traditional method of manual sectioning. Namely, it does not require the destruction of the vertebrae, it permits unlimited multiple virtual sectioning from unlimited angles and perspectives, it eliminates potential sources of variation inherent to manual processing such as section width and location, it eliminates the need to adjust light source and light angle during vertebrae age reading, and it reduces sample processing time with vertebral sections able to be scanned in an uncleaned state.

MicroCT technology, however, proved to be too expensive for broader application in the present study. Nonetheless, it is anticipated that costs will decrease over time as this method becomes more widely accessible.

7.2.4 Life-history characteristics

Both contrasts and consistencies in age and growth characteristics were observed among *C. obscurus*, *C. brevipinna* and *C. plumbeus* in south-eastern Australian waters. Longevity and modelled growth parameters varied markedly among the three species, with growth coefficient estimates purporting *C. obscurus* to be a slow-growing species, *C. brevipinna* as having a slow-moderate rate of growth and *C. plumbeus* to be a moderate-rapidly growing species. This notwithstanding, all three sharks were demonstrated to be long-lived species exhibiting a range of common inter-specific attributes. Namely, all three demonstrated rates of growth to be greatest in the years immediately after birth, decreasing progressively over time, and males to grow more rapidly than females during the juvenile phase. In addition, all three sharks exhibited sexually dimorphic growth, with females growing larger, living longer and being generally larger at a given age. Such trends are consistent among species within the Carcharhinidae family (Cortés 2000).

Aspects of the growth of *C. obscurus*, *C. brevipinna* and *C. plumbeus* reported here challenge those of conspecific populations in other parts of the world – suggestive of different growth characteristics in south-eastern Australian waters. More specifically, we extend current estimates of maximum age for *C. brevipinna* and generally report markedly less conservative growth coefficients (k) for *C. obscurus* and *C. plumbeus* of those previously defined. Confounding factors such as sample biases and variations in ageing methodology, interpretation and candidate growth models, however, render most inter-study comparisons overly speculative (Cailliet et al. 1990, Carlson et al. 2006). Nevertheless, the biologically-realistic growth parameters reported in this study serve as a contrast to most growth parameters reported to date for *C. obscurus* and *C. plumbeus* in particular. In light of this, and on the bases of genetic validation and sample size and distribution, we propose our growth parameters to be among the more robust currently available for these species. In addition,

when considering previous studies from other geographic regions, our results tentatively propose comparatively positive implications for the sustainable management of these species in NSW waters in the form of greater reproductive potential for *C. brevipinna* and a faster rate of growth to asymptotic length for *C. obscurus* and *C. plumbeus*.

With respect to reproductive biology, *C. obscurus*, *C. brevipinna* and *C. plumbeus* where each demonstrated to be relatively late-maturing species of low fecundity and lengthy gestation – highlighting their overarching low productivity and hence susceptibility to stock depletion off the NSW coast. Sex-specific lengths- and ages-at-maturity varied among species, however males and females of all three sharks matured at similar sizes and life-stages proportional to their observed attainable lengths and longevities in NSW waters, respectively; the sole exception being *C. obscurus* which matured comparatively later in life. While length-at-birth varied, fecundity, gestation period, embryonic sex ratio and time of parturition were all either very similar or shared among the three species. Common attributes such as these suggest that potential management restrictions incorporating maximum rates of harvest and parturition periods may to some degree be suitable across the three target species.

While the vulnerability of these three species to the effects of targeted fishing are well established in the literature, and upheld in this thesis, our reproductive parameters generally challenge those reported from other parts of the world for all three species (with notable exceptions), in turn raising important implications relating to their comparative abilities to withstand targeted-fishing pressure. Our data suggest that *C. obscurus* and *C. plumbeus* mature at younger ages in south-east Australian waters compared to other regions. Earlier onset of maturity implies greater reproductive potential and hence a greater resilience to population decline off the NSW coast compared to other areas. Similarly, our estimates of fecundity for *C. obscurus* and *C. brevipinna*, being consistent with most southern hemisphere regions but higher than all northern hemisphere populations, support greater productivity in

the present study region, suggesting in turn a greater tolerance to harvest pressure and increased potential for stock recovery following excessive mortality. Furthermore, empirical lengths-at-birth suggest further differences between south-east Australia and other regions; *C. plumbeus* appears to be born at considerably larger size in NSW waters, and our results for *C. brevipinna* support previous findings hinting at larger size-at-birth in the southern compared to the northern hemisphere. Larger size-at-birth is believed to have positive implications for neonate survivorship and hence population growth via reduced predation risk and natural mortality (Cortés & Parsons 1996, Cortés 1998). Not all comparisons between this study and conspecific populations, however, reflect positively on the comparative abilities of these species to resist overexploitation in the study region. Observations of gravid females provide some evidence that pregnancy occurs at larger mean lengths in NSW waters, particularly in *C. obscurus* and *C. brevipinna*. Large mean length- and age-at-pregnancy suggests low reproductive output, and hence an elevated susceptibility to population decline, in NSW waters. Furthermore, observations of *in utero* embryos provide some, albeit weak, evidence that gestation may be longer in NSW waters than in other regions for *C. brevipinna* and *C. plumbeus*; this compounds the apparent vulnerability of these species to the detrimental effects of fishing pressure in the study area.

In the same way as for growth characteristics as previously discussed, however, such comparisons of reproductive characteristics between conspecific populations are subject to a range of confounding factors and thus should be treated with considerable caution. Perceived geographic differences in maturity parameters may stem from sampling biases, variations in species' maximum attainable lengths, differing maturity-stage criteria and methodology, or combinations thereof. Maturity comparisons involving estimates of age (i.e. age-at-maturity and mean age-at-pregnancy) may be compromised by variations in technique of preparation and reading of vertebrae, and/or reader accuracy and precision. Furthermore, studies

determining age-at-maturity via substitution of length-at-maturity into published growth curves are subject to the biases inherent in the respective growth parameters.

Nevertheless, the life-history characteristics of *C. obscurus*, *C. brevipinna* and *C. plumbeus* elucidated in the present study generally raise serious (but not unexpected) implications for their management and conservation in temperate eastern Australian waters. As long-lived, relatively late-maturing and slow-growing species of low reproductive output, all three exhibit considerable vulnerability to overexploitation. These species (but particularly *C. obscurus* and *C. plumbeus*) have long been recognised, on the basis of their life-history traits, as among the least resilient to fishing mortality and, therefore, among the most susceptible to stock depletion and low subsequent rates of recovery (Smith et al. 1998, Cortés 2002, McAuley et al. 2007a).

While the results of this study appear to challenge many of the findings emanating from other parts of the world – illustrating the importance of measuring local life-history parameters for accurate population modelling and demographic analyses – they nonetheless broadly suggests that south-eastern Australian stocks of all three species are similarly sensitive to harvest pressure and low rebound potential (Sminkey & Musick 1996, McAuley et al 2007a, Romine et al. 2009). Moreover, the fact that the NSW OTLF lands individuals representative of the entire size range and all life stages of each of the three species, with a focus on large adult individuals, is cause for considerable concern. Demographic modelling by McAuley et al. (2007a) demonstrated that the harvesting of only a small number of juvenile age classes has distinct benefits for the exploitation of K-selected shark stock, such as *C. obscurus* and *C. plumbeus*. The life-history parameters of these species, coupled with poor historical track-records of management, serve to highlight the critical importance of protecting neonate survivorship as well as mature female biomass in fisheries targeting *C. obscurus*, *C. plumbeus* and *C. brevipinna*, such as the NSW OTLF.

7.3 Limitations & future directions

With the exception of the reproductive biology chapter, demonstrably robust sample numbers, generated from high-intensity sampling effort in NSW waters, highlight this study's greatest strength. This permitted comprehensive evaluations of the genetic diversity, population structure and age and growth parameters of *C. obscurus*, *C. brevipinna* and *C. plumbeus* off Australia's south-east coast. It also facilitated the estimation of arguably among the more accurate growth parameters of those published to date for the three study taxa. Nevertheless, this study was subject to a range of limitations, discussions of which reveal logical directions for future research.

A consequence of the abovementioned sampling effort was tissue sample biases strongly weighted towards NSW waters in genetic structure analyses for all three species. Random re-sampling simulation and rarefaction analysis were employed to test the influence of said biases on pairwise *F*-statistics. Both techniques raised doubt as to the reliability of various population comparisons presented in this study, hence encouraging a level of circumspection in the reporting of our findings. More specifically, random re-sampling simulations provided evidence that detections of population genetic differentiation in *C. obscurus* (between NSW and WA) and *C. brevipinna* (between NSW and QLD, and NT) in Australian waters were driven in large part by the strong biases in NSW sample size. That is, as the NSW sample size was gradually reduced towards a more balances analysis, the likelihood of finding a non-significant result increased. This technique highlighted either the weak nature, or uncertainty surrounding the actual existence, of fine-scale genetic subdivision within Australian waters for both *C. obscurus* and *C. brevipinna*. Furthermore, rarefaction analyses suggested that NSW, as well as South Africa in the case of *C. brevipinna,* were the only locations at which adequate proportions of the available genetic diversities had been sampled. In this way, these two were the only locations for which haplotype relative frequencies were able to be

discerned with any confidence. With much of the available diversities seemingly unsampled from QLD, NT, WA and Indonesia, it is appropriate that findings emanating from comparisons involving these locations should be treated with considerable caution.

The exclusive use of mitochondrial sequence data in the present study was equally limiting. It inhibited the testing of a null hypothesis that gene flow between putative populations is equal between males and females. Conflicting patterns of genetic structure between mitochondrial and bi-parentally inherited nuclear data (or mito-nuclear discordance) has been widely documented in shark species (Pardini et al. 2001, Schrey & Heist 2003, Keeney et al. 2005, Portnoy et al. 2010, Karl et al 2011, Daly-Engel et al. 2012, Tillett et al. 2012b, 2012c), and has been typically attributed to male-mediated gene flow (Portnoy & Heist 2012, Toews & Brelsford 2012). Persistent male dispersal despite constrained female gene flow, as is implied in the latter hypothesis, has significant implications relating to interpretations of population subdivision and, in turn, the allocation of appropriate management units. Reservations associated with the mutation rate of the ND4 region and hence its suitability in effectively discerning genetic structure, as well as the use of only one mitochondrial marker, present additional limitations of this study requiring consideration.

With respect to our investigations into life-history, gear-selectivity and fishing grounds translated to a general paucity of small-medium sized, as well as maturing, individuals for all three study species. These biases towards large, adult sharks not only influenced candidate growth model fitting, and hence resultant growth parameters, but also rendered high-resolution estimates of length- and age-at-maturity, length-at-birth, fecundity and gestation period difficult to discern. Compounding this, the range of lengths (and hence ages) over which all maturity stages (but especially immaturity and intermediate maturity) were allocated, particularly for females, was indicative of some observer inaccuracy. Furthermore, our estimates of fecundity, mean length- and age-at-pregnancy and gestation period were

based on conspicuously low sample numbers of gravid females, warranting circumspection in our findings.

In light of the abovementioned limitations, and within a context of considerable vulnerability to overexploitation as reinforced in the present study, we strongly encourage future work aimed at achieving greater resolution of reproductive parameters in NSW waters (i.e. size- and age-at-maturity and timing and duration of development) through more robust sample numbers and assessments of placental scars and number and size of ova in females, as well as the presence/absence of spermatozoa in the epididymis for males. We also urge studies with a dedicated focus on evaluating connectivity in *C. obscurus*, *C. brevipinna* and *C. plumbeus* around Australia. Future studies incorporating genetic microsatellite techniques, as well as physical tagging and tracking, would greatly improve stock structure resolution and hence the appropriate allocation of management units. Our genetic structure analyses for *C. obscurus*, for example, highlighted a knowledge gap regarding gene flow through southern Australian waters. While some insight was provided by a recent satellite-tracking study by Rogers et al. (2013), demonstrating the movement of *C. obscurus* between southern and south-western Australian waters, no definitive information pertaining to movement (or lack of) between east and west Australia via southern or northern waters is currently available. Genetic sampling of *C. obscurus* in southern waters, as well as more intensive sampling effort expended in locations other than NSW for all three species, would greatly improve interpretations of the data presented here. Similarly, a general lack of published information relating to *C. brevipinna* suggests further focus on this species across its global range is to be encouraged. Finally, further work incorporating the application of the life-history parameters as determined in this study in the development of population models, demographic analyses and stock assessments in eastern Australian waters would be of great benefit to the NSW OTLF in striving towards demonstrably sustainable carcharhinid shark catches.

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Appendix A: Chapter 3 Published Manuscript

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PLOS ONE

Population Expansion and Genetic Structure in *Carcharhinus brevipinna* in the Southern Indo-Pacific

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Abstract

Background: Quantifying genetic diversity and metapopulation structure provides insights into the evolutionary history of a species and helps develop appropriate management strategies. We provide the first assessment of genetic structure in spinner sharks (*Carcharhinus brevipinna*), a large cosmopolitan carcharhinid, sampled from eastern and northern Australia and South Africa.

Methods and Findings: Sequencing of the mitochondrial DNA NADH dehydrogenase subunit 4 gene for 430 individuals revealed 37 haplotypes and moderately high haplotype diversity (h = 0.6770 ±0.025). While two metrics of genetic divergence (Φ_{ST} and F_{ST}) revealed somewhat different results, subdivision was detected between South Africa and all Australian locations (pairwise Φ_{ST}, range 0.02717–0.03508, p values ≤ 0.0013; pairwise F_{ST} South Africa vs New South Wales = 0.04056, p = 0.0008). Evidence for fine-scale genetic structuring was also detected along Australia's east coast (pairwise Φ_{ST} = 0.01328, p < 0.015), and between south-eastern and northern locations (pairwise Φ_{ST} = 0.00669, p < 0.04).

Conclusions: The Indian Ocean represents a robust barrier to contemporary gene flow in *C. brevipinna* between Australia and South Africa. Gene flow also appears restricted along a continuous continental margin in this species, with data tentatively suggesting the delineation of two management units within Australian waters. Further sampling, however, is required for a more robust evaluation of the latter finding. Evidence indicates that all sampled populations were shaped by a substantial demographic expansion event, with the resultant high genetic diversity being cause for optimism when considering conservation of this commercially-targeted species in the southern Indo-Pacific.

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Introduction

Patterns of genetic variability in extant taxa have been generated by events and processes occurring over evolutionary time scales. Genetic bottlenecks and demographic expansions, coupled with associated fluctuations in effective population size, are examples of such events, respectively manifesting as low and, eventually, high levels of genetic diversity [1-8]. Evolutionary processes that influence genetic variability, however, need not be characterised by pronounced reduction or elevation in diversity. In a range of taxa, barriers to dispersal and gene flow caused by geographic separation or long-term behavioural traits have led to spatial partitioning of genetic diversity. Cessation of gene flow results in spatial genetic differentiation [9-13], and ultimately, speciation due to natural selection, genetic drift and mutation [14-16]. Quantifying genetic diversity and metapopulation structure, therefore, can provide insight into the evolutionary history and behaviour of a species and, in turn, the most appropriate strategy for its management.

In the marine environment, generating accurate, representative estimates of genetic diversity and population structure can be challenging. Cryptic barriers to dispersal and inherent uncertainties pertaining to the spatial extent of gene

flow within a species make the most informative experimental designs difficult to determine, notwithstanding the practical issues associated with the collection of highly-vagile marine taxa. For example, various members of the Carcharhinidae represent large, cosmopolitan shark species occupying predominantly continental-shelf waters [17]. Species such as the dusky (*Carcharhinus obscurus*), sandbar (*Carcharhinus plumbeus*), bull (*Carcharhinus leucas*) and common blacktip (*Carcharhinus limbatus*) shark are capable of travelling considerable distances, and are suspected to undertake long-range migrations [18-23]. These species are also dependent on shallow coastal habitats for birthing and offspring development [22,24-27], with mounting evidence demonstrating philopatric behaviour in juveniles and, more notably, in gravid females [12,28-31]. This trait suggests that, for some carcharhinid sharks, spatial genetic connectivity may be lower than otherwise predicted based on vagility and demonstrated patterns of movement. The contrast between long-range dispersal ability and the potential for sex-specific disruption of gene flow between geographically proximate locations provides a complex context within which to decipher genetic structure. Given the implications for management and conservation, however, this same dichotomy highlights the importance of an understanding of spatial genetic subdivision in shark species.

Genetic structure has been investigated in several carcharhinids at a range of geographic scales [32]. Studies on global phylogeography have consistently shown that large oceanic expanses are robust barriers to gene flow [33-38]. Genetic subdivision has also been documented over finer spatial scales and attributed to either philopatric behaviour or historic events causing geographic isolation [12,28,30,31,35,39-42].

The spinner shark (*Carcharhinus brevipinna*) has thus far been neglected in the population genetic literature. No research on genetic diversity or stock structure has been conducted in any part of its cosmopolitan range, which includes much of the world's tropical and warm-temperate continental shelf waters [17]. *Carcharhinus brevipinna* is predominantly a by-catch or secondary target species, but is nevertheless an important component of commercial catches in multi-species shark fisheries around the world [43-50]. Furthermore, owing to confusion with the 'blacktip' shark, commercial catch records of *C. brevipinna* are most likely gross underestimates in some regions. Recreational catch rates are also suspected to be substantial, however, as for most shark species, they remain unquantified. In Australian waters, considerable numbers of *C. brevipinna* are landed along the eastern, northern and western coastlines where they are harvested using demersal longlines, demersal and pelagic gillnets, and handlines [51-55]. In eastern Australia, a fishery-observer study revealed this species to be the third most abundant large shark caught in the New South Wales Ocean Trap and Line Fishery (NSW OTLF) [53].

Carcharhinus brevipinna is a schooling species known to frequent nearshore waters as adults and utilise inshore nursery habitats as juveniles [24,56-59]. As such, *C. brevipinna* is considered highly vulnerable to fishing pressure and human-induced habitat alteration, and is hence globally IUCN listed as 'near threatened' [60]. Despite this, long-term catch-data sets have provided evidence for stock stability in *C. brevipinna*. Carlson et al. [50] proposed that growth overfishing had not occurred on this species in the heavily fished western North Atlantic, with the average landed size remaining stable from 1994–2009. Furthermore, the abundance of *C. brevipinna* in this fishery appears to have remained largely unchanged, with some evidence for increase over the same period [50]. Similar findings were reported by Dudley and Simpfendorfer [45] from the western Indian Ocean, who revealed stable catch per unit effort (CPUE) and stable/increasing size at capture from 1978–2003. Having experienced comparatively lower targeted-fishing pressure on a global scale, *C. brevipinna* has not been subject to the same concern or scrutiny regarding the status of its populations as that levelled at species such as *C. obscurus* and *C. plumbeus* [61-64]. However, the life-history characteristics of *C. brevipinna* suggest a similar vulnerability to overfishing and to slow intrinsic rates of population recovery [44,48,65-69]. Furthering our understanding of global *C. brevipinna* populations, therefore, may be considered prudent.

Here we assess genetic structure and diversity in *C. brevipinna* using mitochondrial DNA (mtDNA) sequence data. We test a null hypothesis of genetic homogeneity throughout Australian and South African waters, and discuss the evolutionary history of the species in the region. We generate an estimate of scientific-observer accuracy in identifying *C. brevipinna* in an eastern Australian large-shark fishery, and also discuss the implications of our findings for fisheries management and conservation.

Materials and Methods

Ethics Statement

Tissues were sampled from New South Wales (NSW) waters according to a protocol approved by the NSW Government Primary Industry (Fisheries) Animal Care and Ethics Research Authority (Permit ACEC REF 07/03 – CFC).

Sample collection

Shark tissues were collected from a range of locations in the southern Indo-Pacific (Figure 1) using a variety of fishery-dependent methods. From NSW waters, tissues were harvested during 2007–2010 from landed catch by scientific observers on-board commercial shark-fishing vessels within the NSW OTLF. These samples were taken from individuals spanning the entire size range of the species (Figure 2). A small quantity (<2 g) of white muscle tissue was excised from each specimen, immediately preserved in 95% reagent grade ethanol, and stored at room temperature. Additional samples, collected during 2000–2010, were obtained from more distant locations, including from the waters of north-western Northern Territory (NT), Gulf of Carpentaria (GoC) and Queensland (QLD) in northern Australia, as well as from the east coast of South Africa (Figure 1). Tissues from north-western NT, GoC and QLD were sampled from predominantly neonate and small-juvenile individuals from landed catch by observers within their respective commercial shark fisheries (Figure 2), and were preserved in 20% dimethylsulphoxide (DMSO) solution. Fin-clip samples from South Africa, preserved in 100% ethanol,

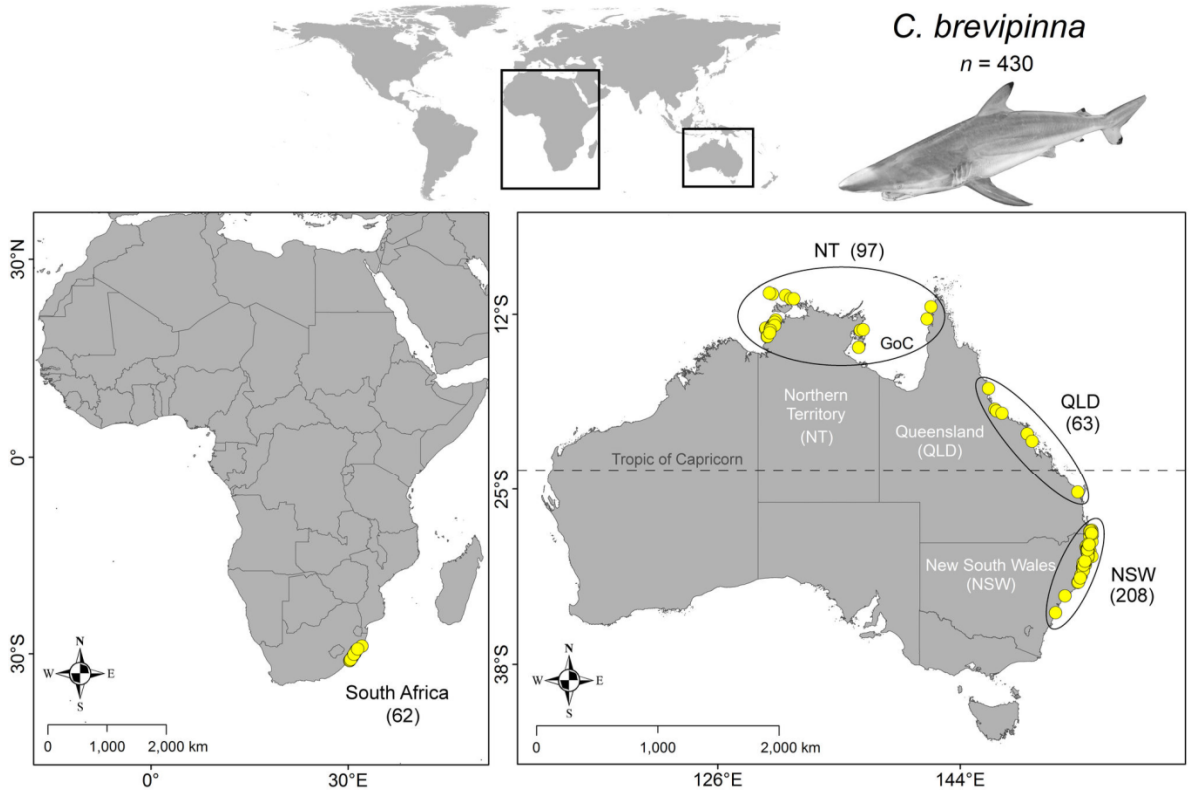

Figure 1. Collection locations for tissues included in genetic structure and diversity analyses. Sample numbers for each putative population are in parentheses. GoC = Gulf of Carpentaria.

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were collected from adult and sub-adult sharks caught in the Kwazulu-Natal beach protection nets (Figure 2). For South African specimens, pre-caudal length (PCL) measurements were converted to total length (TL) using the morphometric equation published in Allen and Wintner [67]. Additional samples were obtained from QLD and NSW waters by sampling sharks caught in government bather protection programs [59,70].

DNA extraction, amplification and sequencing

To obtain mtDNA sequence data, total genomic DNA was extracted from 5 mg of tissue using a modified salting-out protocol [71]. Samples were digested with 10 µl of Proteinase-K (10 mg·ml^{-1}) in 580 µl of TNES [50 mM Tris.HCl (pH 7.5), 400 mM NaCl, 20 mM EDTA and 0.5% SDS] by incubation overnight at 55 °C. Proteins were precipitated by adding 170 µl of 5 M NaCl followed by microcentrifugation at 14,000 rpm for 5 min. Supernatant (600 µl) was recovered into a fresh tube and the DNA precipitated by adding 600 µl of ice-cold 100% ethanol. Tubes were stored at −20 °C for approximately 1 h. DNA was then recovered by microcentrifugation at 14,000 rpm for 15 min, and the ethanol decanted. The resulting DNA pellet was washed with 200 µl of 70% ethanol, 100 mM sodium acetate solution, and microcentrifuged at 14,000 rpm for 3 min. Following decanting, all remaining ethanol was removed using a micropipette. DNA was air-dried, resuspended in 100 µl of TE buffer [10 mM Tris.HCl (pH 7.6) and 1 mM EDTA] and stored at −20 °C. DNA yield was checked on a 1.0% agarose TBE (90 mM TRIS-borate and 2 mM EDTA) gel run at 110 V.

Polymerase Chain Reaction (PCR) was used to amplify the mitochondrial DNA NADH dehydrogenase subunit 4 (ND4) gene from all tissue samples. Reactions were carried out in 50 µl volumes containing 1 µl of DNA template, 1× GoTaq Colourless reaction buffer [consisting of 1.5 mM $MgCl_2$ and 200 µM deoxynucleoside triphosphates (dNTPs)] (Promega, Madison, WI, USA), 0.5 µl of RNase (1 mg·ml^{-1}), and 0.5 µM of each of the primers ND4 (5' CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC) [72] and H12293-LEU (5' TTG CAC CAA GAG TTT TTG GTT CCT AAG ACC) [73]. Amplifications were performed in an Eppendorf ep gradient S Mastercycler (Eppendorf AG, Hamburg, Germany), using thermal cycling conditions consisting of an initial denaturation (94 °C for 3 min), followed by 35 cycles of 94 °C for 15 s, 60 °C for 30 s and 72 °C for 1 min, with a final extension step of 72 °C for 10 min, and soak/finish at 4 °C. PCR products were visualised on a 2.0% agarose TBE gel, run at 110 V, and stained with GelRed

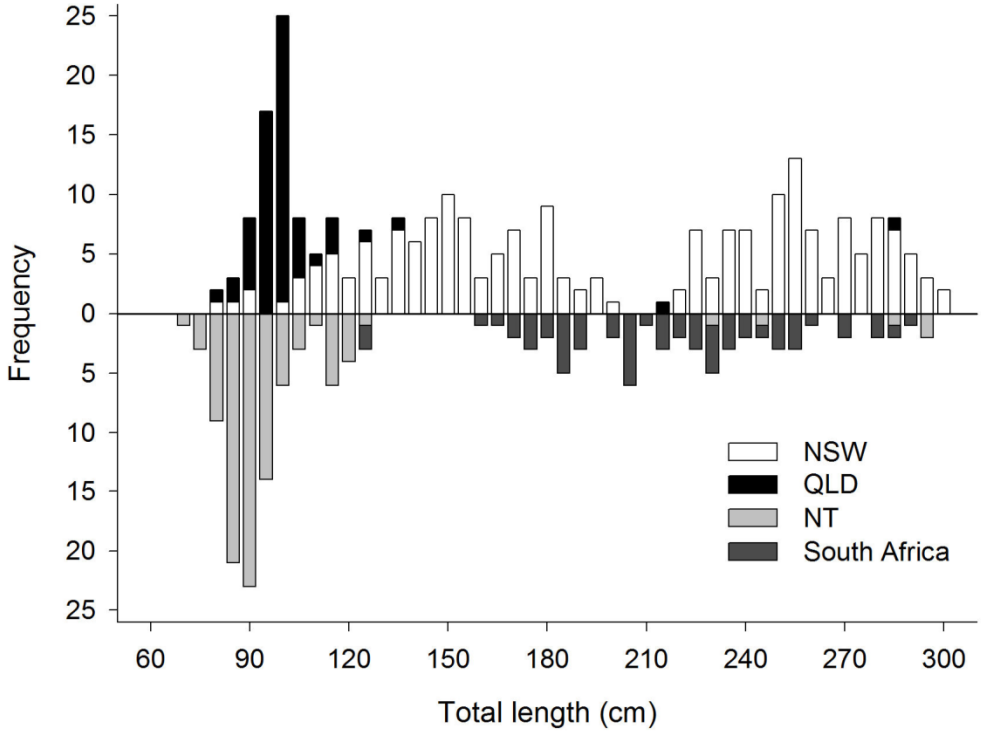

Figure 2. Length-frequency distribution of individuals from which tissues were sampled.
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(Biotium Inc., Hayward, CA, USA). PCR products were purified prior to sequencing using Exosap-IT (USB Corporation distributed by GE Healthcare Bio-Sciences, Rydalmere, Australia). Sequencing was performed with an Applied Biosystems 3130xl Genetic Analyzer 16-array capillary sequencer (Life Technologies, Carlsbad, CA, USA). Sequencing reactions and analyses were carried out by the Macquarie University (MQ) DNA Sequencing Facility using Big Dye Terminator reactions and the forward PCR primer only.

Sequence alignment and ID validation

Sequences were trimmed and edited manually. Edited sequences were entered into Biomanager (https://biomanager.info) and aligned using the ClustalW (accurate) algorithm [74]. GenBank reference sequences for *C. brevipinna* were available for the cytochrome oxidase I (CO1) gene, but not for ND4, prior to this study. Therefore, to validate that the study species had been correctly identified and also to determine the species identity of any misidentified individuals, randomly-selected representatives from each separate haplotype determined from the alignment output were amplified for the CO1 gene using the primers Fish F1 (5' TCA ACC AAC CAC AAA GAC ATT GGC AC) and Fish R1 (5' TAG ACT TCT GGG TGG CCA AAG AAT CA) [75]. PCRs were carried out as above, with thermal cycling conditions consisting of an initial denaturation (95 °C for 5 min), followed by 30 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 1 min, with a final extension step of 72 °C for 7 min, and soak/finish at 4 °C. PCR products were purified and sequenced following the same protocol outlined for the ND4 locus. Resultant CO1 sequences were compared to reference sequences in Genbank for species recognition.

ND4 sequence analysis

To identify and characterise mitochondrial haplotypes, aligned *C. brevipinna* ND4 sequences were imported to Arlequin 3.5.1.2 [76]. A sequence representing each haplotype was lodged in GenBank (Accession codes KF612545-KF612581). The frequency of, and mutational steps between, haplotypes were assessed by generating statistical parsimony haplotype networks in TCS 1.21 using the default settings [77]. Phylogenetic relationships among haplotypes were inferred using the maximum likelihood method based on the Tamura-Nei model [78], and generated in MEGA 5 [79] with 1,000 bootstrap replicates. The best-fitting model of nucleotide substitution, as offered by MEGA 5, was determined by likelihood ratio tests and calculations of Akaike and Bayesian Information Criteria performed in jModelTest 2.1.1 [80]. To assess the ability of the ND4 region to differentiate between carcharhinids, the phylogram was rooted with a range of morphologically similar species, as well as with two sphyrnid species as outgroups.

Genetic diversity indices were obtained with Arlequin using the Tamura-Nei substitution model [78], and included polymorphism statistics, number of haplotypes, haplotype diversity (h) and nucleotide diversity (π). Harpending's raggedness index (H_{RI}) was estimated from nucleotide mismatch distributions constructed in Arlequin under the sudden demographic expansion model with 20,000 bootstrap replicates [81]. Tajima's *D* and Fu's *F* neutrality indices were also estimated in Arlequin, and are indicative of departures from mutation-drift equilibrium or patterns of selection [82,83]. In conjunction with H_{RI}, the latter two analyses can be used to determine if a population has undergone an expansion event (possibly following a genetic bottleneck). Mismatch distributions will be multi-modal (or ragged) in a stable population, where the generation of new mutations is offset by random drift, and uni-modal for expanding populations, where new mutations accumulate faster than their loss due to drift [81]. For Tajima's *D* and Fu's *F*, signals of population expansion are denoted by significant negative test statistic values. Statistical significance was assessed here, following 20,000 simulated samples, at $\alpha = 0.05$ and $\alpha = 0.02$ for *D* and *F* values respectively [83].

Population genetic structure

To test the null hypothesis of panmixia (genetic homogeneity) in Australian and South African waters for *C. brevipinna*, an analysis of molecular variance (AMOVA) [84] was implemented in Arlequin to evaluate the overall extent of net genetic subdivision between sample locations. We employed two *F*-statistic metrics of genetic divergence: Φ_{ST} [84] and F_{ST} [85]. While Φ_{ST} has been regarded as the superior metric on the basis of its incorporation of a measure of genetic distance between haplotypes, frequency-based F_{ST} has been proposed as potentially a more appropriate measure of genetic differentiation among locations where migration is theoretically occurring at a faster rate than mutation [86]. Φ_{ST} was calculated via the computing of a distance matrix using the Tamura-Nei model [78] for estimation of genetic distance between sequences, while F_{ST} used haplotype frequencies only. AMOVA partitioned genetic variance among, and within, sample locations, and calculated overall Φ_{ST} and F_{ST} fixation indices. Genetic differentiation between each pair of locations was also measured by calculating pairwise Φ_{ST} and F_{ST} estimates. Statistical significance was determined following 20,000 permutations of the sequence data and, in the case of pairwise Φ_{ST} and F_{ST}, assessed at an initial critical significance level of $\alpha = 0.0083$ (adjusted from $\alpha = 0.05$) following sequential Bonferroni correction for six simultaneous comparisons [87,88]. The AMOVA structure consisted of one group made up of the following four putative populations: NSW ($n = 208$), QLD ($n = 63$), NT ($n = 97$) and South Africa ($n = 62$) (Figure 1). The analysis outlined above is henceforth referred to as the 'original analysis'. Prior to conducting this large-scale AMOVA, we investigated the extent of genetic subdivision on a finer scale between GoC ($n = 43$) and north-western NT ($n = 54$) waters. This analysis indicated genetic homogeneity (fixation indices: $\Phi_{ST} = 0.00035$, $p > 0.39$; $F_{ST} = 0.00151$, $p > 0.31$), hence providing justification for pooling GoC and north-western NT samples to create one northern population termed 'NT'.

Carcharhinus brevipinna sample sizes were clearly biased towards NSW (Figure 1), where 208 samples were collected compared to 62, 63 and 97 samples from the other three locations. We evaluated the influence of this sampling bias on the *F*-statistics of pairwise population comparisons involving NSW via random re-sampling simulations. Ten thousand replicate random sample-sets of $n = 60$ (for comparison with QLD and South Africa, but not NT owing to its larger original sample size), $n = 100$ and $n = 150$ were selected without replacement from the NSW population, while QLD, NT and South African sample sizes were kept unchanged. Population pairwise Φ_{ST} and associated *p* values were generated for each replicate random sample-set in Arlequin using the batch processing function and permutation settings as outlined above. Resultant Φ_{ST} and *p* value distributions were plotted, and the likelihood of producing a result contradictory to that of the original analysis was calculated as either the proportion of *p* values ≤ 0.05 or > 0.05, depending on the result of the original analysis. That is, if the original pairwise *p* value was significant ($p \leq 0.05$), the likelihood of a contradictory result equals the absolute number of *p* values $> 0.05/10{,}000$.

The 'Isolation by Distance' (IBD) hypothesis was also tested to determine if inter-population genetic distances increased linearly with geographic distance. Genetic (Φ_{ST}) and geographic (km, by sea) distances between the four putative populations were calculated in GenAlEx [89] and ArcMap 10.0 (ESRI), respectively. Pairwise genetic and geographic distance matrices were correlated using a Mantel test, with a test for a significant relationship by 9,999 random permutations, also implemented in GenAlEx.

Rarefaction analysis

To determine whether sample sizes adequately represented population genetic variability, rarefaction exact curves were generated to qualitatively assess the proportion of haplotypic diversity sampled at each of the four locations. The expected number of haplotypes found for a given sample number (from one to the total sample size obtained at each location) was calculated using the rarefaction formula of Hurlbert [90], and executed in the statistical package R [91]. A trend towards an asymptotic relationship infers haplotype saturation, i.e. that the majority of the available genetic diversity was likely sampled at that location and that more intensive sampling is likely to yield few additional haplotypes. In contrast, a steep slope suggests that a large fraction of the available haplotype diversity remains unsampled.

Results

Fishery-observer accuracy in NSW waters

The ND4 gene region proved to be capable of distinguishing a range of morphologically-similar carcharhinids (Figure 3), as previously shown by Tillett et al. [55]. Genetic validation was possible for a total of 190 sharks identified by scientific observers as *C. brevipinna* in the NSW OTLF from 2007–2010. Of these, 187 were genetically confirmed to be *C. brevipinna*,

translating to an observer-accuracy estimate of 98.4% for the identification of this species in the fishery (Table 1). Misidentified individuals (n = 3) comprised two *C. limbatus* and one *C. obscurus* (Table 1).

Table 1. Fishery-observer identification accuracy.

Genetic identification	Observer identified *C. brevipinna* (n = 190)
C. brevipinna	98.4 (187)
C. limbatus	1.1 (2)
C. obscurus	0.5 (1)

Percentage (individual counts in parentheses) of each genetically-identified shark species from observer-identified *Carcharhinus brevipinna* in the New South Wales Ocean Trap and Line Fishery.

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Genetic diversity and summary statistics

An 857 base pair mtDNA ND4 sequence was obtained for 430 *C. brevipinna* individuals collected from Australian and South African waters (Figure 1). A total of 37 haplotypes were defined, characterised by 41 polymorphic sites composed of 40 transitions and one transversion (Table S1). A phylogenetic tree placed all haplotypes into a single, shallow clade (Figure 3). One haplotype (SP1) clearly dominated the sample set, and was found in all four populations in reasonably similar proportions (Table 2). The same number of haplotypes (n = 23) was found in NSW and NT waters, despite NSW having over double the sample size (Table 3). NSW exhibited six haplotypes endemic to the area, whereas NT displayed five. Almost identical sample sizes revealed 17 haplotypes from QLD waters and 11 from South African waters, with two unique haplotypes defined from each location (Table 3). Haplotype (h) and nucleotide (π) diversities were very similar, and high in the case of the former and low in the case of the latter, across three of the four putative populations (QLD, NT and South Africa; h, range 0.7279–0.7493; π, range 0.0015–0.0016) (Table 3). Comparatively lower diversity was observed in NSW waters (h = 0.5984, π = 0.0010). All mismatch distributions were consistent with the sudden population expansion model, with no significant deviation from a uni-modal distribution (H_{RI}, range 0.054–0.099) (Table 3). In support of this, all four putative populations displayed significant negative neutrality indices (D, range -2.245 – -1.506; F, range -23.626 – -4.464) (Table 3).

Rarefaction and optimum sample size

Rarefaction exact curves indicated trends towards asymptotic relationships for both the NSW and South African locations (Figure 4), despite markedly different sample sizes. This suggests that the majority of the haplotypic diversities available at these two locations were most likely sampled. Steeper slopes were observed from QLD and NT waters (Figure 4), suggestive that some proportion of the available genetic diversities remained unsampled. Optimum sample size for the adequate representation of levels of genetic variation present in a given *C. brevipinna* population appears to be site dependent.

Population genetic structure

The haplotype network incorporating the four putative populations was shallow and shaped in a distinct 'star-burst' pattern, characterised by one central haplotype (SP1) surrounded by an array of low, or lower, frequency variants (SP2-SP27) (Figure 5). A high degree of haplotype sharing was observed among the four geographically-distinct populations, with the dominant haplotype (SP1) being common at each of the four locations and ~58% (or n = 21 of n = 36) of lower frequency haplotypes being shared between two or more locations (Figure 5, Table 2).

Despite this, AMOVA fixation indices detected significant levels of genetic differentiation between the four putative populations for both F-statistic metrics (Φ_{ST} = 0.01634, p = 0.0001; F_{ST} = 0.01493, p < 0.0035) (Table 4). We therefore reject the null hypothesis that *C. brevipinna* are panmictic in Australian and South African waters. Pairwise results, however, revealed some differences between the two measures of divergence. The Φ_{ST} metric detected genetic subdivision between South Africa and all Australian locations (pairwise Φ_{ST}, range 0.02714–0.03508; p value, range 0.0000–0.0013), with all three comparisons significant after Bonferroni correction (Table 5). Φ_{ST} also detected genetic differentiation, albeit weaker, between NSW and QLD waters (pairwise Φ_{ST} = 0.01328, p < 0.016) which was also significant after sequential Bonferroni adjustment, as well as some evidence for genetic subdivision between NSW and NT (pairwise Φ_{ST} = 0.00669) which was significant at p < 0.05 but not after Bonferroni correction (Table 5). In contrast, the haplotype-frequency based analysis indicated significant genetic differentiation between the NSW and South African locations only (pairwise F_{ST} = 0.04056, p = 0.0008) (Table 5). All other pairwise F_{ST} comparisons, with the exception of QLD vs NT, were only marginally non-significant (pairwise p value, range 0.0510–0.0845). The finding of genetic homogeneity between QLD and NT was concordant between both F-statistics. A strong positive relationship, with high goodness-of-fit (r^2 = 0.86), was observed between pairwise genetic and geographic distances for *C. brevipinna*. This relationship, being driven entirely by differences between Australian locations and South Africa, was not statistically supported by a mantel test (p = 0.091).

Simulation was used to test the effect of a bias in the numbers of *C. brevipinna* sampled from NSW on the F-statistics analysis of pairwise population comparisons. Random re-samplings demonstrated an increasing likelihood of finding a non-significant result between NSW and QLD, and between NSW and NT, with decreasing NSW sample size (Figure 6). More specifically, for NSW vs QLD, 21.08% of replicate pairwise comparisons where sample size was set to 150 for NSW (and left at 63 for QLD) did not provide statistical support for the original analysis, for which sample size was 208 for NSW and 63 for QLD. This increased to 48.29% and 71.8% as the NSW sample size was reduced further to 100 and 60, respectively. Considering NSW vs NT, the likelihood of producing a contradictory result to that of the original analysis was high as NSW sample size was reduced. Where sample size was set to 150 for NSW (and left at 97 for NT), 61.32% of

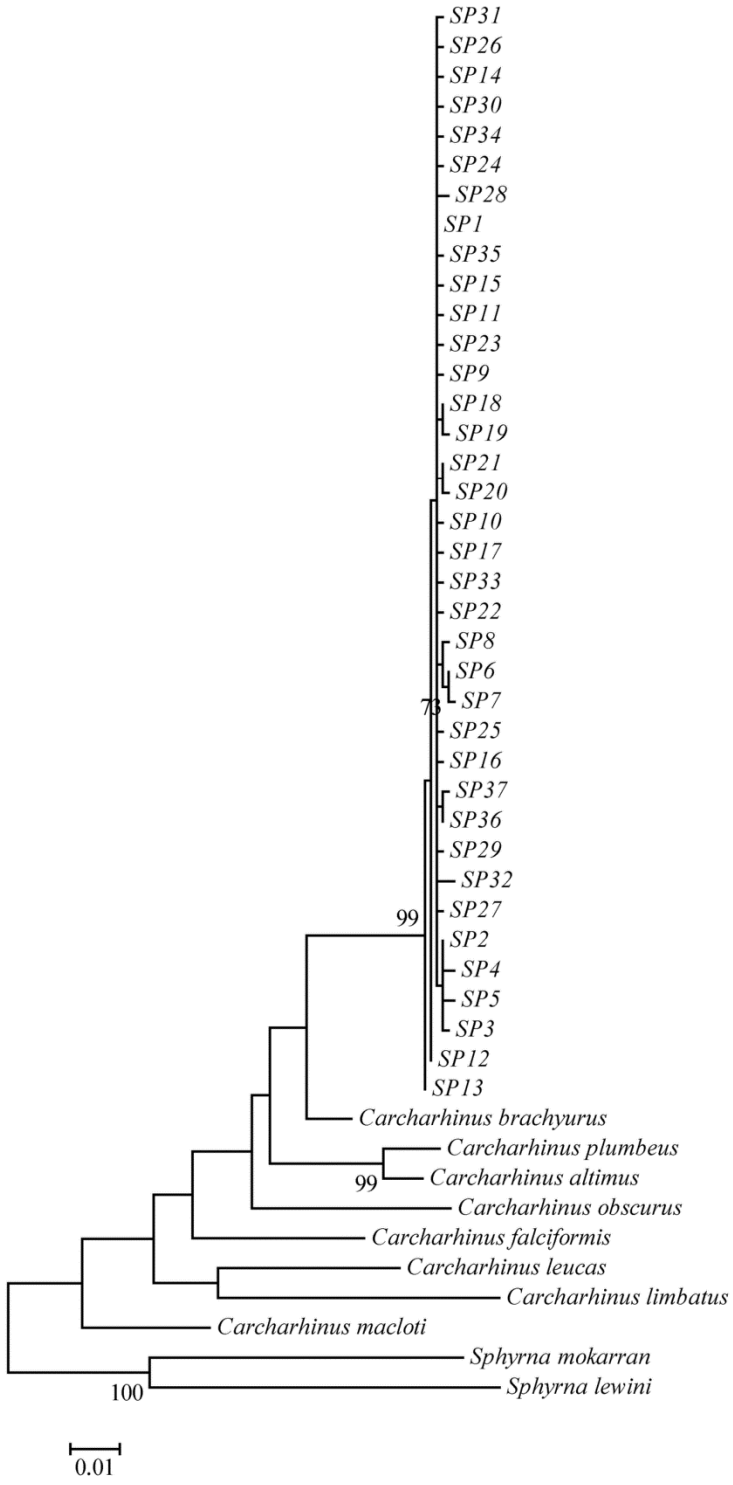

Figure 3. Maximum likelihood phylogenetic tree for *Carcharhinus brevipinna* haplotypes. Nodal bootstrap support is displayed where ≥ 70%. Scale represents the proportion of polymorphic sites between haplotypes.

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Table 2. Haplotype relative frequencies observed from each sampling location.

	Relative frequency				
Haplotype	NSW (*n* = 208)	QLD (*n* = 63)	NT (*n* = 97)	South Africa (*n* = 62)	GenBank Accession
SP1	0.625	0.492	0.505	0.468	KF612545
SP2	0.082	0.111	0.124	0.065	KF612546
SP3	0.005	0.032	0.010	–	KF612547
SP4	0.010	0.063	0.041	0.016	KF612548
SP5	–	–	0.010	–	KF612549
SP6	0.019	–	0.010	–	KF612550
SP7	–	–	–	0.065	KF612551
SP8	0.005	0.016	0.031	–	KF612552
SP9	–	–	–	0.032	KF612553
SP10	–	0.016	–	0.145	KF612554
SP11	–	–	0.010	0.016	KF612555
SP12	–	–	0.021	0.048	KF612556
SP13	–	–	0.010	0.016	KF612557
SP14	–	–	0.010	–	KF612558
SP15	–	–	0.010	–	KF612559
SP16	–	–	0.010	–	KF612560
SP17	0.019	0.016	0.021	0.032	KF612561
SP18	0.053	0.048	0.021	–	KF612562
SP19	–	–	0.010	–	KF612563
SP20	–	0.016	0.010	–	KF612564
SP21	0.038	–	0.041	–	KF612565
SP22	0.024	0.063	0.021	–	KF612566
SP23	0.005	–	0.010	–	KF612567
SP24	0.005	–	0.010	–	KF612568
SP25	–	0.016	–	–	KF612569
SP26	–	0.016	–	–	KF612570
SP27	0.005	0.016	–	–	KF612571
SP28	0.005	0.016	–	–	KF612572
SP29	0.019	0.016	0.010	–	KF612573
SP30	0.010	0.016	–	–	KF612574
SP31	0.010	–	–	–	KF612575
SP32	0.010	–	–	–	KF612576
SP33	0.014	–	–	–	KF612577
SP34	0.005	–	–	–	KF612578
SP35	0.005	–	–	–	KF612579
SP36	0.019	0.032	0.041	0.097	KF612580
SP37	0.010	–	–	–	KF612581

SP1-37 = Observed *Carcharhinus brevipinna* mitochondrial DNA ND4 haplotypes.

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replicate pairwise comparisons did not provide statistical support for the original analysis, for which sample size was 208 for NSW and 97 for NT. This increased to 74% when the NSW sample size was reduced to 100. Further illustrating this point, as NSW sample size was reduced, pairwise Φ_{ST} and *p* value distributions revealed increasing variability in conjunction with decreasing mean Φ_{ST} and increasing mean *p* value relative to the output of the original analysis (Figure 7). This pattern was observed for both sets of locations. In contrast, replicate pairwise comparisons between NSW and South Africa displayed an unchanging, and zero percent, likelihood of generating a different result to that of the original analysis as NSW sample size was altered (Figure 6).

Discussion and Conclusions

Observer identification accuracy in an east Australian shark fishery

Observer accuracy was high (98.4%) in the identification of *C. brevipinna* in the NSW OTLF. This estimate is comparable to other target species within this same fishery; *C. obscurus* and *C. plumbeus* were correctly identified by fishery observers to accuracies of 96.6% and 99.4%, respectively (PT Geraghty, unpublished data). Given the fundamental importance of accurate catch-composition data in fisheries (and species) management [55,92,93], this high level of accuracy in the

Table 3. Genetic diversity indices observed for *Carcharhinus brevipinna* sample locations in the southern Indo-Pacific.

Location	n [a]	n_H [b]	n_{Hq} [c]	h [d]	π [e]	H_{RI} [f]	D [g]	F [h]
NSW	208	23	6	0.5984 (±0.040)	0.0010 (±0.0008)	0.074	− 2.245***	− 23.626***
QLD	63	17	2	0.7424 (±0.056)	0.0015 (±0.0011)	0.057	− 2.056**	− 13.080***
NT	97	23	5	0.7279 (±0.047)	0.0015 (±0.0010)	0.054	− 2.163**	− 22.072***
South Africa	62	11	2	0.7493 (±0.050)	0.0016 (±0.0011)	0.099	− 1.506*	− 4.464*
Pooled	430	37	•	0.6770 (±0.025)	0.0013 (±0.0009)	0.064	− 2.252***	− 29.294***

a. Sample size (n ,

b. number of haplotypes (n_H),

c. number of unique haplotypes (n_{Hq}),

d. haplotype diversity (h ,

e. nucleotide diversity (π),

f. Harpending's raggedness index (H_{RI}),

g. Tajima's (D) and [h] Fu's (F) tests of selective neutrality, Values in parentheses represent standard deviations (s.d.).

• value not applicable.

* denotes significance at the $p \leq 0.05$ level, **$p \leq 0.01$, ***$p \leq 0.001$.

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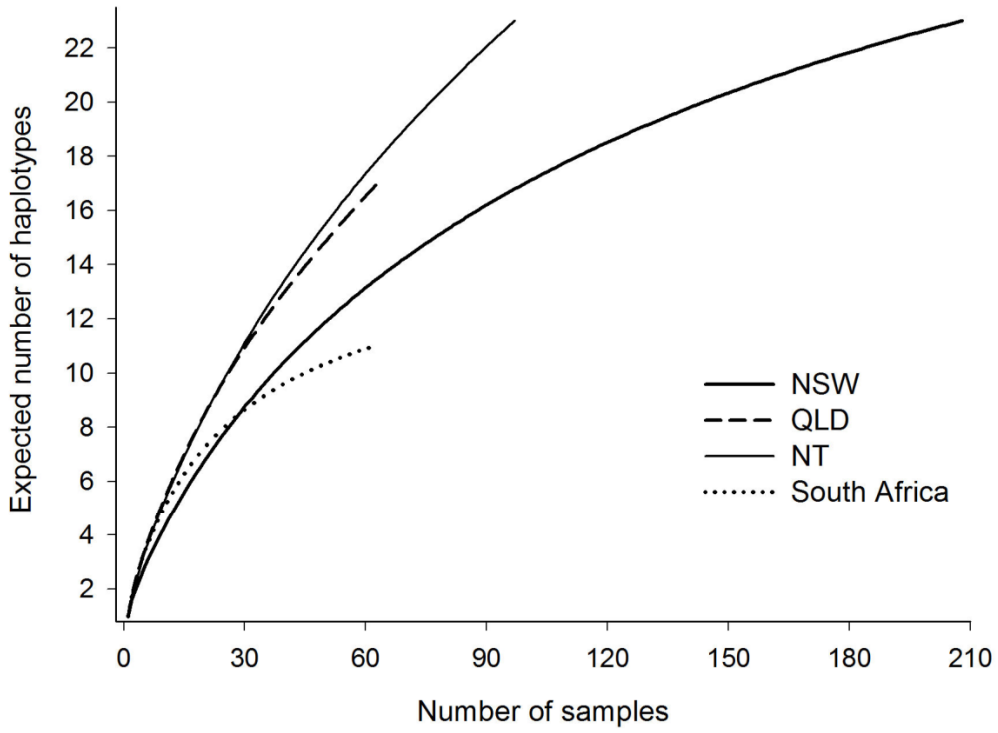

Figure 4. Rarefaction exact curves for sample locations.

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recognition of the three most harvested shark species (by number) in the NSW OTLF [53] confirms the usefulness of fishery-observer data in the management of this eastern Australian large shark fishery.

Our measure of observer accuracy (98.4%) for *C. brevipinna* in the NSW OTLF was higher than that reported for the same species by Tillett et al. [55] in the Northern Territory Offshore Net and Line Fishery (NT ONLF), for which observer accuracy was estimated at 87.2%. Higher identification accuracy in the NSW OTLF compared to the NT ONLF was not unexpected for this particular species given the difference in size class targeted by the two fisheries. The vast majority of the landed

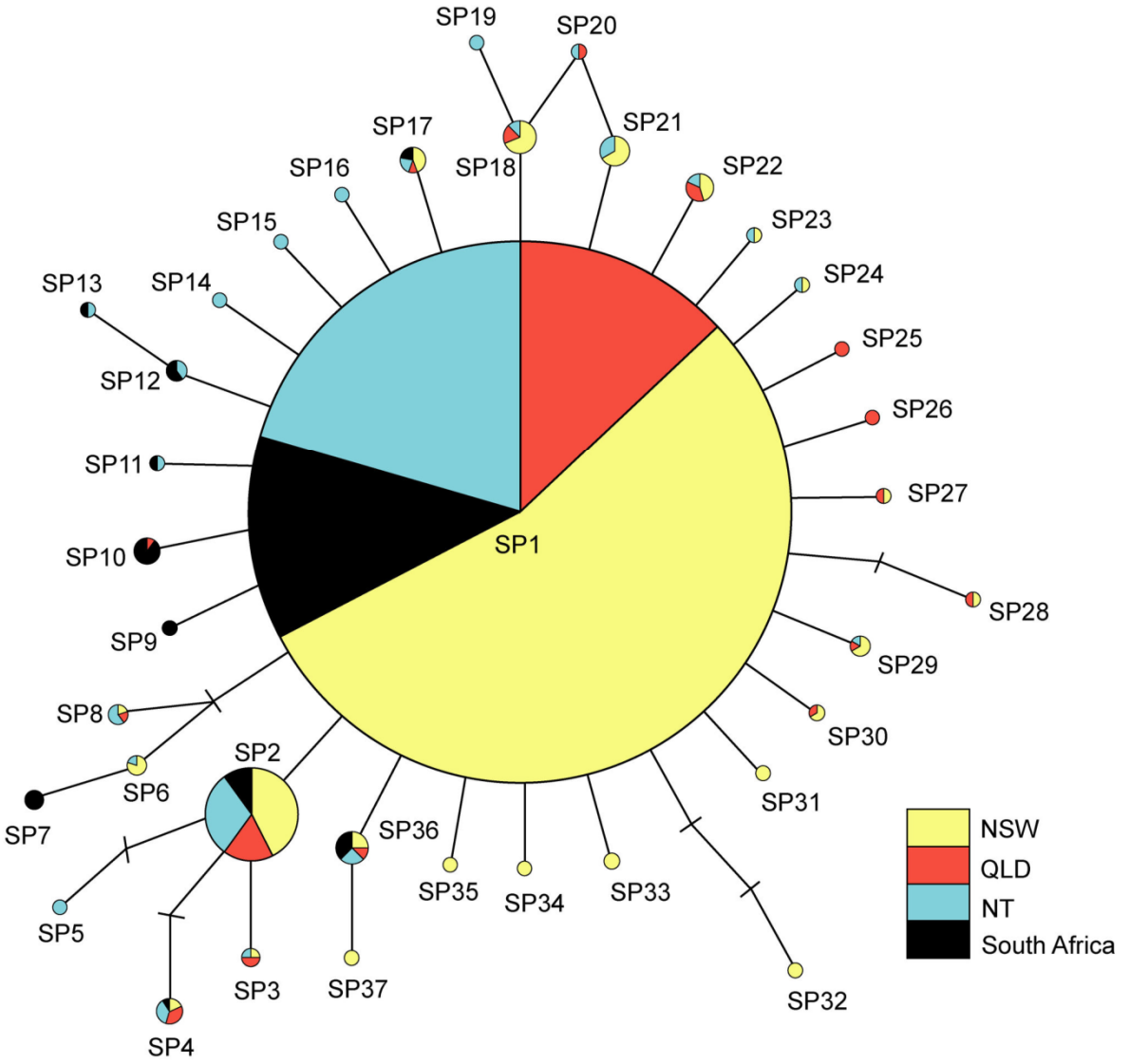

Figure 5. *Carcharhinus brevipinna* mitochondrial DNA ND4 haplotype network. Sizes of circles correspond to the number of individuals displaying each haplotype. Shading indicates the proportion observed from each of the four putative populations. (−) = mutational steps/missing haplotypes.

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shark catch in the NSW OTLF is in the form of mature, adult individuals [53]. In contrast, the NT ONLF targets predominantly neonate and small juvenile life stages, illustrated by the fact that all sharks identified as *C. brevipinna* by observers in the latter fishery were ≤ 1.2m TL [55]. Size-at-capture is important as *C. brevipinna* is characterised by diagnostic traits that become increasingly discernible as an individual grows larger, most notably tooth shape and fin pigmentation [17]. At small sizes, *C. brevipinna* can be difficult to distinguish from a range of other morphologically-similar carcharhinid species [17].

Evolutionary history in the southern Indo-Pacific

The *C. brevipinna* haplotype network was distinctly star-shaped, characterised by a single dominant haplotype surrounded by a high number of low, or lower, frequency variants. This central, and presumably ancestral, haplotype was prominent in all three Australian sample locations, as well

Table 4. AMOVA analyses of spatial genetic variation for *Carcharhinus brevipinna* from Australian and South African waters.

Source of variation	d.f.	Test statistic	Sum of squares	Variance components	Percentage of variation (%)
Among populations	3	Φ_{ST}	4.304	0.00916	1.63
	3	F_{ST}	2.475	0.00508	1.49
Within populations	426	Φ_{ST}	234.819	0.55122	98.37
	426	F_{ST}	142.742	0.33507	98.51
Fixation indices		Φ_{ST} = 0.01634; p = 0.00010 (±0.00007)			
		F_{ST} = 0.01493; p = 0.00345 (±0.00041)			

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Table 5. Comparison of pairwise *F*-statistic values between putative populations.

	NSW (n = 208)	QLD (n = 63)	NT (n = 97)	South Africa (n = 62)
NSW		0.01151 (0.0601)	0.00921 (0.0531)	***0.04056*** (0.0008)
QLD	***0.01328*** (0.0151)		−0.00704 (0.9099)	0.01306 (0.0845)
NT	0.00669* (0.0387)	−0.00507 (0.8166)		0.01411 (0.0510)
South Africa	***0.03494*** (0.0000)	***0.03508*** (0.0009)	***0.02717*** (0.0013)	

Observed Φ_{ST} values are below the diagonal, and F_{ST} values are above diagonal, with p values in parentheses. Bold italics indicate values significant after sequential Bonferroni correction (initial α = 0.0083). * Statistically significant at $p \leq 0.05$, but not following Bonferroni adjustment.

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as off the coast of South Africa - evidence that Australian and South African waters share common ancestry in this species.

The pattern of genetic diversity observed here in *C. brevipinna* is indicative of a contemporary demographic expansion event having occurred throughout the southern Indo-Pacific. This hypothesis is supported by a range of evidence: the distinctly 'star-burst' haplotype network denoted by numerous low-frequency mutations, mismatch distributions and neutrality test statistics suggesting strong departures from mutation-drift equilibrium for all four putative populations and the observed combination of generally high haplotype and low nucleotide diversities [82,83,94-96]. Attempts at dating this population expansion event were abandoned in the absence of mutation-rate estimates for ND4 in elasmobranchs.

It must be noted here, however, that spatial sample coverage in the present study is limited to only a very small area of this species' global distribution range, which includes much of the world's tropical and warm-temperate continental shelf waters [17]. Therefore, in the absence of genetic analysis of samples representative of the entire distribution of the species, we are unable to determine whether or not this rapid population growth was a worldwide event or was restricted to the southern Indo-Pacific.

The strong signal of population expansion reported here in *C. brevipinna* is unprecedented among sharks, with comparable signals more commonly associated with taxa such as humans [2] and teleost fishes [6,97,98]. Evidence for population expansion has, however, been presented for some shark species through analyses of mismatch distributions [8,99], star-like haplotype networks [40,100,101], or combinations of the latter two supported by neutrality indices [102,103].

Contemporary genetic structuring

This study marks the first dedicated assessment of genetic structure in *C. brevipinna*. The application of two metrics of genetic divergence (Φ_{ST} and F_{ST}) demonstrated that population genetic findings can be dependent on the *F*-statistic employed - especially pertinent where subdivision is at the margins of statistical significance [98]. We therefore encourage the concurrent use of both metrics as standard practice in population genetic studies.

With this in mind, genetic differentiation was detected over a broad spatial scale between Australian and South African waters. This finding based on mtDNA was not unexpected and, being consistent with a range of other shark population genetic studies [35-37,99,104-107], re-iterates that large oceanic expanses (in this case the Indian Ocean) represent robust barriers to contemporary gene flow in coastal shark species.

Evidence for genetic subdivision, albeit weak, was also detected over finer spatial scales within Australian waters, i.e. between NSW and both QLD and, to a lesser degree, NT. Genetic homogeneity was observed between QLD and NT waters. These results tentatively suggest that gene flow is restricted to some degree along Australia's eastern continental margin as well as between the south-eastern and northern coastlines, and that gene flow is unencumbered between north and north-eastern Australian waters. These findings were somewhat unexpected given *C. brevipinna*'s potential for active dispersal. That said, however, genetic differentiation has previously been detected in similar and related shark species over comparable geographic scales in Australian waters [31,42,108,109], as well as those of the Gulf of Mexico and north-western Atlantic [12,30].

Reproductive philopatry, or the fidelity of gravid females to nursery areas, is typically invoked to explain fine-scale genetic structuring (based on maternally-inherited mtDNA) in the absence of barriers to dispersal for highly-vagile sharks [12,30,31,34,41,109]. Confidently discerning this sex-biased behavioural trait, however, is complex and relies on a robust experimental design involving the exclusive sampling of neonates, or adult females at time of parturition rather than during dispersal, from spatially discrete areas [12,32]. The collection of tissues in the present study was generally reliant on both spatial and temporal opportunistic sampling, rather than according to a dedicated experimental design. Nevertheless, tissues from NT and QLD were almost exclusively sampled from neonates and small juveniles, with

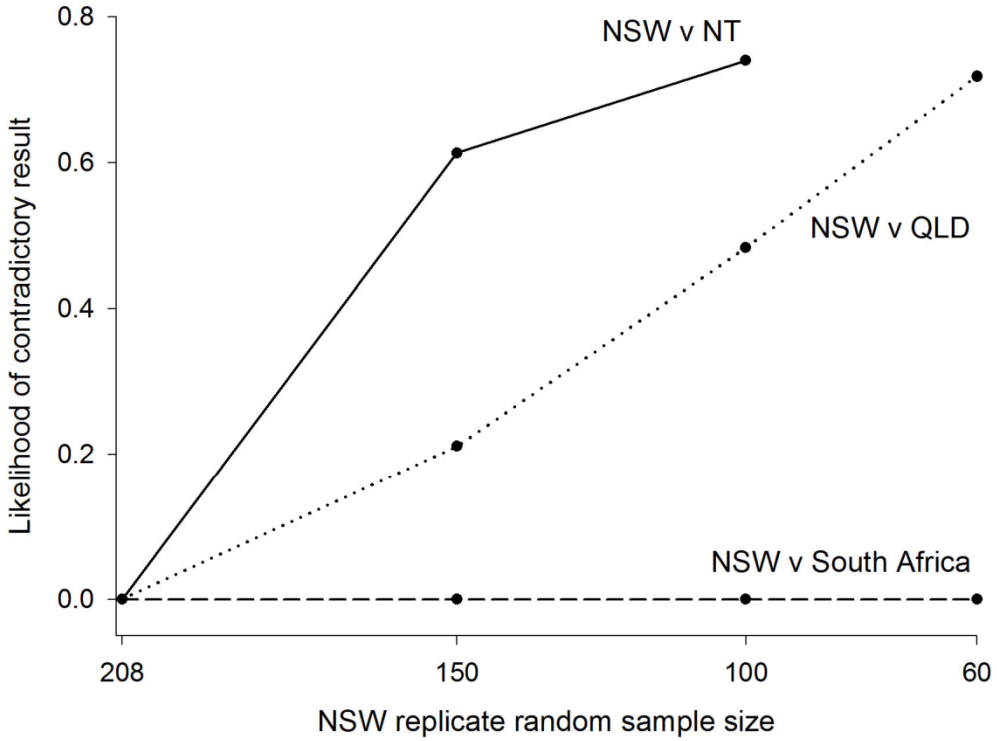

Figure 6. Likelihood of pairwise result contradicting that of the original analysis. Likelihoods computed based on 10,000 replicate random re-samples of the NSW population at varying sample sizes. Y-intercept represents original NSW population (*n* = 208).

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length-frequency modes at 90 cm and 95–100 cm TL respectively (Figure 2). While it is conceivable that the fine-scale genetic structuring observed in this study reflects signs of reproductive philopatry, the only meaningful test of this hypothesis would be a comparison of the NT and QLD locations between which our data failed to detect genetic differentiation.

Consideration of our results in light of those by Ovenden et al. [40], however, would suggest that an affinity for nearshore habitat for nursery purposes in *C. brevipinna* has influenced our findings of fine-scale genetic differentiation to some degree. In their study, Ovenden et al. [40] failed to detect evidence for genetic subdivision along Australia's east coast in milk sharks (*Rhizoprionodon acutus*) using ND4 sequence data. *Rhizoprionodon acutus*, a considerably smaller-bodied and presumably less-vagile species than *C. brevipinna*, conforms to a population model characterised by permanent habitation of nearshore waters without the use of discrete nursery areas [110]. In contrast, the exclusive use of nearshore habitat by *C. brevipinna* for parturition and juvenile development is well documented (24, 56-59). Differing life-cycles denoted by varying usage of nearshore habitat, therefore, may account for these contrasting genetic structures observed along Australia's east coast.

Alternatively, genetic differentiation between NSW and NT may be a relict signature of repeated periods of temporary isolation due to the rise and fall of the Torres Strait land bridge caused by fluctuating sea levels during the Pleistocene epoch [111,112]. This physical, yet temporary, barrier to movement (and hence gene flow) in marine taxa between the east coast and areas west of the Cape York Peninsula was hypothesised to account for contemporary genetic subdivision in pigeye sharks (*Carcharhinus amboinensis*) [42] which, like *C. brevipinna*, have a distribution restricted to northern regions in Australian waters [17]. Under this hypothesis, however, one would anticipate a similar level of genetic differentiation between QLD and NT, rather than genetic homogeneity as observed.

Similarly, a marked change in marine environment coinciding with the Tropic of Capricorn (Figure 1) represents an alternative hypothesis explaining restricted contemporary gene flow between south-eastern and more northern Australian waters [108]. This latitudinal line discretely separates the NSW population from both QLD and NT populations (with the exception of one individual from southern QLD waters), and delineates a shift from temperate and subtropical continental shelf waters, rocky coastline and drowned river valleys to a largely reef and lagoon-dominated tropical ecosystem.

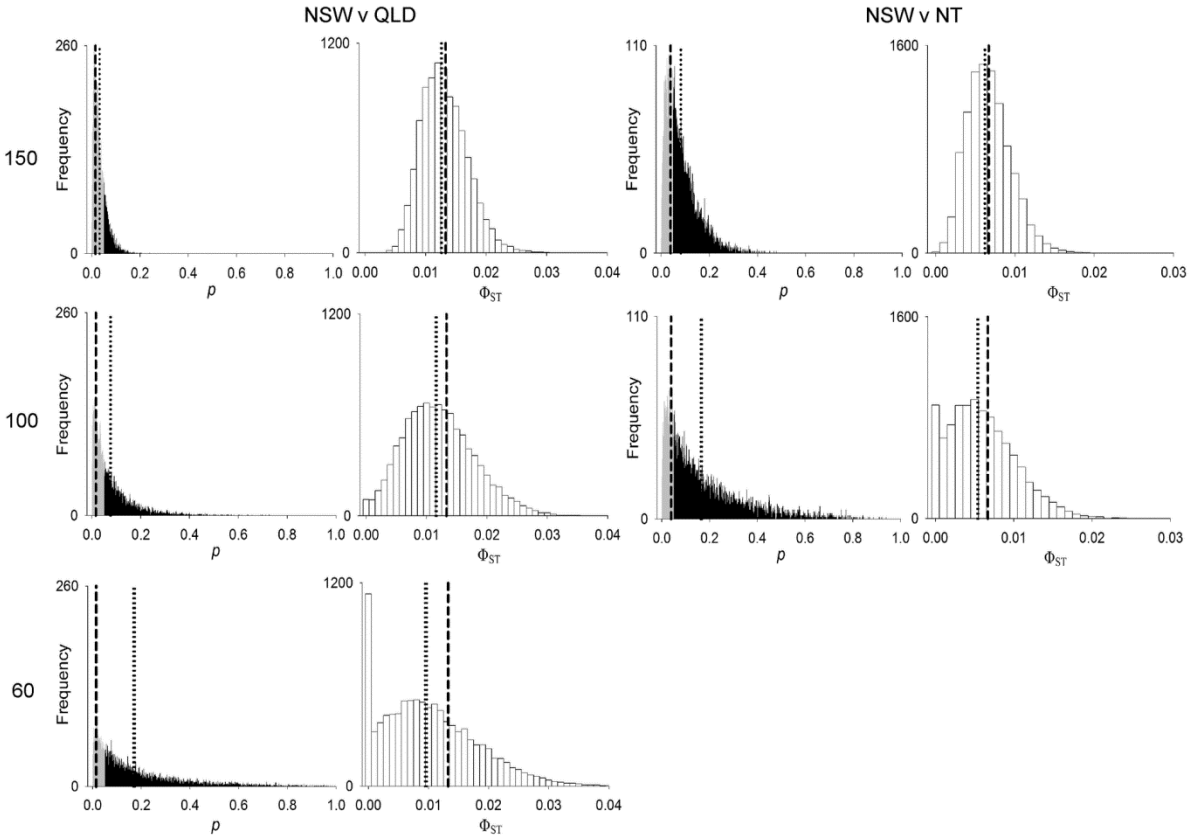

Figure 7. Pairwise Φ_{st} and *p* value distributions following random re-sampling simulations. NSW versus QLD and NT pairwise distributions based on 10,000 replicate random re-samples of the NSW population at *n* = 150, 100 and 60. Grey and black zones on *p* value distributions represent $p \leq 0.05$ and $p > 0.05$ respectively. Dotted lines denote upper and lower 95% confidence intervals around sample means. Dashed lines indicate pairwise Φ_{ST} and *p* values generated by the original analysis.

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Project limitations

This study was subject to a range of limitations requiring careful consideration. To begin with, very low values for both Φ_{ST} and F_{ST} metrics (resulting from high incidence of haplotype sharing of both ancestral and recently derived haplotypes among all four putative populations, coupled with generally shallow divergence between mutational variants) is suggestive of a slow rate of mutation in the ND4 gene region. This raises considerable doubt as to the ability of ND4 to effectively discriminate population structure in *C. brevipinna*. For example, pairwise *F*-statistic estimates involving the South African population were demonstrably low in the present study (range, 0.01306–0.04056) compared to others reporting genetic differentiation in sharks over comparable spatial scales (range, 0.18–0.991) (Table 6). Given that these previous studies were all based on analysis of a different mitochondrial locus (i.e. the control region), a slower rate of mutation in the ND4 region may account for the comparatively low *F*-statistics observed here. However, a hypothesis based on low ND4 mutation rate is challenged by the findings of both Dudgeon et al. [113] and Ovenden et al. [114] who demonstrated that for *C. limbatus*, Australian blacktip (*Carcharhinus tilstoni*) and zebra (*Stegostoma fasciatum*) sharks, ND4 was the most polymorphic of a range of mtDNA markers, including the control region. Alternatively, therefore, low *F*-statistic values associated with observed genetic structuring between Australia and South Africa, as well as within Australian waters, may reflect continued low-level gene flow, or a recent cessation of gene exchange, between subdivided locations. Until the relative mutation rates of ND4 and CR are determined for *C. brevipinna*, however, or this study is reassessed via sequencing of CR, it is impossible to confidently support or refute the abovementioned hypotheses. Moreover, this issue emphasises the limitations inherent in the analysis of only one mtDNA locus.

The clear bias in sample sizes weighted towards the NSW population represents another major limitation of this study. Random-resampling simulations provided some evidence that

Table 6. Mitochondrial divergence metrics for population pairwise comparisons involving Australia and South Africa.

Pairwise comparison	Species	Gene	F_{ST}	Φ_{ST}	Reference
AUS v SA	*Carcharhinus brachyurus*	CR		0.97	[36]
	Carcharhinus obscurus	CR		0.18	[37]
	Carcharodon carcharias	CR	0.81		[104]
	Carcharhinus brevipinna	ND4		0.03216	Present study
EAUS v SA	*Carcharias taurus*	CR	0.813		[105]
	Carcharhinus brevipinna	ND4	0.04056	0.03494	Present study
NEAUS v SA	*Carcharhinus plumbeus*	CR		0.588	[35]
	Carcharhinus brevipinna	ND4	0.01306	0.03508	Present study
WAUS v SA	*Carcharias taurus*	CR	0.676		[105]
	Carcharhinus plumbeus	CR		0.6165	[35]
	Sphyrna lewini	CR		0.991	[99]
	Sphyrna lewini	CR	0.45		[107]
SAUS v SA	*Galeorhinus galeus*	CR		0.34	[106]

CR = control region, ND4 = NADH dehydrogenase subunit 4.

AUS = Australia (general), EAUS = eastern Australia, NEAUS = north-eastern Australia, WAUS = western Australia, SAUS = southern Australia, SA = South Africa

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the detections of significant genetic differentiation within Australian waters (i.e. between NSW and QLD, and NSW and NT) were driven in large part by this bias. Replicate pairwise comparisons for both sets of locations indicated an increasing likelihood of finding a non-significant result as the NSW sample size decreased towards a more balanced analysis. This either serves to emphasise the weak nature of the observed fine-scale genetic subdivisions within Australian waters, or draw their actual existence into question. Conversely, replicate pairwise comparison between NSW and South Africa returned a significant difference independent of the NSW sample size, hence reinforcing the strength of the genetic subdivision between the latter two regions, and indicating that the original analysis was robust to the bias in sample size in this instance.

Rarefaction analysis added further uncertainty regarding the reliability of our fine-scale findings reported in the present study. NSW and South Africa were the only two locations at which adequate levels of the available genetic diversities were likely sampled, hence confirming the robustness of the comparison between these two putative populations. In contrast, a proportion of the available diversity appeared to have remained unsampled from QLD and NT, suggesting that findings emanating from comparisons involving the latter two locations should be treated with some degree of caution. Rarefaction curves demonstrated that the optimum sample size required to accurately represent levels of haplotypic variation, and in turn to confidently discern haplotype relative frequencies, within any given *C. brevipinna* population is site dependent. For Australian locations, sample sizes in excess of 100 were required for robust comparisons, whereas a sample size of ~60 appeared sufficient for South African waters.

Implications for management and future direction

The generally high genetic diversity reported here in *C. brevipinna* is cause for optimism when considering the management and conservation of this commercially-targeted species in southern Indo-Pacific waters. *Carcharhinus brevipinna* exhibited high haplotype numbers and similar or high haplotypic diversity (n_H = 23, h = 0.5984, n = 208) compared to *C. obscurus* (n_H = 12, h = 0.5224, n = 301) and *C. plumbeus* (n_H = 11, h = 0.2826, n = 440), two closely-related species, off Australia's east coast (PT Geraghty, unpublished data). Comparatively high haplotype numbers implies that *C. brevipinna* may display a greater resilience to a loss of genetic diversity, as a result of high-intensity fishing pressure, than these other commercially-targeted shark species in Australian waters.

The lower genetic diversity observed in *C. brevipinna* from the south-eastern zone (h = 0.5984), compared to QLD (h = 0.7424) and NT (h = 0.7279), may be accounted for by NSW representing sampling of the species' southern-most distribution limit [17]. Range limits are associated with extreme and/or unstable environmental conditions, and have been hypothesised to result in low population density, increased genetic drift and inbreeding and, consequently, lower genetic diversity [115,116]. Alternatively, lower genetic diversity in NSW may be a consequence of greater harvest pressure in the region. This hypothesis, however, is difficult to support given the absence of robust data permitting a direct comparison of historical harvest levels of *C. brevipinna* between NSW, QLD and NT, as well as a lack of knowledge pertaining to original population sizes and periods of time required to affect quantifiable reductions in genetic diversity.

Our genetic structure results indicate the delineation of two management units for *C. brevipinna* in the southern Indo-Pacific – Australia and South Africa. The most appropriate boundary between these management units, however, is unknown and would require more detailed spatial sampling within the Indian Ocean basin. Our data also suggest, albeit tentatively, two management units within Australian waters – south-eastern (NSW) and northern (QLD and NT) Australia. This implies that, in the event of a population collapse in south-eastern Australia, recovery of genetic diversity would rely largely on reproduction by surviving local individuals in NSW waters. Currently, each Australian state is independently responsible for the management of shark fishing operations occurring within its respective waters, with little to no collaboration across jurisdictional borders. Our results suggest that the independent management of NSW and QLD *C. brevipinna* populations is perhaps appropriate, but that cooperation between QLD and NT would be prudent.

In light of the limitations of the present study, however, we recommend this work be considered as a starting point for

evaluations of genetic structure in this commercially-important species, rather than a study upon which definitive management decisions are made. Moreover, we strongly urge future studies to focus on achieving greater population structure resolution via more extensive sampling within Australian waters, as well as throughout this species' global distribution range, in conjunction with analysis of nuclear and/or additional mitochondrial markers. Such studies, conducted in association with active tagging and tracking, would assist with more robust allocations of management units, and hence the sustainable exploitation of this target species.

Supporting Information

Table S1. Polymorphic sites for mitochondrial DNA ND4 haplotypes defined for *Carcharhinus brevipinna*.
(DOC)

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Author Contributions

Conceived and designed the experiments: PTG JEW WGM JRO MRG. Performed the experiments: PTG. Analyzed the data: PTG. Contributed reagents/materials/analysis tools: WGM SPW AVH JRO MRG. Wrote the manuscript: PTG. Participated in manuscript review: JEW SPW AVH JRO MRG.

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Due to copyright laws, the following articles have been omitted from this thesis. They appear in the page range 251-274. Please refer to the following citations for details.

Geraghty, P. T.; Jones, A. S.; Stewart, J. and Macbeth, W. G. 'Micro-computed tomography: an alternative method for shark ageing' Journal of Fish Biology, Vol. 80, Issue 5, p. 1292–1299.

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Appendix D: Animal Care & Ethics Certificate

NSW GOVERNMENT | Primary Industries

ANIMAL RESEARCH AUTHORITY

Names of Applicants:	Location of Research:	Conditions of Authority:
WILL MACBETH SHANE MCGRATH LACHLAN ROBERTS GLEN CUTHBERT DAMIAN YOUNG MAT BIRCH PASCAL GERAGHTY	Cronulla Fisheries Centre	As per application

are authorised by

NSW Primary Industries

to conduct the following type of research

OBSERVER-BASED SURVEYS OF NSW COMMERCIAL FISHERIES
ACEC REF 07/03 – CFC

as approved by and in accordance with the establishment's
Animal Care and Ethics Committee

Primary Industries (Fisheries) Animal Care & Ethics Committee

This authority remains in force from

6 JUNE 2011 to 6 JUNE 2012

unless suspended, cancelled or surrendered.
(Major three yearly review due in 2013)

JO PICKLES
EXECUTIVE OFFICER

N.M. Otway
NICK OTWAY
CHAIR

13 July 2011