

THE BIOLOGY AND FISHERIES OF ANGEL SHARKS AND
SAWSHARKS IN SOUTH-EASTERN AUSTRALIA

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MACQUARIE
University



**Primary
Industries**

DEDICATION

In memory of my grandfather, Robert Raoult, who instilled a passion for all things marine into me and believed in me more than myself.



‘Impossible n’est pas Français’

Napoleon Bonaparte

DECLARATION

I certify that the work in this thesis entitled “The biology and fisheries of angel sharks and sawsharks in south-eastern Australia” has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University. I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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ABSTRACT

Shark populations are under threat from commercial fisheries, and many studies suggest that shark fisheries are not sustainable. Angel sharks (*Squatina australis*, *Squatina albipunctata*) and sawsharks (*Pristiophorus nudipinnis*, *Pristiophorus cirratus*) have been caught by commercial fisheries in south-eastern Australia since the beginning of the 20th century. Despite indications from research trawlers that their populations have declined, no studies have been conducted to examine the effects of commercial fisheries or the biology of these sharks. This project aimed to examine the impacts of commercial fisheries on sawshark and angel shark populations, and to contribute to a better understanding of the life-history traits of these sharks. With this information, it should be possible to assess the sustainability of sawshark and angel shark fisheries in south-eastern Australia.

A collection of commercial and scientific fishery data sets were combined to determine whether fisheries have had an impact on angel shark and sawshark populations. Data sets were also used to re-define the distributions of the four species that occur in the area. In both cases, it appears that current fishing rates are sustainable, but shark populations are at significantly lower levels than when fisheries commenced. It is therefore recommended that all four species are classified as ‘sustainably overfished’ and that they require ongoing monitoring.

Historically, angel sharks have been difficult to age due to their unique vertebral structure. Sawsharks have similar vertebrae with no apparent band deposition. A scanning x-ray fluorescence microscope (SXFm) was used to observe the elemental distribution of shark vertebrae, and to determine the age of angel sharks and sawsharks. Traditional age bands were more correlated with strontium rather than calcium, suggesting that strontium is the driver for band deposition. These results suggest that vertebral banding may be related to environmental variables such as salinity.

Due to the difficulty of accessing SXFMs and their rarity, it would be beneficial if other, more accessible ageing techniques were developed that yielded similar results. A micro computed tomography (microCT) ageing technique was developed that successfully replicated the results of the SXFM. These results suggest that microCT could be further developed into a widely-applicable elasmobranch ageing tool, which replicates results from a less-accessible SXFM and enables analytical ageing analysis.

Morphometrics and reproductive data were collected for angel sharks and sawsharks caught by fisheries in south-eastern Australia. Female-biased dimorphism was apparent, as well as similar male and female growth curves. Morphometrics suggest that larger *S. albipunctata* have larger eyes than *S. australis*, and may be the reason *S. albipunctata* are more suited to deeper waters. Results also suggest that anterior pectoral margins can be used as a proxy for total length when examining truncated specimens.

Muscle samples were analysed for isotopic analysis to determine the trophic levels of sawsharks, and whether co-occurring *P. cirratus* and *P. nudipinnis* avoided competition. Results suggest that these two sawshark species avoid competition through resource partitioning, and that they have significantly different trophic levels. Isotope level comparison to other studies suggests that *P. cirratus* has an invertebrate diet, while *P. nudipinnis* has a piscivorous diet. For both species, diet shifted to higher trophic levels during ontogeny.

This study suggests that fisheries have had a significant impact on the populations of sawsharks and angel sharks, but that current fishing levels can continue if the objective is not to return shark populations to their pre-industrial levels. Results are also of use to increase the effectiveness of management protocols.

CHAPTER DECLARATIONS

Chapter One: Introduction

Written by myself with guidance and corrections from my supervisors, Jane Williamson and Vic Peddemors.

Chapter Two: Exploring the possibilities of long-term sustainable fishing of elasmobranchs: a case study with angel sharks (*Squatina* sp.) in south-eastern Australia

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Chapter Three: Long-term deep ocean sawshark fishery in south-eastern Australia

Data were obtained directly from NSW Department of Primary Industries and the Australian Fisheries Management Authority. Those data were collected from commercial fishermen logbooks, observer programs, and scientific trawlers. Dr. Ken Graham extracted data from the *FRV Kapala* trawl spreadsheets. Data analysis and write-up were performed by myself, with guidance and corrections from my supervisors Jane Williamson, Vic Peddemors, and Kevin Rowling.

Chapter Four: Strontium mineralization of shark vertebrae correlates with age-related banding

Samples were obtained from contractors working for the Department of Primary Industries who monitored shark nets, from angel sharks caught by Richard Bagnato and the *Maybelle*, and from sawshark obtained by the *AMC Bluefin*. The preparation method was developed by myself, David Zahra, Nicholas Howell, and Vic Peddemors. SXFM was controlled by Martin de Jonge and Daryl Howard. The analytical program was provided by Daryl Howard. Post-analysis was performed by myself, David Zahra, and Nicholas Howell. Write-up was performed by myself, with guidance and corrections from all the co-authors.

Chapter Five: Biology of angel sharks (*Squatina sp.*) and sawsharks (*Pristiophorus sp.*)
caught in south-eastern Australian trawl fisheries

Samples were obtained from the NSW Shark Meshing Program and from the *AMC Bluefin*. Dissections and data collection and data analyses were performed by myself. Document was written by myself with guidance and corrections from Jane Williamson and Vic Peddemors.

Chapter Six: Not all sawsharks are equal: species of co-existing sawsharks show plasticity in trophic consumption both within and between species

Samples were obtained from the *AMC Bluefin*. Muscle tissue was analysed for isotopic deltas by Rene Diocares at Griffiths University. Data analysis and write-up was performed by myself, with guidance and corrections from Jane Williamson and Troy Gaston.

Chapter Seven:

Written entirely by myself with guidance and corrections from my supervisors, Jane Williamson and Vic Peddemors.

Appendix I: Localized zinc distribution in shark vertebrae

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Appendix III: Preliminary age analysis of angel sharks and sawsharks

Similar to Chapter Four.

Appendix II: MicroCT as a global elasmobranch ageing tool

The analyses and experiments were done by myself, David Zahra and Nicholas Howell. The manuscript was written by David Zahra and Nicholas Howell, with corrections and guidance from myself.

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I have an immeasurable amount of gratitude for my supervisors: Vic, Jane, and Kevin. Jane has spurred me onwards towards research since my undergraduate degree, and done her best to ensure my success from here onwards. Vic has kept me grounded with his typical South-African bluntness, and his passion for this area of research. Kevin has an incredible knowledge on the fisheries and fish of south-eastern Australia, and it still baffles me that he was forced into retirement.

Many thanks to the crew of the *FRV Kapala*, and to the Australian Maritime College who allowed me to collect data whilst having amazing experiences. A lot of my gratitude goes to Richard Bagnato, who helped a great deal in obtaining samples and welcomed me into the world of commercial fishermen.

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PRESENTATIONS DELIVERED DURING CANDIDATURE

Conferences

Australasian Isotope Conference (2015): *Co-occurring species of sawshark avoid competition through trophic plasticity both within and between species*

Sharks International, Durban, South Africa (2014): *Sharks and Particle Accelerators*

Macquarie University Postgraduate Conference (2011) *Biology and Fishery of Angel Sharks and Sawsharks in south-eastern Australia*

Macquarie University Postgraduate Conference (2012): *Biology and Fishery of Angel Sharks and Sawsharks in south-eastern Australia*

Macquarie University Postgraduate Conference (2013): *Sharks and Particle Accelerators*

Australian Society for Fish Biology Conference, Townsville, Australia (2011): *Personality-associated learning strategies in Mulloway, (Argyrosomus japonicus)*

Presentations & Lectures

Newington College Science Festival (2012, 2014): *Shark talk and dissection*

Temperate Marine Biology, 3rd year Macquarie University lecture (2013): *Elasmobranch biology and ecology*

AMC Bluefin student talk (2012): *Sawshark biology & research*

PUBLISHED CHAPTERS PRESENT IN THIS THESIS

These published chapters have been reformatted to better integrate into this thesis:

Chapter 4: V. V. Raoult, V. M. Peddemors, D. Zahra, N. Howell, D. L. Howard, M. D. de Jonge, J. E. Williamson (2016) Strontium mineralization of shark vertebrae. *Scientific Reports* (in press)

Chapter 5: V. Raoult, T. F. Gaston, J. E. Williamson (2015) Not all sawsharks are equal: species of co-existing sawsharks show plasticity in trophic consumption both within and between species. *Canadian Journal of Fisheries and Aquatic Sciences*. 72 (11): 1769-1775.

Chapter 6: V. Raoult, V. Peddemors, J. E. Williamson (2016) "Biology of angel sharks (*Squatina* sp.) and sawsharks (*Pristiophorus* sp.) caught in south-eastern Australian trawl fisheries and the New South Wales shark meshing (bather protection) program. *Marine and Freshwater Research* (in press)

CHAPTER ONE:

INTRODUCTION



Pristiophorus nudipinnis embryos

Sharks under threat

The world's oceans are currently facing a multi-pronged threat from a number of anthropogenic sources (Halpern *et al.* 2008): pollution (Lancelot *et al.* 2007; Naim 1993), ocean acidification (Doney *et al.* 2009; Hoegh-Guldberg *et al.* 2007; Orr, Fabry *et al.* 2005), climate change (Chin *et al.* 2010), habitat destruction (Walker and Kendrick 1998), and introduced species (Grosholz 1996; Grosholz and Ruiz 1996). While all these threats may have synergistic ecosystem-changing effects, arguably the hazard that has had the greatest isolated impact has been that of commercial and recreational fisheries (Cooke and Cowx 2006; Pauly *et al.* 2005). On a global scale, commercial fisheries have repeatedly caught over 90 million tonnes of fish products per year during the last two decades (FAO 2014). As a result of these fisheries, catches of many marine species have declined to less than 10% of their pre-industrialisation levels (Myers and Worm 2003). These declines are exacerbated by shifting baselines: generations of fishermen have never experienced 'natural' levels of fish, and perceptions of catch sizes and quantities have shifted (Jackson *et al.* 2001; Pauly 1995): current fishermen are therefore less likely to recognize the significance of their lowered catch rates.

Elasmobranchs are arguably under the greatest pressure from commercial and recreational fisheries. Unlike many species of bony fishes, elasmobranchs are characterised by their K-selected life histories, with low reproductive rates, low fecundity, and delayed onset of maturity (Stevens *et al.* 2000). Sharks are commonly apex predators in their respective environments, and can thus have disproportionate, cascading impacts on communities and ecosystems if their abundances are altered (Baum and Worm 2009; Myers *et al.* 2007; Pandolfi *et al.* 2005). Effective monitoring and management of fisheries impacts on elasmobranchs is difficult due to the large proportion of sharks caught as bycatch (Bonfil

1994; Francis, Griggs *et al.* 2001; McKinnell and Seki 1998) or from illegal fisheries (Agnew, Pearce *et al.* 2009).

Although the majority of shark species are declining globally, it is commonly thought that the rate of decline can be reduced, or even halted with appropriate management and a more thorough understanding of shark biology (Walker 1998; Worm and Branch 2012). Stability of abundances in shark fisheries has already been shown on species with higher productivity, such as *Mustelus antarcticus* (Prince 2005). The keys to such sustainable fisheries are significant scientific information on species' biology, selective fisheries that mainly capture juveniles, and reliable catch data (Walker 1998).

Shark bycatch fisheries in south-eastern Australia

Commercial fisheries in south-eastern Australia commenced in 1915 with steam trawlers targeting tiger flathead (*Neoplatycephalus richardsoni*), and progressively evolved into a Danish seine fleet in the 1950s (Klaer 2001). That fishing fleet rapidly expanded to 180 vessels in the 1970s due to government subsidy and the introduction of diesel power, which allowed trawling to occur at greater depths than before (>200m) (Rowling 1979). Since then, the total number of fishing vessels has declined to 109 vessels in 1999 (Tilzey and Rowling 2001). The total tonnage of fishing vessels has increased, however, along with technological advancements that may increase catch efficiency. As a result of the reductions in fishing vessels, the total number of hours trawling in Commonwealth waters in 2013 has been reduced by over half from the levels observed in 2000 (Penney *et al.* 2014). Although there are currently a number of line fisheries in south-eastern Australia that specifically target sharks, demersal trawlers, Danish seine, gill-netting and long-lining in the area catch a large number of sharks as bycatch.

Bycatch is generally referred to as the un-intentional catch of fish during commercial fishing that targets other species (Hall *et al* 2000). The issue of bycatch in south-eastern Australia has been well documented (Kennelly 1995). Demersal trawlers in particular are unselective and capture many non-targeted species. Until recently, south-eastern Australian fisheries did not collect adequate catch data, and quantifying the quantity and type of bycatch was therefore impossible (Kennelly 1995). Although the accuracy of catch data has since been improving, fisheries in the area still rely on the accuracy of fishermen reports, and shark species were still grouped together (i.e. unspecified angel shark) until 2010. It is thus difficult to determine the sustainability of fisheries on specific shark species, and the impacts of fisheries were best assessed by scientific research trawls in the 1970s and 1990s that used fisheries scientists to identify, measure and sex catches in the area (Tilzey and Rowling 2001). Of the ten elasmobranch species frequently caught as bycatch by demersal trawlers, eight have catch per unit efforts (CPUE) that declined significantly from 1976 to 1996 (Table 1). These declines have drawn attention from fisheries scientists who have suggested that elasmobranchs caught as bycatch require further scrutiny, and recommended a more precautionary approach from commercial fisheries (Tilzey and Rowling 2001). Species targeted by commercial fisheries (i.e. gummy sharks, *Mustelus antarcticus*) and species that showed declines over 99% (i.e. Harrison's dogshark, *Centrophus harrisoni*) have been studied as a result of these surveys and given accurate stock assessments (Walker *et al.* 1994; Forrest and Walters 2009). Two groups of shark that declined significantly, were frequently caught as bycatch, and sold at local markets: sawsharks (*Pristiophorus* spp.) and angel sharks (*Squatina* spp.), neither of whom had their biology or catch frequencies examined.

Table 1 Catch rates of elasmobranchs caught by the FRV Kapala in 1976 and 1996 in all locations combined (Sydney, Ulladulla, Eden-Gabo Islands). * indicate significant differences in mean catch. Taken from Andrew *et al.* (1997).

Species name	Common name	1976 mean catch ± S.E. (kg/h)	1996 mean catch ± S.E. (kg/h)	Percent change
<i>Centrophorus harrissoni</i>	Harrison's dogshark	126.3 ± 17.7	0.4 ± 0.1	-99.6 *
<i>Squalus chloroculus</i>	Greeneye dogshark	44.8 ± 5.5	1.2 ± 0.3	-97.3*
<i>Squalus blainville</i>	Longnose dogshark	15.7 ± 4.1	1.4 ± 0.4	-91.1*
<i>Centrophorus moluccensis</i>	Endeavour dogshark	12.3 ± 1.8	0.2 ± 0.1	-98.3*
<i>Squalus acanthias</i>	Spiky dogshark	64 ± 7.4	75.5 ± 22.3	+17.9
<i>Squatina</i> spp.	Angel shark (undifferentiated)	32.6 ± 5.3	1.3 ± 0.4	-96*
<i>Pristiophorus</i> spp.	Sawshark (undifferentiated)	4.8 ± 0.9	2.5 ± 0.8	-47.9*
<i>Mustelus antarcticus</i>	Gummy shark	5.5 ± 1.1	2.5 ± 0.8	-54*
<i>Galeorhinus galeus</i>	School shark	3.4 ± 1	2.5 ± 0.8	-26.5
<i>Rajidae</i> spp.	Skate (undifferentiated)	32.7 ± 3	5.5 ± 0.8	-83.2*

Angel sharks and sawsharks

Angel sharks

Angel sharks (*Squatina* spp.) are cryptic dorsoventrally compressed sharks (flat sharks) that conceal themselves in shallow sandy substrate to ambush demersal prey (Fouts and Nelson 1999). Their diet consists predominantly of teleosts and cephalopods, with a small proportion of crustaceans (Baremore *et al.* 2008; Bridge *et al.* 1998). While their reproductive cycle remains largely unstudied, particularly in Australia, most angel sharks from the eastern Pacific and the Atlantic are thought to reproduce annually, with the size of litters ranging from one to twelve (Capapé *et al.* 1990; Natanson and Cailliet 1986). While there has been no direct study on movement patterns for *Squatina* spp., some studies suggest that there is a size

segregation of larger individuals in deeper waters due to larger numbers of juveniles being caught in shallower areas (Bridge *et al.* 1998), but little large-scale migration (Gaida 1997). The reproductive biology and growth characteristics of some *Squatina* spp. have been documented (i.e. *S. pacifica*, *S. tergocellata*), yet one aspect that is not well understood is their age-to-length relationships.

Without a thorough understanding of age and growth parameters, the effects of fisheries on *Squatina* spp. are difficult to quantify. A number of studies have attempted to determine the age of angel sharks using traditional vertebral section analysis (Baremore *et al.* 2009; Natanson and Cailliet 1990). In this genus, vertebrae do not form vertebral bands of the type that are used for ageing in other species of sharks (Goldman *et al.* 2012; Simpfendorfer *et al.* 2002). Distinct *intermedialis* connective tissue layers are visible, but they appear to be more related to size than age (Baremore *et al.* 2009). The only accurate estimation of age comes from a study by Natanson and Cailliet (1990), which used an extensive tagging and recapture program, and estimated that female *S. californica* did not reproduce until at least 10 years of age. While recapture programs are theoretically ideal, they are difficult to organise without dedicated research vessels and require long-term commitments. Tagging studies where individuals are held in aquaria are also difficult for larger species where there is little understanding for their optimal holding requirements, as is the case for angel sharks that grow to nearly 1.5m total length and over 20kg in weight.

Squatina spp. occur in environments ideal for benthic trawling (sandy flats) and are frequently caught as bycatch by these fisheries. Consequently of the nineteen known species, twelve are considered threatened to critically endangered by the IUCN Red List of Threatened Species, and some populations have become regionally extinct (Dulvy and Forrest 2010). Angel sharks are also frequently caught by bather protection programs in both Australia (Reid and Krogh 1992) and South Africa (Shelmerdine and Cliff 2006). Three species of angel shark occur in

south-eastern Australia: *Squatina australis*, *S. albipunctata*, and *S. tergocellata* (Last and Stevens 2009). *S. tergocellata* (found in South Australian waters) has been studied by Bridge *et al.* (1998), but the two species that occur in New South Wales and Tasmanian waters remain unstudied. These latter two species have been caught by trawl fisheries in the area since their inception. Due to the the potential impacts of these fisheries, and the declines recorded by the fisheries research trawler *FRV Kapala*, New South Wales (NSW) Fisheries have designated *S. australis* and *S. albipunctata* as species of interest that require further scrutiny.

Sawsharks

Sawsharks (*Pristiophoridae*) are small (less than 1.5m total length), cylindrical sharks found mainly in the Indo-Pacific region . *Pristiophorus* spp. are characterised by their saw-like rostrums (Last and Stevens 2009). Unlike any other elasmobranch, sawsharks have ‘barbels’ that occur roughly halfway between their mouths and rostrum tip and likely serve a tactile function as they lack sensory structures such as “taste buds” (Nevatte 2015). Sawshark rostral teeth are frequently replaced, while *Pristid* spp. (commonly known as sawfish, and frequently confused with sawsharks) rostral teeth are permanent (Slaughter and Springer 1968). Due to the similarities between *Pristidae* and *Pristiophoridae* rostrums, it has been inferred that sawshark predate in a similar fashion as sawfish: they aggressively sweep their rostrums in schools of fish to maim/kill prey (Wueringer *et al.* 2009). There are currently three identified species of sawshark that are endemic to Australia: *P. delicatus* (Yearsley *et al.* 2008), *P. cirratus*, and *P. nudipinnis* (Last and Stevens 2009). *P. cirratus* and *P. nudipinnis* occur in south-eastern Australia, while *P. delicatus* occurs in the waters of Queensland.

Pristiophorus spp. occur mainly on coastal shelves down to depths of 600m (Last and Stevens 2009). Due to their rostral saws, which easily entangle and break in nets, sawsharks are frequent but undesired bycatch for ocean trawlers and gillnets. *P. nudipinnis* and *P. cirratus* are believed to have been caught since trawling started in south-eastern Australia. The FRV *Kapala* recorded a marked decrease in the catch rates of sawsharks in south-eastern Australia between 1976 and 1996 (Table 1), though the majority of catches of sawshark are outside NSW territorial waters where the *Kapala* operated, and therefore, the data collected from that vessel may not be representative of the rest of the population.

Sawsharks are caught as bycatch in a wide range of fisheries, yet they have received little interest from the scientific community aside from a stock assessment in Commonwealth waters in 2005 (Walker *et al.* 2005). These sharks are not generally considered as charismatic as other species, and due to their presence in deep seas and the difficulty of obtaining samples in those areas, studying them requires a significant diversion of resources. Such gaps in knowledge for deeper-ocean and less charismatic species of shark is a known issue that needs to be addressed, given the potentially larger and longer-term impacts of fishing pressures on more benthic species (García *et al.* 2008). Despite the potential threats, behaviour, movement patterns (if any), diet, and life-history traits of sawsharks remain largely unknown. Due to these apparent gaps in knowledge and the significant quantities of sawsharks caught in south-eastern Australia, NSW fisheries have designated *P. cirratus* and *P. nudipinnis* as species of interest that require research.

Thesis rationale

There are gaps in knowledge on sawsharks and angel sharks in south-eastern Australia that prevent fisheries managers and conservationists from determining whether they are being

caught sustainably. Barker and Schluessel (2005) outlined a number of strategies necessary to effectively manage shark fisheries: participation in management by stakeholders, increased understanding of shark resources, regulation of fishing intensity, and reduced bycatch. In south-eastern Australia, stakeholders are actively managed, and fishing intensity is usually regulated by limiting the numbers of fishing vessels. The principle management issues, therefore, are the lack of species-specific biological data on angel sharks and sawsharks, and an understanding of effective bycatch mitigation methods. Unfortunately, the latter cannot be done without the former.

The overarching objective of this thesis is to develop an understanding of the biology and fisheries of angel sharks and sawsharks in south-eastern Australia that will facilitate effective management of their populations. Fisheries information includes gear selectivity, catch trends over time and space, while biological parameters include age, growth, reproductive parameters, diet, and some aspects of their ecology. Such information will elucidate the sustainable fishing practices and lend insight into and management decisions that can be made to protect populations of these species in south-eastern Australia. Moreover, biological characteristics and the subsequent effective management of these species within Australia may be extrapolated over time to other areas on a global scale.

Angel sharks and sawsharks have vertebrae that do not appear to allow age determination using traditional techniques. Current shark ageing methods rely on the use of an analogous technique used in fish, whereby growth bands are counted on the *corpus calcareum* of dorso-ventral vertebral sections (Branstetter and Stiles 1987). Despite the difficulties associated with these techniques (Cailliet *et al.* 2006) and the recent discoveries of inaccuracies with ages determined from traditional ageing related to the difficulties in separating growth bands in older animals (Francis *et al.* 2007; Hamady *et al.* 2014), shark vertebral bands are still used to determine age without any underlying understanding of elasmobranch vertebral

development. Consequently, an additional objective of this thesis was to more accurately determine the elemental composition of shark vertebrae. Such research would allow the development of techniques that could be broadly applied to a number of shark species.

The following data chapters were compiled in order to achieve those objectives:

Chapter 2 examined historical and current commercial and research catch data on angel sharks from federal and state fisheries to assess whether catches of *Squatina* spp. are declining, and to increase the resolution of current known distribution patterns of *S. australis* and *S. albiguttata* in south-eastern Australia. This section is currently under review in *Fisheries Management and Ecology*.

Chapter 3 collected catch data from commercial and research fisheries in south-eastern Australia to determine whether *P. nudipinnis* and *P. cirratus* catches are declining, and to increase the resolution of distribution patterns of these species in south-eastern Australia.

Chapter 4 assessed the elemental distributions of shark vertebrae, and the associations of these elements with age bands. This was done by using novel technology, a scanning x-ray fluorescence microscope, and suggested that traditional ageing methods need to be re-examined. This section is currently under review in *Scientific Reports*.

Chapter 5 compiled morphometric data from *S. australis*, *S. albiguttata*, *P. nudipinnis* and *P. cirratus* samples collected from a variety of sources in south-eastern Australia. These data were used to determine growth parameters, reproductive development, and how these morphometrics could be used by fisheries monitors. This section has been published in *Marine and Freshwater Research*.

Chapter 6 assesses the co-occurrence of *P. nudipinnis* and *P. cirratus*, and the hypothesis that competition may be reduced due to resource partitioning in these species. This chapter

also assessed the relative trophic position of sawsharks in south-eastern Australian waters through stable isotopes analysis. This section has been published in *Canadian Journal of Fisheries and Aquatic Sciences*.

Chapter 7 provides a synthesis on whether the populations of the four species of sharks studied in this thesis are being caught sustainably in south-eastern Australia. It also collates what is known of the biology of these species to facilitate effective management and conservation of these species.

Appendix I provides a draft of a short communication on the distribution of zinc within shark vertebrae using a scanning x-ray fluorescence microscope. It suggests that zinc may be absorbed from the maternal uterine fluid, rather than directly from the yolk or placenta, and that zinc is not distributed evenly within vertebral structures.

Appendix II provides a draft of a manuscript principally written by my colleagues at AINSE, aiming to develop a more widely-applicable microCT technique for ageing shark vertebrae that can also reproduce the results of the SXFM.

Appendix III provides a draft of a short communication on the ageing of sawsharks and angel sharks using SXFM.

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CHAPTER 2: Exploring the possibilities of long-term sustainable fishing of elasmobranchs: a case study with angel sharks (*Squatina sp.*) in south-eastern Australia

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Squatina australis hiding in sand, photo taken at Jervis Bay, NSW, Australia by Nathan Bass

Abstract

Historical and current commercial and scientific catch data sets were used to determine the population status of the two species of angel shark (*Squatina australis*, *S. albigutta*) occurring in south-eastern Australia. These data indicate that the historically assumed distributions for both species need to be refined. Records highlight catch hotspots south of Sydney and west of the Bass Strait. Depth distributions for both species overlapped and no *Squatina* species were caught in waters greater than 500 m depth. Length frequency distributions indicated that the population contains a reasonable proportion of reproductively mature individuals and that fishing pressure on smaller individuals is lower. Significant seasonal variations in catch rates were observed and may be linked to changing sea temperatures and yearly migrations. Catches through shark meshing and scientific trawling suggest populations have declined by 60-90% since the 1950s in NSW near-shore and continental slope environments. Commercial catch records from 1990 to 2012 indicate no significant declines in catch efficiency, suggesting these species may be sustainably harvested at current rates, unlike the majority of fished elasmobranchs. Due to lower historical trawl intensity in Victorian and Tasmanian waters, it is likely that angel shark populations in those waters have been less impacted by commercial fisheries. Further biological studies must be done to verify and understand these species' apparent resilience to fishing pressures.

Keywords: Management, sustainability, conservation, benthic, migrations

Introduction

The rate of loss of global biodiversity is now several orders of magnitude greater than historically recorded extinction rates. Such loss negatively impacts on ecosystem processes and services (Díaz, Fargione *et al.* 2006). Much of this escalation in biodiversity loss is detrimental to the contemporary health of oceans and can be attributed to anthropogenic

drivers such as overfishing (Pikitch 2012). Large marine predators are particularly vulnerable to overfishing due to their 'K-selected' life history characteristics: it takes many years for these predators to mature and they have low reproductive rates (Musick, Burgess *et al.* 2000). Moreover, coastal marine habitats of such predators are suffering from increasing rates of exploitation and degradation (Worm, Barbier *et al.* 2006).

Populations of sharks worldwide have declined to less than 10% of their baseline levels (Baum, Myers *et al.* 2003). Pressure from commercial and recreational fishing is largely responsible for these reductions, and some species of shark have been fished to near extinction (Casey and Myers 1998). Changes in predator diversity have the potential to affect ecosystems on a large scale (Finke and Denno 2004), and elasmobranchs impact on a wide range of trophic levels (Ferretti, Worm *et al.* 2010). Despite these trends, fishing pressure has continued to increase in waters that have declines in stock, and informed management using species-specific data is needed to address the situation (Worm and Branch 2012).

Research has shown that some shark populations may tolerate commercial fishing with sufficient management although the majority of shark fisheries remain unsustainable.

Management techniques such as limiting fishing to juveniles or sub-adults while protecting adults have been successful with some species such as *Mustelus antarcticus* (Prince 2005).

Walker (1998) suggested that with good management techniques and a thorough understanding of shark life histories and biological characteristics, any species of shark has the potential to be sustainably caught. Unfortunately, the majority of shark fisheries lack the appropriate resolution in detailed biological information.

Ironically, information collated from the mechanism responsible for declines in elasmobranch populations may also be used as an effective assessment tool: fishery data. Although studies conducted by scientific vessels are usually more thorough and precise, they rarely provide the

long-term or large-scale data that commercial fishery records can deliver. For some species such as *Raja batis* (common skate), historical fishing records are available from the early 20th century (Philippart 1998). Despite the inherent difficulties of interpreting results due to changes in effort and/or techniques, commercial fishery data allow researchers to gain a historical understanding of the extent and pattern of catches that can lead to more accurate predictions of long-term stock availability and sustainability.

Early catch data available for angel sharks in south-eastern Australia are broad, contains grouped species, and in many cases do not record any data on by-catch (Tilzey and Rowling 2001). Moreover, these data provide little information regarding location, fishing effort, or biological parameters of individuals caught. Modern catch data (post 1980s) are much improved, providing GPS coordinates, depth, effort and, if fisheries observers were present, reliable biological observations on the catch. Such biological factors can be used to determine biological trends such as trophic interactions (Ward and Myers 2005), chronological behaviours (Driggers, Campbell *et al.* 2012), and seasonal variations in abundance (Barnett, Stevens *et al.* 2010).

Angel sharks (*Squatinae*) are bottom-dwelling, flat-bodied Chondrichthyans that spend the majority of their time buried in the substrate. While their morphology is somewhat similar to that of stingrays, their cryptic and aggressive foraging techniques separate them (Fouts and Nelson 1999). Angel sharks occur throughout the Atlantic and Pacific Oceans, where several species of *Squatinae* have been comprehensively studied (Baremore, Murie *et al.* 2010; Cailliet, Mollet *et al.* 1992; Capapé, Diatta *et al.* 2005). In contrast, there is a dearth of research on the two species that occur in south-eastern Australia (*S. australis* and *S. albipunctata*), which are believed to occur from northern NSW to the southern border of Victoria (Last and Stevens 2009). To illustrate this point, one of these species (*S. albipunctata*) was only named in 2009 (Last and Stevens). Both species have been caught

incidentally off south-eastern Australia by commercial trawling for over 90 years, with an average catch of more than one hundred tons per year in recent decades, for a monetary value of over AUD \$400,000 per annum in the New South Wales (NSW)/Victorian markets (AFMA and NSW collated data). In addition, a small number of both species are regularly caught in the NSW Shark Mesh Program (SMP) (Reid *et al.* 2011).

The south-east trawl fishery is one of the oldest fisheries in Australia. During its existence, the trawl fleet has seen a number of boom and bust cycles, driven primarily by fish stock downturns and the discovery of new resources; the most recent leading to an increase in fishing effort during the 1970s (Tilzey and Rowling 2001). Fisheries research and management lagged behind increases in fishing effort, and it was not until the early 1990s that quota management was introduced to the trawl fishery. Stock assessment became a research priority to ameliorate the lack of knowledge of species that were being harvested in substantial quantities. Unfortunately predictive models for some species are still hampered by a lack of biological data (Tilzey and Rowling 2001). The more recent (2001) availability of longer-term data sets has allowed the development of a greater number of population models for many species of commercial interest (i.e. *Carcharhinid* spp. with a value of ~\$19.6 million in 2009-2010), with the exclusion of species of less commercial value, such as angel sharks. As a result quota controls have never been introduced for any *Squatina* species in Australia.

A number of iconic species have declined in number due to intensive trawling in the south-east trawl fishery (Graham, Andrew *et al.* 2001; Klaer 2001). In particular, species of dogsharks of the genus *Centrophorus* have shown strong declines in catch since trawling started on upper continental slopes in the 1970s (Graham, Andrew *et al.* 2001). Despite significant depletions of other elasmobranch species, angel sharks continue to provide

relatively stable incidental catches and appear to have been less affected by the operation of the trawl fishery in continental shelf waters than other species during the past 90 years.

In this study, we determine the status of populations of two species of angel shark found in south-eastern Australia, *Squatina australis* and *S. albipunctata*, using a number of commercial and scientific catch data sets. We refine the current known spatial distribution of these species, investigate any seasonal or annual patterns in angel shark presence, and determine whether current anthropogenic activities may have had an impact on the stocks of both species.

Methods

Data sets

All commercial fisheries data were zero-corrected (catch reports with 0 catch of the species involved were removed) to present more realistic appraisals of CPUE. Four separate data sets were analysed to assess the populations of *Squatina australis* and *S. albipunctata* at various scales of resolution off south-eastern Australia: (1) The Australian Fisheries Management Authority (AFMA) data set, which covers the entire study area, ranging from New South Wales (NSW) to waters around Tasmania and through the Bass Strait into the Great Australian Bight (GAB); (2) The NSW Fisheries data set, which includes the NSW Ocean Fish Trawl and Ocean Prawn Trawl fisheries and provided mid-scale (North-South NSW) data. Finer-scale data were provided by (3) the NSW Shark Meshing (Bather Protection) Program (SMP), and by (4) the results from research trawling by the NSW Fisheries Research Vessel *Kapala*. The latter two data sets provided high-resolution spatial and temporal data and included length-frequency information for all caught animals. Unfortunately, only the *Kapala* data set was species-specific, while the SMP data only distinguished between the two species since 2009. As such, long-term descriptions of catch trends are only at the generic level.

The AFMA data set ranged from 1999-2012 and included all Commonwealth fisheries in the area. A number of different fishing methods were involved: gill-netting, trawling and long-lining. Due to the geographic scope of this data set, it was separated into three areas: South Australia (all the area west of the Victorian/South Australian border), Bass Strait/Tasmania, and the East coast (all the area east and north of the NSW / Victoria border). The AFMA data set includes a large amount of data from the GAB, and consequently we expect a majority of the sharks reported there to be *S. tergozellata*, as the range of *S. albipunctata* does not extend to the west and *S. australis* are more confined to inshore waters.

NSW trawl catch data encompassed 1990 to 2008. As with the AFMA records, data were primarily collected via monthly catch returns from fishermen. They included days of fishing effort per month, the method of fishing, species caught (to genus level), processed weight and area code (area codes were separated north to south for every degree of latitude as per Australia's National Code of Practice, and were used to determine where fishing occurred). Observer information was not part of this data set.

The NSW SMP data were collected for the region netted as part of the Shark Meshing (Bather Protection) Program. Shark nets are set at an average of 500m offshore from a subset of 51 beaches between Stockton and Wollongong on sandy substrate in water depths between 10-12m (Reid *et al.* 2011). These nets are set below the surface and above the substrate to lower the chances of capturing marine mammals and reptiles. Catch data used were from 1950 to 2010.

The FRV *Kapala* was a research vessel that conducted a series of trawl surveys on the upper continental slope and shelf off the NSW coast: East of Sydney, East of Ulladulla, and East of Eden. The first trawls occurred between 1976 and 1977, on what were then relatively lightly fished grounds. In 1997, the *Kapala* re-trawled the same areas and in the same manner,

leading to high-resolution, comparative data. Fisheries researchers on board the re-trawls recorded depth, species, weights, numbers, sex, and lengths of angel sharks caught (Graham, Andrew *et al.* 2001). In addition, the *Kapala* also undertook some trawl surveys on continental shelf grounds in 1997, which had been subject to commercial trawling since about 1920.

Objectives

Data on the catch, the number of days fishing, position and depth of capture by year and month were variously summarised to determine several trends in CPUE expressed as kg/day. Temporal trends were examined using linear regression analyses that observed pooled yearly (~12 years) and seasonal variations in CPUE for each of the broad fishing regions: South Australia (west of the Victoria-South Australian border), Bass Strait – Tasmania (east of the Victoria-South Australian border and south of the Victoria-NSW border), and east coast (North of the NSW-Victorian border). GIS was used to display CPUE at a 1 degree of longitude by 1 degree of latitude resolution over these three regions. Yearly or monthly trends in CPUE or total catch were determined using linear regression analyses in Excel.

Morphometric parameters in conjunction with CPUE data from the FRV *Kapala* were used to determine depth distribution of *S. australis* and *S. albipunctata*, as well as the length-frequency changes (if any) of *S. albipunctata* between 1976 and 1994. Data were analysed using a t-test conducted in Microsoft Excel with the data analysis tool pack.

Data from the NSW shark meshing (bather protection) program were analysed according to the number of angel sharks caught per year from 1950 to present, and a linear regression was used to determine whether a decline in number of sharks caught in inshore waters (if any) was significantly correlated to year.

Results

Distribution

The vast majority of angel sharks were caught by trawling, with only a minimal number caught in gill nets; only trawl data were included in these results. No individuals were recorded in the AFMA demersal long-line fishing that occurred throughout the area since 2000. The assessment of the AFMA CPUE data indicated that there were three major catch-rate hotspots visible in south-eastern Australia: one south of Sydney, a second on the Western side of the Bass Strait, and the third south of South Australia (Figure 1). Only the CPUE hotspots off South Australia and south of Sydney had significant catches (over 100 tons over ten years).

The *Kapala* data indicated that angel sharks were caught from shallow waters to five-hundred meters water depth off NSW (Figure 2). On a species level, a significantly higher abundance of *S. australis* occurred in waters shallower than 50m, and none was found deeper than 150 metres. The relative abundance of *S. albipunctata* was highest between 250 and 350 meters depth (Figure 2).

Catch data from the NSW Shark Meshing (Bather Protection) Program (SMP) showed that both species were caught at shark net sites along the central NSW coastline (from Newcastle to Wollongong). Lack of early identification of angel sharks to species level by the SMP contractors negates an ability to determine species-specific changes in extreme near-shore (<15m water depth) conditions. Recent (2011) examination of animals caught by the SMP, however, indicated that both species are caught in near-shore mesh nets (Raoult, personal observation).

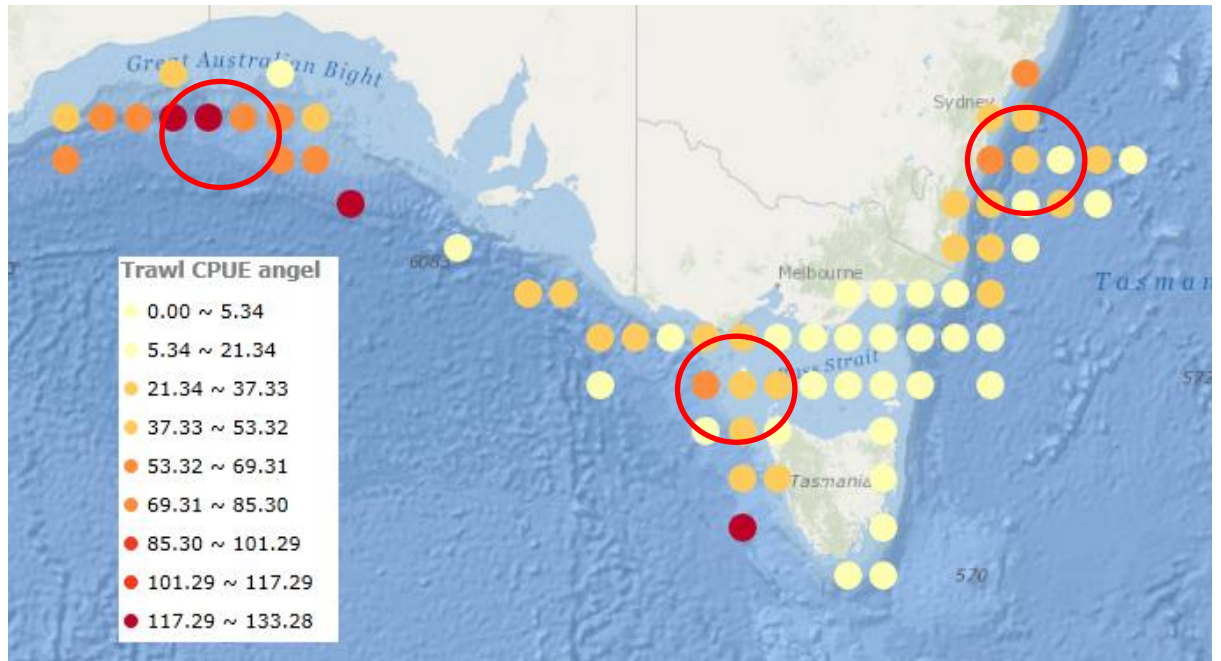


Figure 1: Spatial distribution and mean CPUE (kg day⁻¹) of *Squatina* sp. in south-eastern Australia. Map belongs to ESRI, included with ArcGIS. 'Hotspots' where the majority of angel sharks are caught are highlighted with red circles.

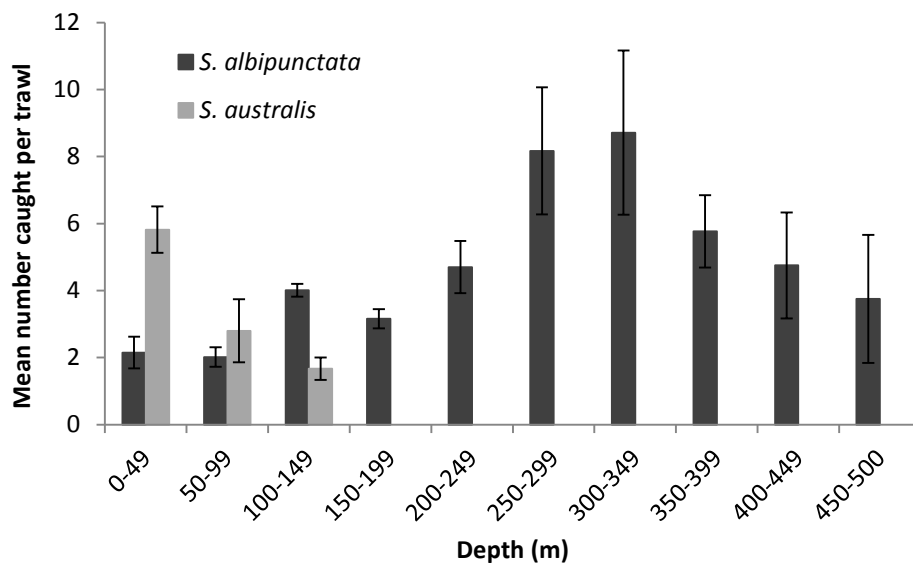


Figure 2: Depth distribution in mean number (± 1 S.E.) of the two *Squatina* species caught using catch data from the FRV *Kapala*.

Population Size Structure

Statistically significant reductions in mean total lengths occurred between 1976 and 1994, both for males ($n_{1976}=384$, 82.8 ± 0.67 ; $n_{1994}=284$, 71.5 ± 1.5 ; $p < 0.01$) and females ($n_{1976}=606$, 88.9 ± 0.77 ; $n_{1994}=469$, 77.7 ± 0.97 , $p < 0.01$; Figure 3). Females grew to larger sizes than the males (126 vs 110cm total length), and few animals smaller than 50 cm were caught, the minimum size being 30 cm total length. Length-frequencies were normally distributed. There was an increase in the proportions of small animals in 1996, with a corresponding decrease in the proportions of large animals.

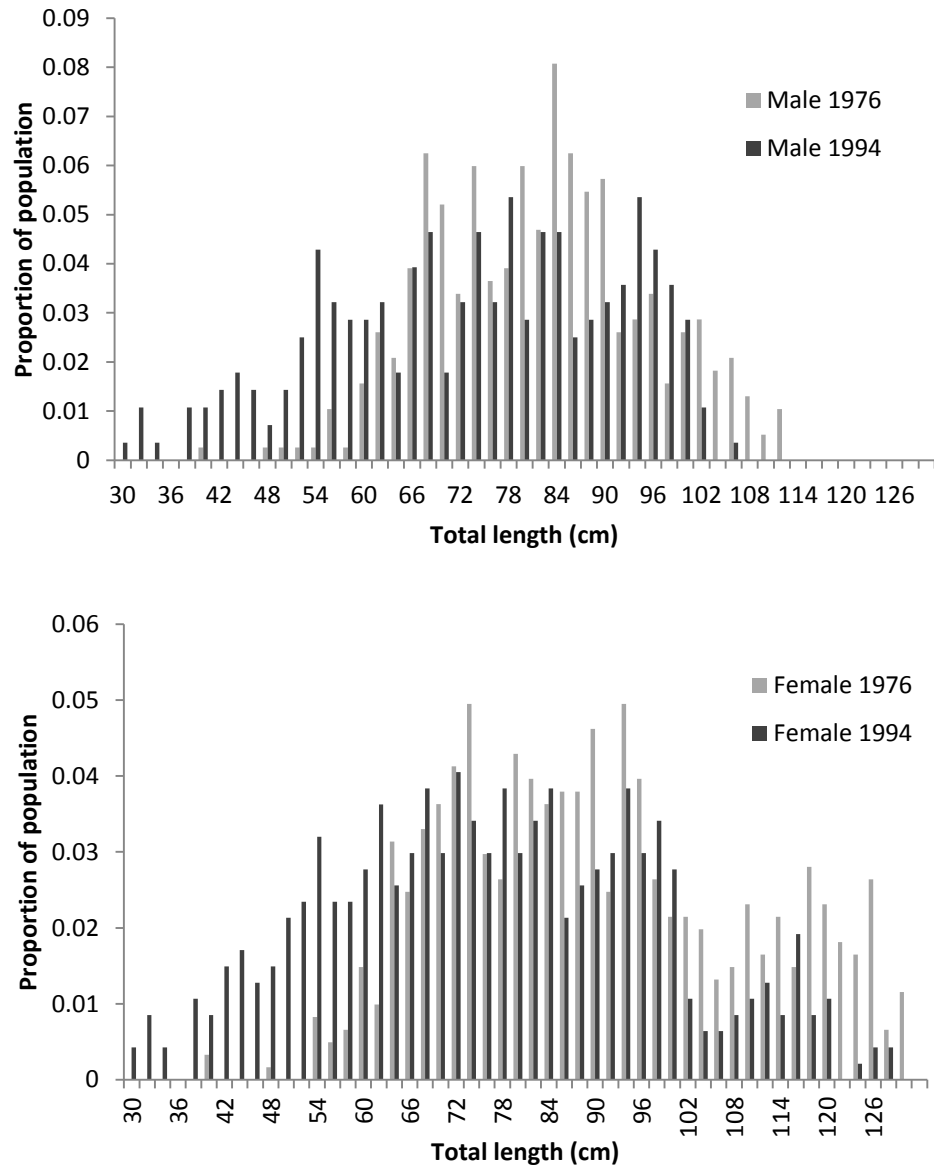


Figure 3: Length frequency distributions of male and female *S. albigata* caught by the FRV Kapala in 1976 and 1996.

Seasonality of catch per unit effort

Angel sharks were caught throughout the year in all data sets, however, variations in seasonal catch per unit effort occurred both on the larger scale of the Commonwealth waters and the smaller scale of NSW waters. In Commonwealth waters (which included a much larger area and a greater variation in latitude and longitude), CPUE varied depending on the area (Figure 4). CPUE was highest in South Australia and lowest around the Bass Strait, Tasmania. Catch

from South Australia showed a distinct temporal pattern, with a six-fold increase from March to May/June and then a continual decrease until the lowest catch in January (Figure 4). CPUE on the east coast increased slightly during the austral winter months (May-September). CPUE from the Bass Strait appeared to mirror the catch on the east coast, with a corresponding decrease in CPUE in one site when the other increased (Figure 4). In the NSW trawl fishery dataset there was a significant correlation between month and CPUE ($p < 0.01$, $df = 1$, $F = 14.9$) in a sinusoidal pattern ($R^2 = 0.91$) in CPUE for both species combined, with the lowest catch efficiency in the austral autumn (January-May) and the highest catch efficiency (30% more) in the austral autumn (August-November) (Figure 5).

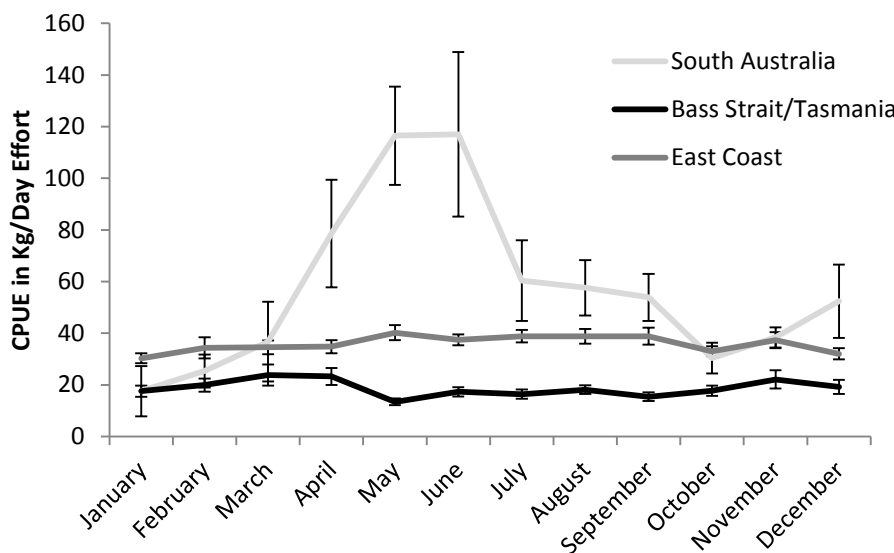


Figure 4: Mean (± 1 S.E.) monthly catch per unit effort in Commonwealth waters for *Squatina* spp. from 2000 to 2011. Note that a large proportion of the South Australian catches are likely to be *S. tergocellata*.

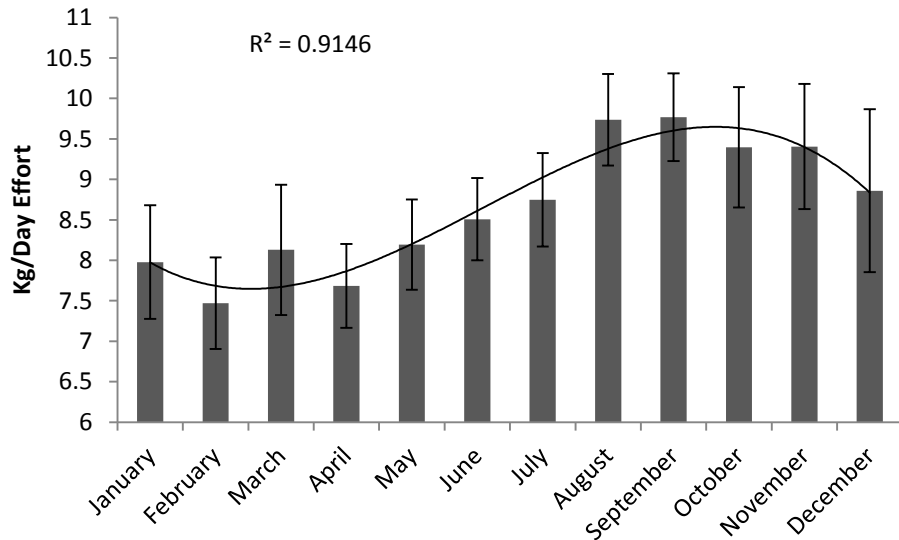


Figure 5: Mean (± 1 S.E.) monthly catch per unit effort in NSW waters for angel sharks (*S. australis* and *S. albipunctata*) between 1990 and 2009. Best fit sinusoidal trend line added.

Sustainability

For nearshore NSW waters, the Shark Meshing (Bather Protection) Program catches for both species combined showed a significant overall decline in the number of angel sharks caught over the 60 years analysed ($p < 0.05$, 1 *df*, $R^2 = 0.56$). However, if this period is subdivided into twenty-year truncheons (vicennial), the last twenty years exhibits a non-significant change in catch rates (Figure 6; $p > 0.05$, 1 *df*, $R^2 = 0.002$). This apparent ‘stability’ in the catch, however, is only ~25% of the catch during the first twenty-year truncheon (1950-1970) (Figure 6).

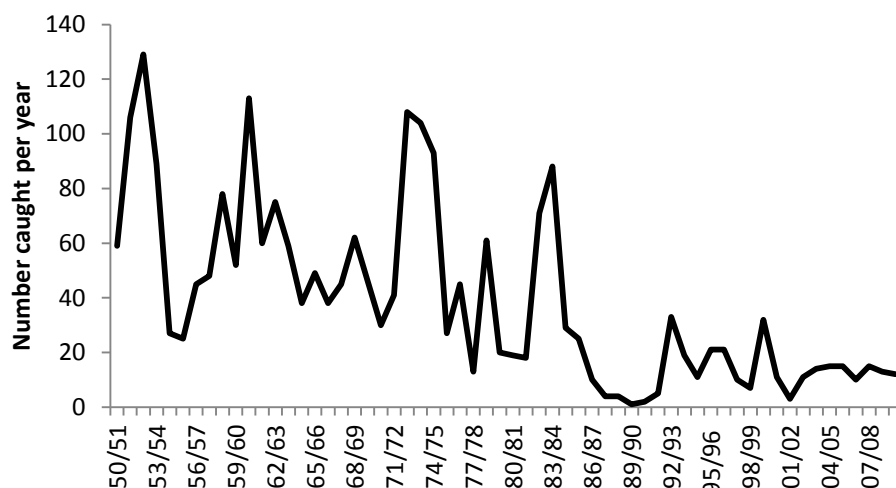


Figure 6: Number of angel sharks (species combined, both *S. australis* and *S. albipunctata*) caught in the NSW Shark Mesh Program per year from 1950 to 2010.

Total catches of angel sharks for combined AFMA and NSW fisheries datasets showed yearly variations with no significant decline in total weight landed (Figure 7; $p > 0.05$, 1 *df*, $R^2 = 0.31$). The Commonwealth data set indicated a slight decline in unstandardized catch rate over the last decade ($p < 0.05$, 1 *df*, $R^2 = 0.34$) and significant inter-annual variations in catch per unit effort (CPUE) of *Squatina* sp. (Figure 8). East coast CPUE declined significantly ($p < 0.05$, 1 *df*, $R^2 = 0.35$). South Australian catches showed the highest CPUE but also the highest variation, and showed no significant trends ($p > 0.05$, 1 *df*, $R^2 = 0.3$). The Bass Strait/Tasmania varied significantly over the years but showed no trend over the time period analysed ($p > 0.05$, 1 *df*, $R^2 < 0.01$). CPUE in the Bass Strait was roughly half that of the east coast (Figure 8).

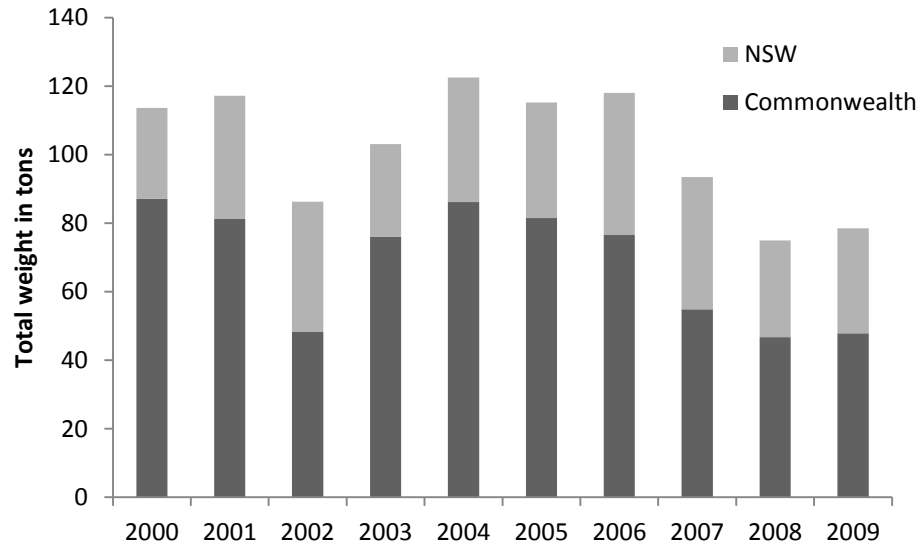


Figure 7: Total tonnage of angel shark landings (trunks only) for all species combined within NSW and Commonwealth waters between 2000 and 2009 (both *S. australis* and *S. albipunctata*). Note that a large proportion of the South Australian catches are likely to be misidentified *S. tergozellata*.

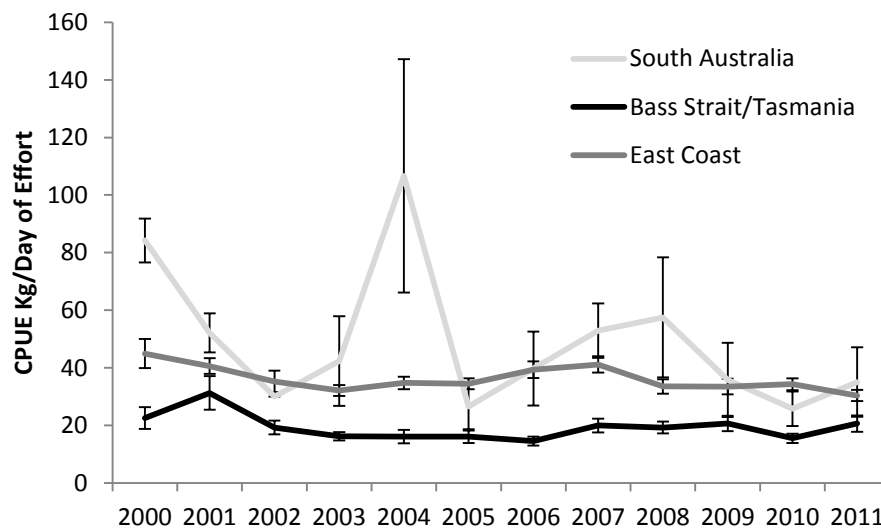


Figure 8: Mean catch per day of effort in kg/day per year \pm 1 S.E. for *S. australis* in Commonwealth waters. Note that a large proportion of the South Australian catches are likely to be misidentified *S. tergozellata*.

In NSW, the trawl CPUE data indicated no decline in CPUE for combined species of angel shark since the 1990's (Figure 9). CPUE varied, sometimes significantly, from year to year, e.g. in 2002 and 2003 when CPUE declined from ~9 to 6 kg per day of effort. The spike in CPUE in 2007 may be due to a change in recording paperwork as the requirements for reporting fishing effort changed in 2006. There is no evidence, however, of a sustained

decline in catch rate from this fishery over the 18 years of records assessed in the current study: there was no significant correlation between year and CPUE ($p > 0.05$, 1 *df*, $R^2 = 0.012$).

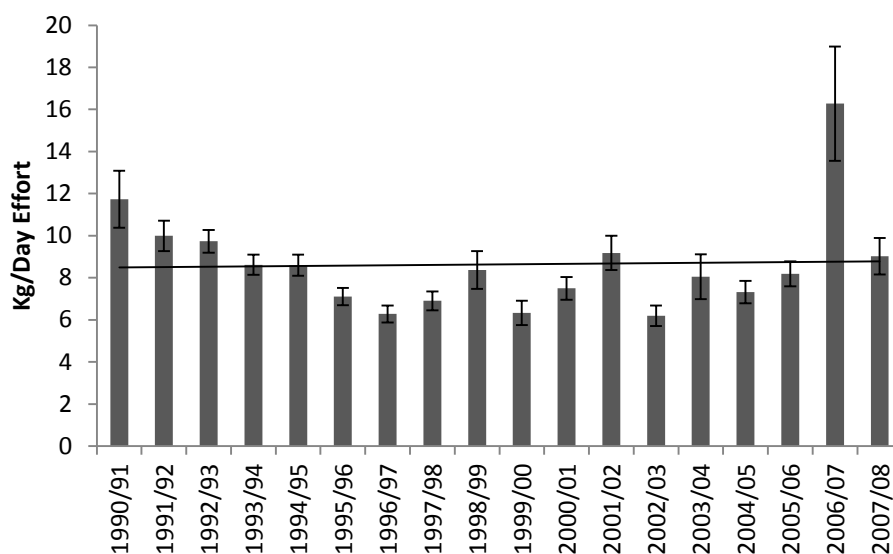


Figure 9: Mean catch per day of effort in kg/day per year \pm 1 S.E. in NSW waters for angel sharks (*S. australis* and *S. albipunctata*). Linear trend line added.

Discussion

Squatina australis and *S. albipunctata* appear to have tolerated significant levels of commercial harvesting over long periods in south eastern Australia. Unlike many European fisheries, the south eastern Australian trawl fisheries are much smaller in scope: there are fewer fishing vessels covering a relatively large area. While North Sea trawlers significantly alter benthic communities through repeated large-scale trawling (Callaway *et al.* 2007), the coverage of south eastern Australian trawlers is relatively less (Graham *et al.* 2001). Thus trawling intensity appears to be a key factor: other species of angel shark (*S. oculata*, *S. guggenheim* from Europe and Brazil, respectively) have shown dramatic declines in abundance under intense fishing pressure and are now classified as endangered or critically endangered on the IUCN Red List (Coelho *et al.* 2005). The more sporadic Australian trawl

fisheries at the current CPUE appear too low in intensity to cause similar levels of continual depletion in south-east Australian populations as seen in these other fisheries. It is also likely that the biological traits of these species are at least partially responsible for their resilience to fishing pressure: the same fishing pressure in the same waters have greatly depleted the stocks of the upper slope dogfishes off NSW and continue to do so (Graham *et al.* 2001)

The *FRV Kapala* data revealed a shift from larger to smaller *S. albipunctata* during the study period. Truncation, or shifting in length and/or age distribution within the stock, is a known sign of overfishing (Berkeley *et al.* 2004), consequently angel sharks are showing signs of strong fishing pressure in certain areas. The increase in the proportion of smaller individuals may be explained by the lowered competitive exclusion as a result of catches of larger angel sharks, or by recruitment: as the fishery removes all sizes of shark, the only sharks added to the population are small, newly-recruited individuals. The operation of the trawl fishery off NSW appears to have led to a significant depletion in angel shark populations (Graham *et al.* 2001). Fishery-independent surveys by the *FRV Kapala* indicated a very significant decline in the abundance (as determined by catch rate) of angel sharks, from over 30kg/hour in 1976 to just above 1kg/hour in 1996. These results suggest a decline of greater than 90% in angel shark abundance on the continental slope at depths of 220-605m in the broad region between Newcastle to ~50nm south of Gabo Island during the period between surveys. Localised depletions have been observed in other angel shark species (*S. oculata*), and are exacerbated by low re-colonisation rates (Vacchi *et al.* 2000). The data presented in this study suggest that, while having declined in the past, catches of *S. albipunctata* have stabilised at substantially lower numbers over the past decade. As *S. australis* appears to be the dominant species in shallow waters, the decline in the catch rate of angel sharks seen by the SMP nets may indicate a similar long term decline in the abundance of this species along the coastline (more a result of the commercial trawl fishery than the SMP itself). Unfortunately, with no

useful species identification of captures during the early years of the NSW Shark Meshing Program, this concept remains speculative.

One of the likely reasons *S. australis* and *S. albipunctata* have shown resilience under current fishing pressure after their initial decline is the difference in depth distribution the species inhabit compared to species targeted by Australian trawlers. Greater numbers of *S. albipunctata* were caught at depths from 250 to 400 meters, and greater numbers of *S. australis* were caught at depths less than 50 meters. The majority of trawling effort in south-eastern Australia prior to the 1990s occurred between 50 and 200 meters (Larcombe *et al.* 2001), however, suggesting that only a portion of the total habitat is being fished for both species. Bathymetry, and in some cases legislation, limit fishing at depths less than 50m, and limited productivity and resource levels reduce the amount of fishing at depths more than 250 meters. Furthermore, while historical trawl intensity in NSW has been high (Graham *et al.* 2001), the relative trawl intensity in Commonwealth waters around Tasmania has been much lower (Larcombe *et al.* 2001). As large areas of *S. albipunctata* and *S. australis* habitat have remained lightly fished, and recent management-induced reductions trawl capacity in the south-eastern trawl fishery have been set in place, future increases in fishing effort in all angel shark habitat is unlikely.

Our data suggest that *S. australis* and *S. albipunctata* may migrate, perhaps inshore during autumn for pupping. At this stage, it is impossible to attribute the scale or cause of any migrations without more data on the life history of the species, such as movement and reproductive data, or data related to ontogeny. Variations in seasonal catch efficiency of *Squatina sp.* were detected that may have coincided with abiotic factors, such as seasonal fluctuations in temperature. Opposite variations in catch efficiency in the Bass Strait and along the East Coast may suggest movement from the Bass Strait to the East Coast in April to warmer waters, and back in September. A migration from the Bass Strait to South Australia,

where catch efficiency also increases, would be less parsimonious: catches in South Australia are more sporadic, and the seasonal CPUE increase is less concordant than CPUE variations of the Bass Strait. Significant sinusoidal trends were visible in NSW. The pattern is slightly offset with mean sea-surface temperatures (lowest in August and highest in March), indicating that catch efficiency may be linked to sea-surface temperature, or to factors associated with sea-surface temperature. Movement studies would elucidate the validity of these hypotheses.

The presence of both species of *Squatina* in the same area poses further ecological questions. Given their similar morphology, competition theory would suggest that both species should not compete with each other for a long period of time (Armstrong and McGehee 1980). It is possible that the two species display food-niche separation or resource partitioning (Barnes *et al.* 2011; Rothhaupt 1988). The different colourations of the two species may also indicate local separation for crypsis according to the available substrate.

Both commercial fishery and research trawl data indicate that the current distributions of *Squatina spp.* in south-eastern Australia need to be redefined. It has previously been assumed that the two species were spatially distinct, with *S. australis* occurring inshore and in shallower waters and *S. albipunctata* offshore and in deeper waters (Last and Stevens 2009). The FRV *Kapala* research trawls indicate an overlap in depth distribution for the two species from 0 to 150 meters' depth. Thus both fishermen and observers should not use depth caught as a reference to differentiate between these species but ideally, the species should be separated using morphological characteristics.

Clear identification guidelines need to be created. The authors suggest the most distinct traits of *S. australis* are the distinct white borders that occur on most fins, as well as the dark dots present on the caudal fins. *S. albipunctata* does not have either of those characteristics and

generally have very defined, small pure white spots present on their dorsal sides in smaller numbers than *S. australis*' more common spots. It is considered likely that a large number of catches entered into the catch database as *S. australis* or *S. albipunctata* in the Great Australian Bight were most likely *S. tergozellata*. Angel sharks caught in relatively deep waters off NSW and identified as *S. australis* were most probably *S. albipunctata*. Therefore, clearer identification guidelines following morphological characteristics such as those recommended for *S. australis* and *S. albipunctata* should also be given to fishermen.

Difficult identification of sympatric species such as *S. australis* and *S. albipunctata* is problematic for management purposes. It was likely that commercial trawlers caught both species in the same trawls, but the species were then misidentified by either the fishermen or the recording authority. The majority of fishermen have made the assumption that only one species occurred offshore. This may have led to a significant species bias in recent catch records. Older catch data in NSW did not include species-specific identification. While both AFMA catch data and post-2009 NSW catch data do separate species, it is highly unlikely that all *Squatina* are correctly identified. The authors strongly advise that species-specific commercial catch data in their current form are not robust for species management when two similar species occur in the same area unless correct identification is taught to fishers and monitors.

Like many shark populations that have declined to less than 10% of their original size (Baum *et al.* 2003), populations of *Squatina* species occurring in south-eastern Australian waters have displayed a steep decline in catch per unit effort that has stabilised over the past two decades. While a shift in mean total lengths was evident, there still appears to be a relatively stable stock of animals present in SE Australia. Recently, lower levels of fishing pressure and the depth distribution of the two *Squatina* species may be responsible for maintaining the populations at a stable level to date.

The reasons behind the stability of *Squatina* sp. populations require further research, but defining the fishery as ‘sustainable’ may be inaccurate. Many fisheries assessments attempt to classify harvesting categorically as sustainable or unsustainable when the effects of commercial fisheries over the last century have caused documented shifted baselines (Baum and Myers 2004; Pauly 1995). Thus, what is deemed ‘sustainable’ has no reference unless it is compared to accurate pre-industrial data, a point underlined by Hilborn (2002). Researchers need to qualify statements of sustainability or unsustainability with regard to such pre-industrial information on a spectrum rather than as categories. Such categories also need to take into account the life history characteristics and longevity of the species, which are relatively unknown for these species. *Squatina* sp. populations in south-eastern Australia are depleted, yet current fishing levels appear sustainable, therefore it is suggested that they are currently classified as ‘sustainably overfished’. Sustainably overfished species may be continually harvested though fishing has lowered population levels and has likely caused associated ecological consequences. Such species should, however, be considered ‘at risk’ and commercial fishing on these species needs rigorous and continual management and monitoring.

Acknowledgements

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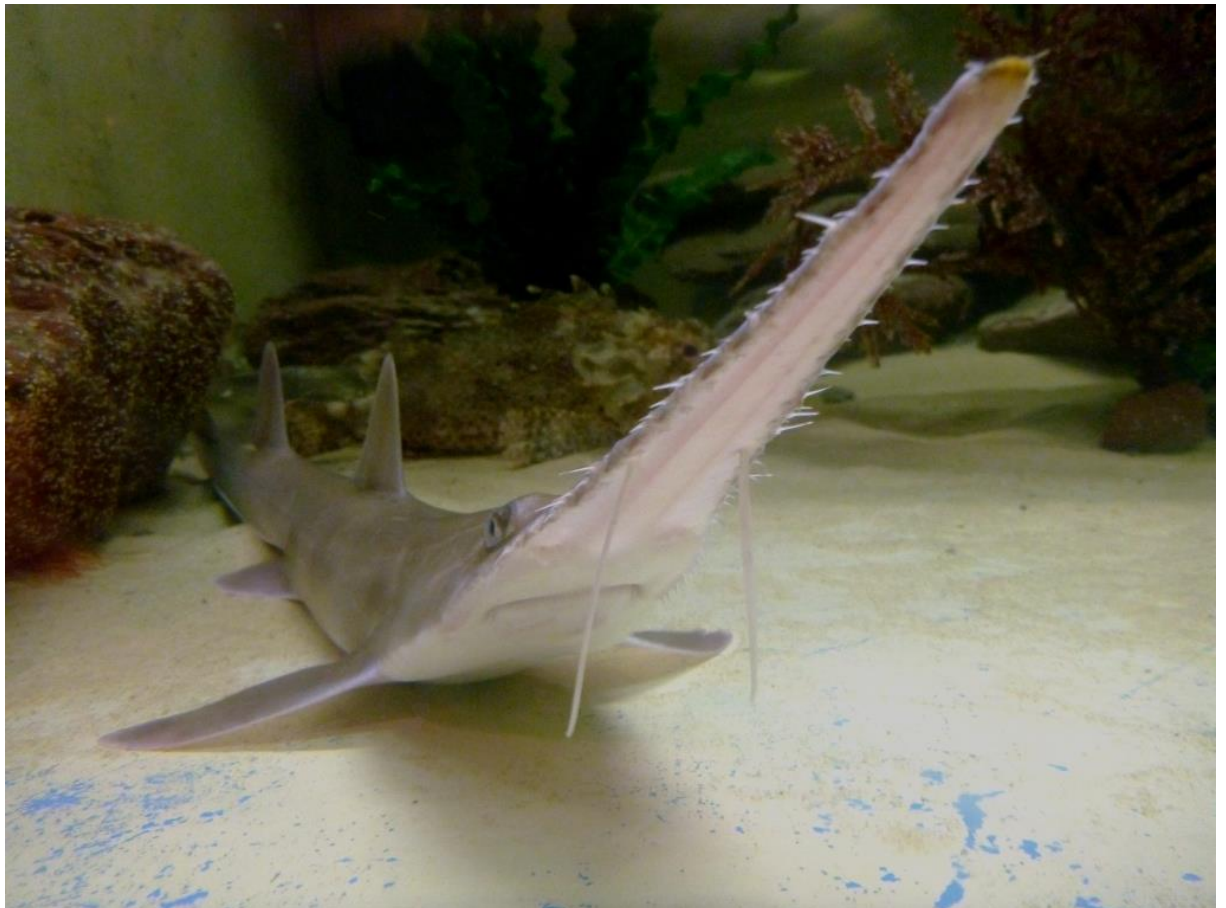
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CHAPTER 3: Examination of a long-term sawshark fishery in south-eastern Australia

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Pristiophorus nudipinnis held in Merimbula Aquarium, NSW, Australia. Photo taken by Tristan Guttridge.

Abstract

Two *Pristiophorus* species occur in south-eastern Australia, and both are frequently caught by commercial trawl and gillnet fisheries. This study aimed to determine whether these sharks were caught sustainably by collating commercial and scientific data sets with various degrees of resolution. It appears that the current biomass of sawsharks is sustainable with current fishing rates; however that biomass is ~60% below pre-industrial levels. Sawsharks occur at shallower and deeper depths than previously thought, and their geographical range is larger than previous studies had found. The depth distributions of both species appear to overlap with no clear pattern. Yearly catch patterns suggest that seasonal migrations occur in autumn (May). These results highlight the need for further biological research on these species.

Keywords: Pristidae, sawfish, Pacific, exploration, management

Introduction

There is a paucity of information on the ecology of organisms occupying deeper oceanic waters due to the technical difficulties of working in waters greater than 100m depth (Mantyla and Reid 1983). Despite this lack of information, large-scale commercial fishing now routinely occurs in deeper waters. Until recently, it was generally assumed that information from shallow water species could be extrapolated to deeper water populations and species, and that harvested stocks were sustainable (Clark 2001). Unfortunately, lessons have been learnt the hard way, with populations of high-value species such as Orange Roughy (*Hoplostethus atlanticus*) collapsing under excessive fishing pressure before adequate biological studies could be done (Clark 2001). Other studies have determined that environments below 100m depth are generally fragile ecosystems not suitable to intensive fishing efforts (Koslow *et al.* 2000).

Although commonly considered ecological stabilisers, sharks are under intense fishing pressure (Baum and Myers 2004; Baum *et al.* 2003; Ward-Paige *et al.* 2010). Elasmobranchs generally grow slowly and have low reproductive rates, which make populations of shark slow to recover from fishing pressures (Stevens *et al.* 2000). Furthermore, when overfished, trophic cascades that affect a number of species in addition to the sharks within food webs may occur (Ferretti *et al.* 2010; Myers *et al.* 2007). Thus, in an effort to reverse the effects of overfishing, sharks have become a target for increased research and management (Worm and Branch 2012).

There are nearly 500 known species of sharks, yet the majority remain unstudied (Compagno 2001). The population status of many shark species listed on the IUCN Red List of Threatened Species remains unknown, particularly the deeper ocean species. Deeper ocean sharks that have been studied, such as dogsharks (genus *Centrophorus*) show rapid declines due to commercial fisheries (Graham *et al.* 2001), suggesting that these species are particularly susceptible to fishing practices.

Sawsharks (*Pristophoridae*) have been consistently harvested as bycatch in commercial trawl fisheries for over 90 years in Australian fisheries (Tilzey and Rowling 2001). Found throughout the world's oceans, three species of sawshark are commonly caught in Australian waters: the common sawshark (*P. cirratus*), the southern sawshark (*P. nudipinnis*) and the delicate sawshark (*P. delicatus*) (Last and Stevens 2009). Often confused with sawfish (*Pristidae*) because of their similar saw-like rostrums, sawsharks are true sharks and thought to have similar life history characteristics like other deep-water sharks that are particularly vulnerable to overfishing (García *et al.* 2008). Aside from an impact assessment conducted on the Commonwealth waters of Australia (Walker *et al.* 2005), the impact of commercial fishing on sawshark populations remains largely unstudied, and this has led researchers to highlight the need for more information on fished species (Wueringer *et al.* 2009). Generic

biological information (Raoult *et al.* 2016) and apparent resource partitioning between *P. cirratus* and *P. nudipinnis* (Raoult *et al.* 2015) have only been recent discoveries.

This study assesses the status of two species of sawshark (*P. cirratus* and *P. nudipinnis*) in south-eastern Australian waters. These species are harvested mainly by the trawl and gillnet fisheries. As data for such fisheries can be relatively difficult to obtain, this study collated several sets of commercial and scientific catch data to increase the temporal and spatial resolution of the population status. Potential migration patterns are proposed by observing seasonal catch data over time. Distribution was re-assessed (Last and Stevens 2009), and yearly catches were used to determine whether fisheries have had a negative impact on sawshark populations.

Materials and Methods

Species identification

Fishermen operating in south-eastern Australia were not obliged to identify sawsharks at a species-level when recording their catches in log-books for either Commonwealth or state fisheries agencies, as a result species were frequently grouped into *Pristiophorus* spp.. Furthermore, the high degree of similarity between the two species would have likely resulted in low confidence with species identification for fisheries data sets. As a result, the two species were grouped together for the purpose of this study.

Data sets

This study assessed three separate data sets. The first data set used, supplied by the Australian Fisheries Management Authority (AFMA), collated data from large-scale catches throughout the Commonwealth authority from Newcastle in New South Wales (NSW) to the western side

of the Great Australian Bight in West Australia. Mid-scale data from NSW Fisheries catch data spanned from Newcastle (32°55'41 S, 151°47'59 E) to Eden (37°03'55 S, 149°57'48 E). Small-scale and long-term data were provided by the NSW Fisheries research trawler *FRV Kapala*: the presence of onboard fisheries scientists ensured that species identifications from the latter source were highly accurate (29-37° S, 149-154° E).

AFMA data were collected from 1999-2012 and comprise data from the three main fisheries in the area: long-lining, trawling and gill netting. This data set relied on fishermen to complete logbooks. Coordinates for catches, along with water depth, the processed weight and number of target species caught, and the weight and number of discarded species were logged. Due to the geographic scope of the Commonwealth data set, it was separated into the East Coast (all the water East and North of the Victorian border), South Australia (all the area to the West of the Victorian border), and the Bass Strait/Tasmania.

The NSW catch data derived solely from the NSW ocean trawling fishery for the period 1990 to 2008. Like the AFMA data set, NSW catch data relied on fishermen to fill out monthly catch returns with information on species caught, location (in the format of seven 'zones' separated evenly from North to South, determined by whole degrees of latitude) and total weight landed. No observer studies were included in this data set, and like the AFMA data recorded weights caught were for headed and gutted animals.

The *Kapala* research trawler data set included a series of research trawls conducted in 1976-77 and subsequently in 1997. Trawling occurred along a number of sites along the NSW coast: East of Sydney, east of Ulladulla, and south-east of Eden. Fishing was done on the upper continental slope and recorded by fisheries scientists. Variables that were recorded include GPS location, start and end of trawl depth, species, individual weights, the number of individuals, sex, and total lengths of all the animals that were caught.

The number of sawsharks caught each year may give a more meaningful analysis than the total weight of processed sharks caught. Morphometric data collected from commercial fisheries catches were compiled, and mean total weight was calculated for both species. Estimated processed weight was then calculated, and the total number of sawsharks caught each year was estimated.

Objectives

Data on the catch, the number of days fishing, position and depth of capture by year and month were variously summarised to determine several trends in CPUE expressed as kg/day. Temporal trends were examined using linear regression analyses that observed pooled yearly (~12 years) and seasonal variations in CPUE for each of the broad fishing regions: South Australia (west of the Victoria-South Australian border), Bass Strait – Tasmania (east of the Victoria-South Australian border and south of the Victoria-NSW border), and east coast (North of the NSW-Victorian border). GIS was used to display CPUE at a 1 degree of longitude by 1 degree of latitude resolution over these three regions.

CPUE or weight landed trends over time (across years and seasons) were analysed using linear regression analyses in Microsoft Excel using the data analysis tool pack.

Results

Sawsharks were caught at different catch per unit effort rates in different areas around south-eastern Australia. In Commonwealth waters, there were three catch efficiency hotspots that also caught more than 10 tonnes a year: just north of Sydney, on the western side of the Bass Strait, and on the western side of the Great Australian Bight (Figure 1). The depth at which catches occurred was highly variable: the lowest mean number of sawsharks caught per depth was from 150-199m, but catch numbers were significantly higher above and below that depth.

Sawsharks were caught from a depth of 9 meters to over 500 meters (Figure 2). There was no clear depth separation of the two species.

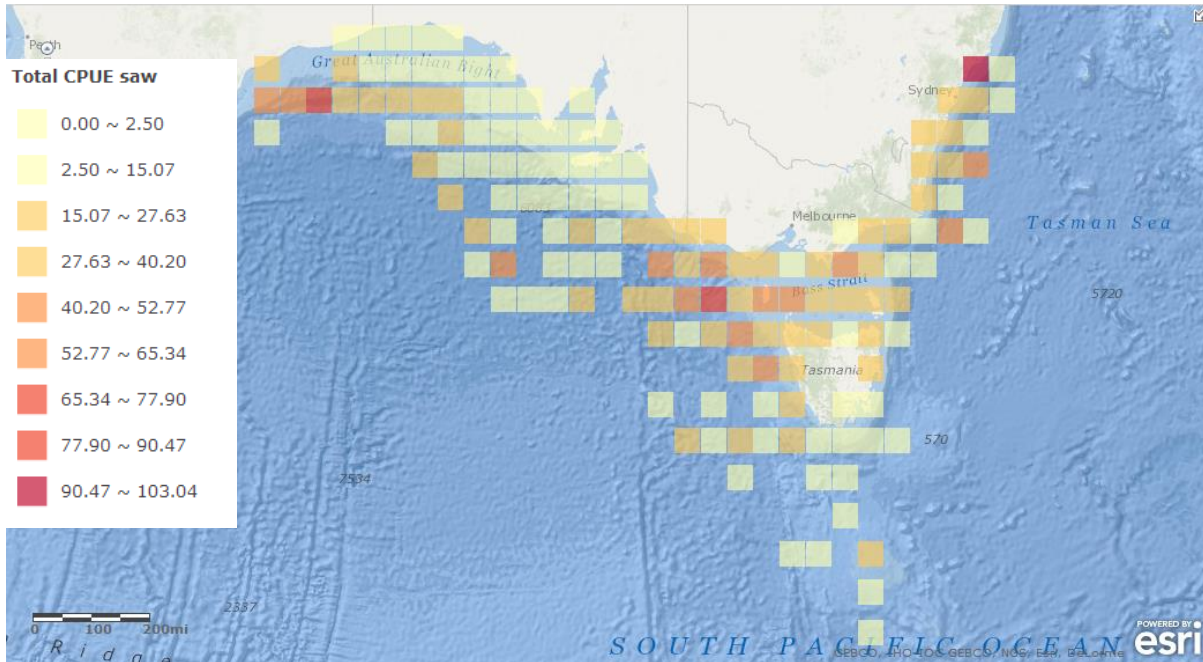


Figure 1: Mean catch per unit effort in kg/day of sawshark species in Commonwealth waters from 2000 to 2011. Taken from ESRI GIS online.

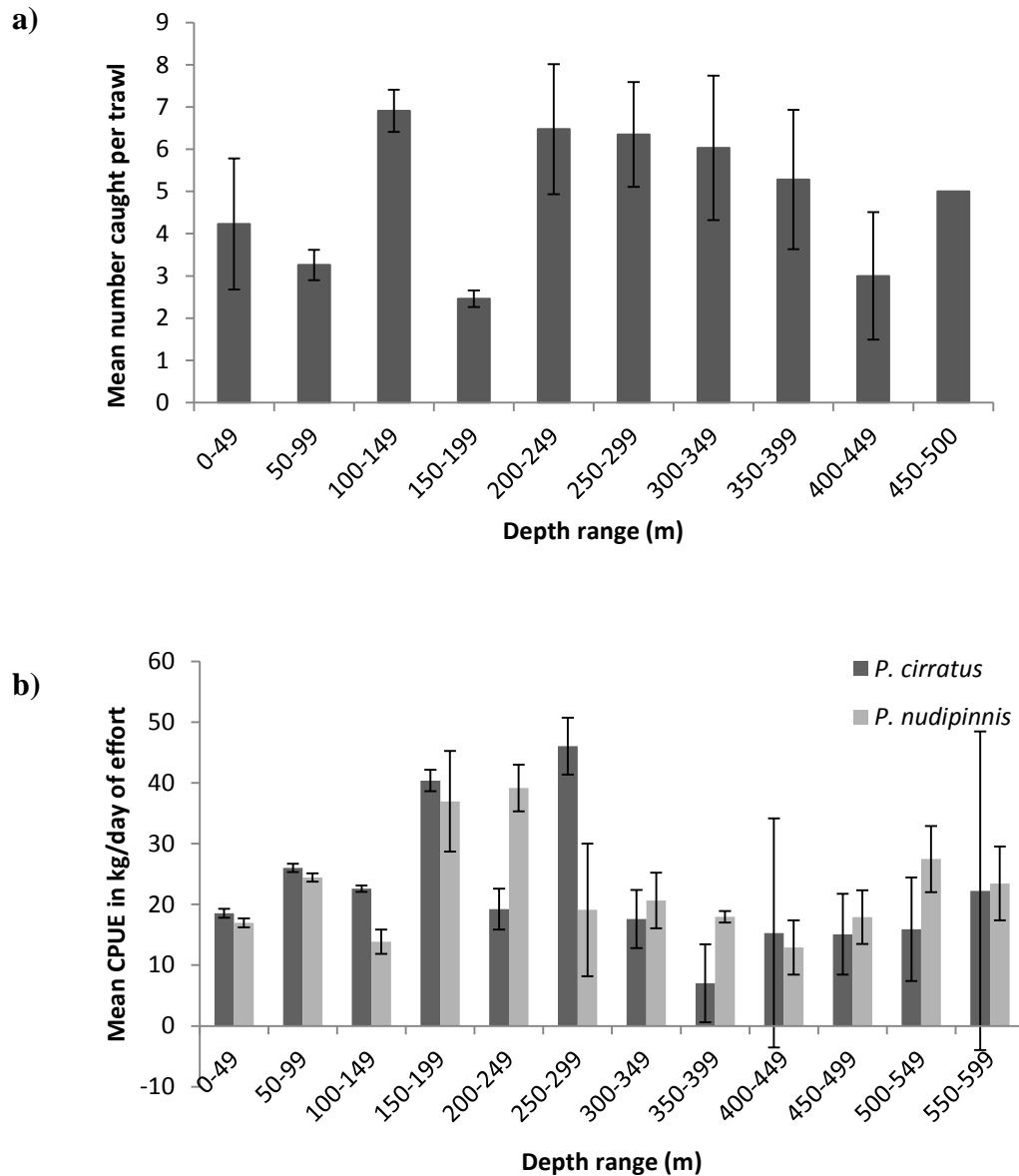


Figure 2: Mean number (± 1 S.E.) of sawsharks (both species) caught by (a) the *Kapala* and (b) mean CPUE of *P. cirratus* and *P. nudipinnis* from Commonwealth fisheries at various trawl depths

Seasonal catch rates of sawshark varied depending on the broad location. In Commonwealth waters, the east coast recorded the highest catch rate in Autumn, before rates dropped in winter and spring. Conversely, in the Bass Strait catch rates were lowest in Autumn before increasing through winter - spring - Summer. In South Australia, catch rates reflected a similar pattern to the east (Figure 3). Catch rates in NSW waters were stable throughout the

majority of the year, with the exception of autumn/early winter, when catch rates peaked (Figure 4).

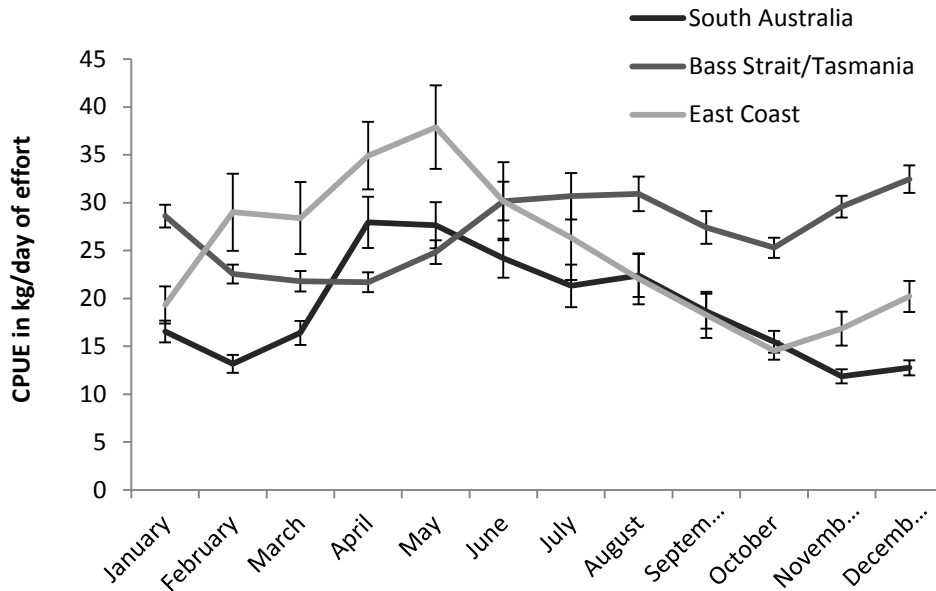


Figure 3: Monthly mean catch per day of effort in kg/day \pm 1 S.E. in Commonwealth waters for grouped *Priostiophorus* sp. from 2000 to 2011.

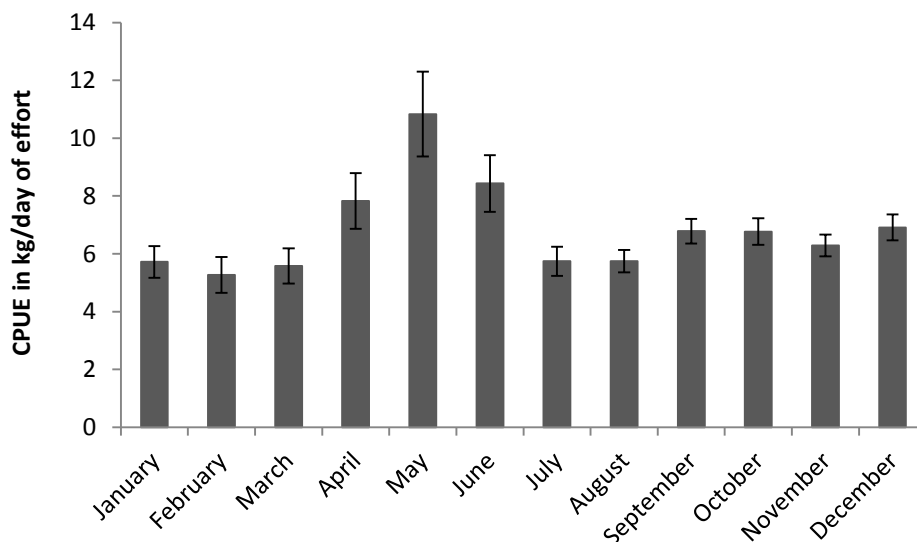


Figure 4: Monthly mean catch per day of effort in kg/day \pm 1 S.E. in NSW waters for grouped *Priostiophorus* sp. from 1990 to 2009.

Yearly catches of sawsharks varied over the 9 years of sampling. Combined total catches of NSW and Commonwealth fisheries increased by 90 tonnes from 2003 to 2006 before returning to levels similar to the pre-2003 spike (Figure 5). At an average total weight of 850 grams (Raoult *et al.* 2016), and considering that reported weights are often processed weights (head and guts removed), the total yearly catch of sawsharks could be of more than 470,000 animals (350 tonnes/(0.85/2)kg, mean weight divided by 2 to reflect processing).

The majority of sawshark catches occurred in Commonwealth fisheries, where over 200 tons per year were consistently caught (fig 5). In Commonwealth waters, South Australian catches varied but were relatively stable over time ($p > 0.05$, $F = 1.56$, 1 *df*). In the Bass Strait, catch per unit effort was not correlated to the year, but was highest in 2004-2006 ($p > 0.05$, $F = 3.5$, 1 *df*). Catch per unit effort on the east coast declined significantly to less than half its initial value from 37 to 17 kg/day ($p < 0.01$, $F = 16.2$, 1 *df*, $R^2 = 0.61$) (Figure 6). Based on these data, the least efficient catch method of the three studied for sawsharks was longlining from 2000 to 2011, while trawling was the most efficient. The catch rates of techniques varied significantly over time, with gillnets declining by a fifth from 2006 to 2011 and trawling declining by a third (Figure 7). Gillnets were more frequently used to catch sawsharks than trawl nets (9171 versus 5300 days of effort) and were responsible for the majority of the catch (1802 and 1273 tonnes, respectively). In NSW waters, catch per unit effort was highly variable, but showed no significant yearly correlation ($p > 0.05$, $F = 0.006$, 1 *df*) (Figure 8).

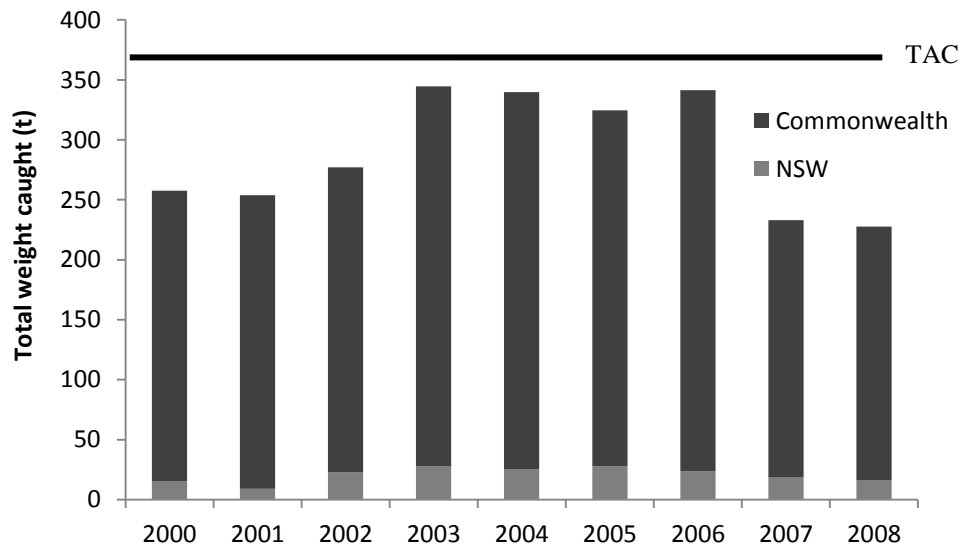


Figure 5: Total carcass weight of grouped *Pristiophorus* sp. landings in NSW and Commonwealth waters, from 2000 to 2008. Total allowable catch (TAC) added for reference.

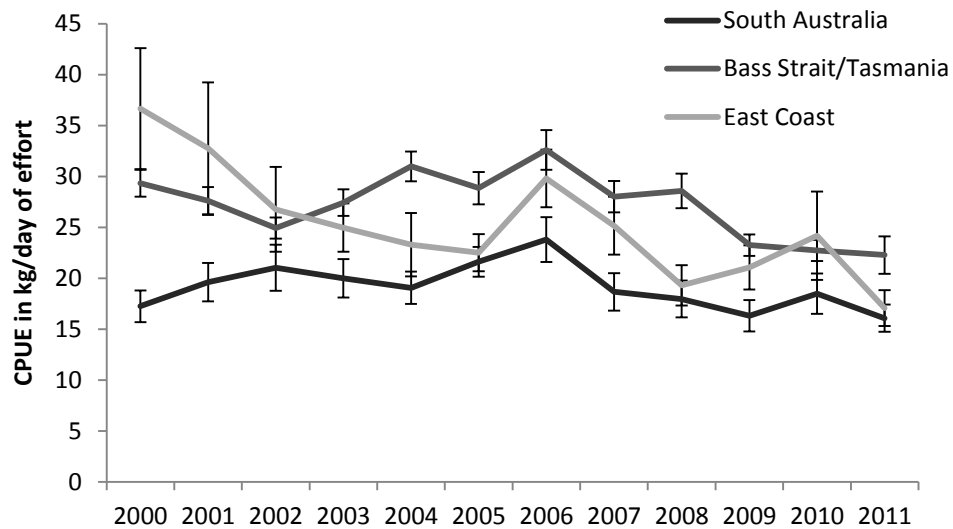


Figure 6: Yearly mean catch per unit effort in kg/day \pm 1 S.E. in Commonwealth waters of grouped *Pristiophorus* sp. from 2000 to 2011. Only the East Coast data showed a significant link between CPUE decline and year.

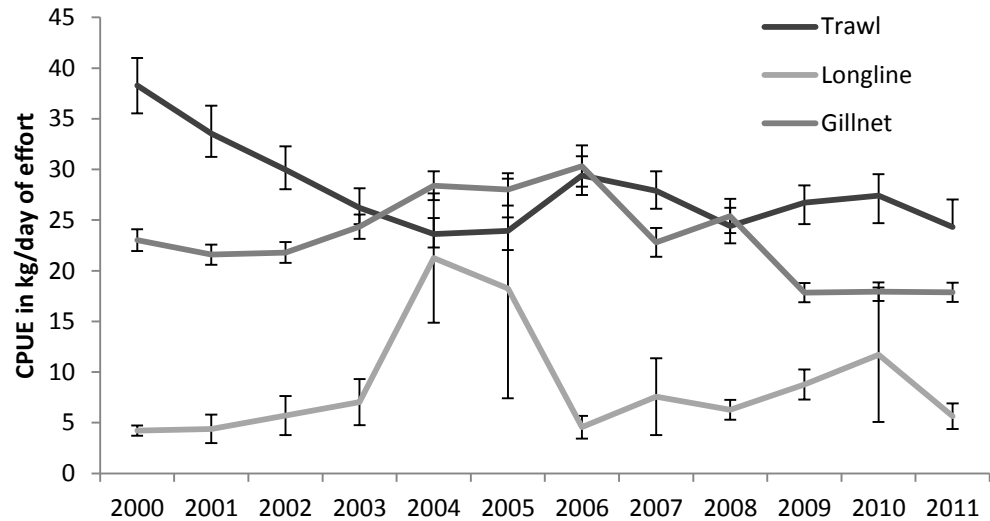


Figure 7: Yearly mean catch per unit effort in kg/day \pm 1 S.E. of grouped *Pristiophorus sp.* in Commonwealth waters for different fishing techniques, from 2000 to 2011.

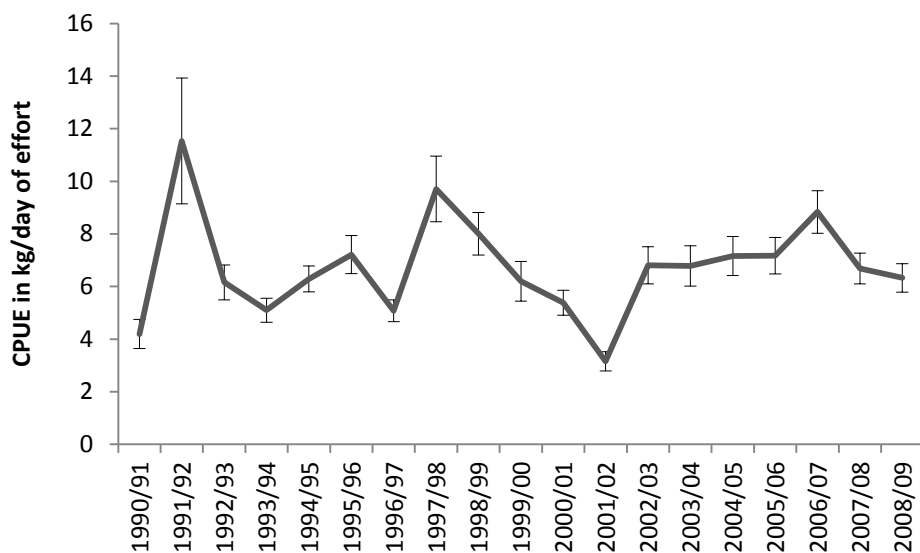


Figure 8: Yearly mean catch per unit effort in kg/day \pm 1 S.E. of grouped *Pristiophorus sp.* in NSW waters from 1990 to 2009.

Discussion

For fisheries managed by NSW, the catch rate of sawsharks was stable over the 18-year data set. In Commonwealth fisheries, catch rates of sawsharks were also generally stable over the period analysed, except for an overall decline in the ‘East’ area. It should be noted that the fisheries catching sawsharks were operating for many years prior to the period for which data

were available to the current study. Seasonal CPUE variations were apparent, with increases in April/May/June in NSW, the East Coast, and South Australia, and an opposed trend in the Bass Strait. Depth data indicated increasing catches to 300 meters.

Historically the highest total weight of sawsharks caught in south-eastern Australia was 359 tonnes in 1995 (excluding NSW DPI fisheries) after rising from 59 tonnes in 1970 (Walker and Hudson 2005). Cumulative catches from 2003 to 2006 were close to reaching that limit, suggesting that 360 tonnes is the maximum catchable weight of the south-eastern fishery. Future periodic decreases from the 360 tonne value would be indicative of an overfished sawshark population. The total weight caught is below the set 364 ton total allowable catch (TAC), TAC was lowered in 2009, 2010 and 2011 to 226 tonnes: it should be noted that this TAC is below total landings in every year data was available. Furthermore, the authors are not convinced that a TAC is an effective measurement tool for these species. Sawsharks are caught as unintentional by-catch that is difficult for fishermen to disentangle from nets. If catch weights were above individual quotas fishermen would be forced to discard the sawsharks, which are known to have very high post-catch mortality upwards of 50% (Braccini *et al.* 2012). Other management efforts may be necessary, such as no-take zones or reducing fishing effort: both management tools require further understanding of sawshark distribution and movement patterns.

Season CPUE data may suggest yearly movement patterns. Seasonal catch per unit effort was generally highest in the autumn, except in Bass Strait where CPUE increased until the summer and was lowest in autumn. Seasonal catch variations are generally accepted to be caused by shifts in behaviour (Pope and Willis 1996). In the autumn, it is possible that sawshark populations migrate to shallower waters for seasonal breeding aggregations that are more easily accessible to fishing vessels. Accurate movement studies would verify migratory

patterns. Another explanation for the increase in CPUE is yearly recruitment: seasonal length-frequency data would confirm this.

Previous sawshark distribution studies indicated that *P. cirratus* occurred from 40 to 600 meters' depth and that *P. nudipinnis* occurred past 70 meters (Last and Stevens 2009). The *Kapala* research trawling and the Commonwealth logbook data indicate that sawsharks occur as shallow as 9 depth, and beyond a depth of 500m. Mean numbers caught decreased past 300 meters, highlighting that the presence of sawsharks may decrease below that depth. Research trawling in Tasmania also indicated that the two species do co-occur in the same depth and area (personal data). The sinusoidal shape of the *Kapala* depth distribution data suggested that there may be two separate distributions: one from 0 to 150 meters and one from 150 to 500 meters, however, Commonwealth data indicated no clear separation of the two *Pristiophorus* spp.. It may be possible that both species' depth distributions overlap throughout their habitable zones, as the two co-occurring species are known to avoid competition through resource partitioning (Raoult *et al.* 2015).

Sawsharks appear to be caught sustainably, though current populations are likely between 40 and 10% of their initial levels, depending on the area. A Commonwealth assessment of sawsharks catches determined that populations were likely at 40% of their initial levels (Walker *et al.* 2005), and the discussion of this study's results must be considered in that context. South Australian catch rates were stable, indicating sustainable fishing rates. The Bass Strait had the most fishing effort due to the high use of gillnets in the area. While ocean trawling had a higher mean catch per unit effort than gillnets, gillnets were responsible for the majority of sawshark catches (gillnets have been banned in NSW waters). Earlier catch data presented at AFMA research assessment groups suggest that the catch levels in the early 2000s were historically high, and that the decline seen in this study is actually stable fluctuations. High concentrations of sawsharks are known to occur in the Bass Strait (Walker

et al. 2005), and decreased catch rates in this area must, therefore, be closely monitored. NSW coastal trawling was stable, but Commonwealth CPUE significantly declined over the study period. The opening of marine protected areas and closures of other fisheries may have had an effect on these declines. Commonwealth vessels generally operate offshore relative to NSW fisheries zones; therefore, it is possible that offshore populations have been depleted. Offshore depletions are also supported by historical data from the fisheries-independent FRV *Kapala*, which indicate that populations of sawsharks (determined from catch rates) offshore of NSW have declined by two-thirds between 1976 and 1996, as the catch per unit effort of those species declined from 3kg/h to <1kg/h (Graham *et al.* 2001). Although catch per unit effort was relatively stable since the 1990's, the *Kapala* data suggest that sawshark populations were significantly depleted between the 1970s and 1990s. These findings suggest that sawsharks populations on the East coast of Australia have declined to less than 10% of their pre-industrial levels, and should be classified as 'sustainably overfished' (current fishing effort is sustainable, but the current biomass of sawsharks is significantly lower than 'natural' levels, and a reduction in fishing effort will likely lead to a rise in both CPUE and the size of sawshark populations), and additional management measures are necessary to prevent further declines.

Due to the difficulties associated with correctly separating *P. nudipinnis* from *P. cirratus*, the two species had to be grouped in this study. These difficulties mirror those found when examining the *Squatina sp.* fishery in the same area (Chapter 2). Commercial fishermen need to be given clear identification guidelines to increase data reliability. Even if those guidelines are available, it is highly likely that the species will be misidentified due to the large numbers of individuals from both species that may be caught concurrently. Data from Walker *et al.* (2005) suggests that 11.5% of demersal trawl catches of sawshark are *P. nudipinnis* and the rest are *P. cirratus*, though extrapolating these factors to the entire range of these species

when the majority of Commonwealth catches are above 80m depth and in the Bass Strait may lead to large discrepancies. Further research should examine the overlap of the depth distributions of these sharks in a way that could allow extrapolation of species identification from fisheries data sets.

This study has provided evidence for depletions of sawshark populations on the East coast of Australia though catch rates on these populations remain relatively stable. Catches in the Bass Strait and South Australia appear to be stable, despite catching larger amounts of sawsharks than the Eastern coastline. Seasonal variations in CPUE highlight possible migrations and catch/depth data suggest that there may be a depth separation of *P. nudipinnis* and *P. cirratus*. In light of these conclusions, the authors suggest that *P. sp.* populations are classified as 'sustainably overfished' and warrant further study and continued monitoring.

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CHAPTER 4: Strontium mineralization of shark vertebrae

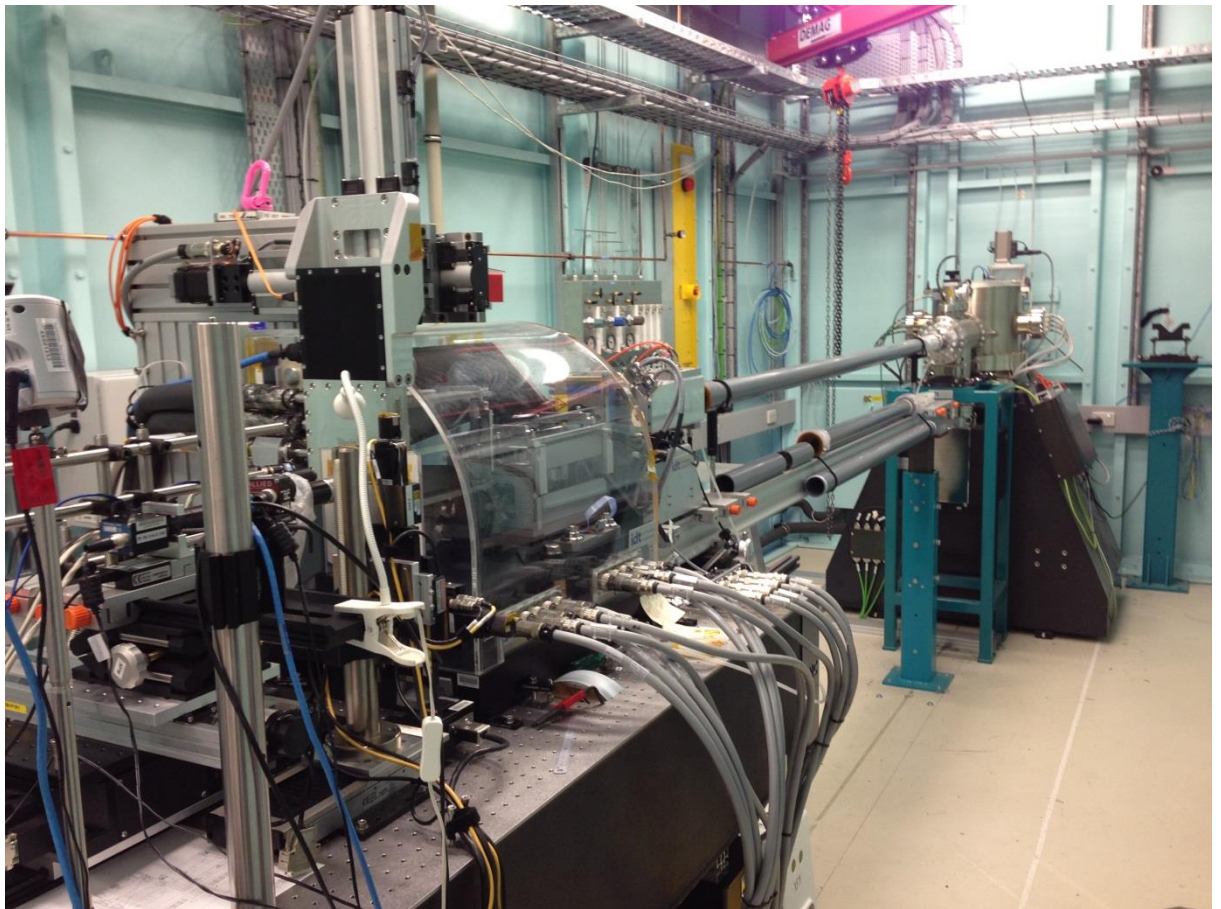
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The Scanning X-Ray Fluorescence Microscope used for these experiments at the Australian Synchrotron, Melbourne, Australia.

Abstract

Determining the age of sharks using vertebral banding is a vital component of management, but the causes of banding are not fully understood. Traditional shark ageing is based on fish otolith ageing premises and assumes that growth bands are a result of varied seasonal calcification rates. Here we investigate these assumptions by mapping elemental distribution within the growth bands of vertebrae from six species of shark representing four different taxonomic orders using scanning x-ray fluorescence microscopy. Traditional visual growth bands, determined using light microscopy, were more closely correlated to strontium than calcium in the majority of species. Elemental distributions suggest that vertebral strontium bands may be related to environmental variations in salinity. These results highlight the requirement for a better understanding of shark movements to correlate with vertebral development if confidence in age readings is to be improved. Analysis of shark vertebrae using similar strontium-focused elemental techniques, once it has been validated for a given species, may allow more successful estimations of age on individuals with little or no visible vertebral bands.

Introduction

Humanity's growing demand for protein has led to substantial pressure on both terrestrial and oceanic ecosystems. Harvesting of large predatory fishes by industrial fishing techniques has possibly lowered populations to less than 10% of their historic numbers in some areas (Dulvy *et al.* 2014; Worm and Branch 2012), though the accuracy of these estimates has been questioned (Burgess *et al.* 2005; Walters 2003). Sharks are especially vulnerable to fishing due to their low reproductive rates and delayed maturity (Baum and Myers 2004; Baum *et al.* 2003; Ward-Paige *et al.* 2010). Their disappearance can have cascading impacts on ecosystems (Myers *et al.* 2007), and considerable resources have been allocated to their

management and conservation. However, basic information about life history parameters such as reproduction, diet and age structure are often lacking for species, making assessment and management of shark stocks difficult.

Ageing sharks is problematic due to large numbers of samples required (often difficult to obtain), the need for cross-validation, the lack of calcified structures in many deep-water species, and discrepancies between growth and age estimates (Goldman *et al.* 2012).

Inaccurate estimates can lead to poor predictions of population growth, and subsequent overfishing (Campana 2001) or under-exploitation of resources. Typically, shark ageing has been based on a modification of fish otolith ageing but using vertebrae instead of otoliths (Goldman *et al.* 2012). Vertebral bands in sharks have been counted as a proxy for yearly growth patterns (Cailliet *et al.* 2006), under the assumption that banding patterns result from changes in calcification rates over time in a manner similar to that of fish (Cailliet *et al.* 1983; Jones and Geen 1977). Although the seasonality of these bands has been validated for many species (Brown and Gruber 1988; Passerotti *et al.* 2010), processes that determine the formation of these bands are not fully understood. Moreover, published methods of shark ageing vary substantially because different preparation methods must be tested to determine the most effective technique for each species that has not been aged previously (Goldman *et al.* 2012). Different ageing methods and subjective differences in readability make comparative studies problematic (Cailliet *et al.* 2006; Francis *et al.* 2007; Geraghty *et al.* 2013).

To investigate the elemental composition of vertebral bands, and by association, the factors that may influence their deposition, elemental distribution within vertebrae was assessed in six diverse species of sharks using Scanning X-ray Fluorescence Microscopy (SXFm) (Lombi *et al.* 2011). Assessments of SXFM images were compared to traditional ageing with light

microscopy (Goldman *et al.* 2012). Implications for shark ageing, development and conservation were then discussed.

Materials and methods:

Collection of sharks

Six species of shark were analysed in this study: *Sphyrna zygaena* (Smooth Hammerhead), *Charcharodon carcharias* (White Shark), *Carcharhinus brevipinna* (Spinner Shark), *Heterodontus portusjacksoni* (Port Jackson Shark), *Carcharhinus obscurus* (Dusky Shark), and *Squatina albipunctata* (Eastern Angel shark; this genus is known to have no visible growth bands in the *corpus calcarum* (Baremore *et al.* 2009; Natanson and Cailliet 1990)). Sharks were caught by the New South Wales Shark Meshing (Bather Protection) Program, with the exception of *S. albipunctata*, which was caught by a commercial fishing trawler near Sydney, Australia. NSW shark nets are set during the summer period along the Newcastle to Wollongong region in New South Wales, Australia (Reid *et al.* 2011). The Animal Ethics Committee at Macquarie University agreed that ethics approval was not required for this study. Tissue samples were retrieved from animals caught by commercial fisheries for sale in local markets and/or the NSW bather protection programs, which occasionally catch and kill local wildlife and thus, sharks were obtained for purposes other than research.

Laboratory preparation of vertebral sections

Vertical slices of vertebrae were prepared by removing the first cervical vertebra from each carcass (this is common practice and produces better results than other vertebrae, see Simpfendorfer *et al.* 2000) and freezing it (-20°C) in a sealed plastic bag until preparation. During preparation, vertebrae were thawed to room temperature, and then manually cleaned of debris with a sterile scalpel before being sectioned. To avoid possible contamination no chemical agents (e.g., sodium hypochlorite, ethanol) were used during the cleaning process,

and the decision was made to avoid using other cleaning techniques (i.e., sonication) because SXFM is less prone to surface contamination than other elemental techniques. Vertebrae were sectioned using an Isomet circular saw with a single 0.1 mm-increment adjustable diamond-edged blade. Sections were cut dorso-ventrically through the centre of each vertebra. Cuts were 0.6mm in thickness or thinner, dependent on the degree calcification (more calcified specimens could be cut thinner). Each cut was immediately placed on Kapton film and covered with Kapton adhesive tape. This created an airtight seal to prevent dehydration of the samples that can cause severe tissue warping. Samples were then flattened between two microscope slides and transported to the Australian Synchrotron in Melbourne.

X-ray fluorescence imaging of vertebral sections

Prepared sections of vertebrae were attached to polycarbonate frames at the Australian Synchrotron's X-ray Fluorescence Microprobe (Paterson *et al.* 2011) using clear double-sided tape, with roughly 15-20 samples per frame. Smaller vertebral sections were scanned at 15 μm intervals (e.g., Eastern angel sharks) while larger sections were scanned at 25 μm intervals (e.g., *Charcarchinidae*). Imaging intervals were chosen to balance scanning time and measurement sensitivity with the expected length scale of elemental variations. Per-pixel dwell times were typically 10 ms, and the scanning time for each frame was between 16 and 22 h, depending on the dwell, the vertebral section, and scanned area. Elemental maps were created and viewed using GeoPIXE™ (Ryan 2000), which uses a detailed specimen model to determine first-order depth-independent elemental concentrations. A series of x-ray fluorescence concentration standards (Micromatter) were measured at regular intervals through the experiment to control for experimental drift if present; none was observed, and so all data were processed using a single fit model.

The quantitative accuracy of elemental mapping relies on the uniform illumination of a narrow column through a specimen, and the measurement of characteristic x-ray fluorescence emitted from that column. The high incident x-ray energy (18.5 keV) ensured uniform illumination; however, the lower energy of the characteristic x-ray fluorescence can result in significant absorption of the x-ray fluorescence for various elements (the fluorescent light is reabsorbed by the element before it can be detected by the detector). The absorption length of calcium fluorescence (~3.7 keV) in water (a crude model for wet cartilage) is around 110 μm , whereas that of strontium (~14.1 keV) is 500 μm , leading to a strong effect of the depth sensitivity on elemental distribution. The matrix correction applied by GeoPIXE corrects for this difference in escape depth, assuming that the elemental distribution is uniform through the thickness of the vertebrae (i.e. parallel to the growth bands). Here we have recorded elemental maps with the incident x-rays closely aligned along the elemental bands, so that this assumption is valid. Any sample misalignment would result in a blurring of the strontium distribution relative to calcium due to the increased penetration of fluorescent light from the strontium spectrum, and such is not observed in our elemental images.

To determine the age of sharks, elemental lineouts were taken from data from the *corpus calcarum*, from the centre to the outer edge. Plots of moving average of six consecutive values were constructed to distinguish a trend from the noise caused by the high sensitivity of the SXFM. Elemental band counts were assigned for calcium and strontium separately using a moving framework on the 6 point moving average, and defined as a peak variation of 2.5% from the continuous averages after the birthmark (defined as the change in angle on the face of the centrum of the vertebra (Goldman *et al.* 2012))

Light microscopy imaging

Light microscopy ageing was done on 0.6mm sections of the vertebrae directly adjacent to the vertebra used for the SXFM analysis. Vertebrae were manually cleaned with a scalpel and sectioned using an Isomet diamond saw. Excess tissue remaining after the sectioning was also removed with a scalpel. No chemicals were used when preparing these vertebrae to enable effective comparison with the SXFM samples. Vertebrae were observed in saltwater under a high-contrast binocular microscope immediately after sectioning. Images were taken via USB camera and saved with ImageJ software. Images of the samples were then independently aged by two experienced readers. A growth band consisted of one opaque and one translucent band (Goldman, Cailliet *et al.* 2012). Only vertebrae where the growth bands were well defined and visible were scored. Regression analyses were then performed to determine whether calcium peak counts were more correlated with visual counts than strontium peak counts.

Data analysis

To compare how well strontium and calcium peak counts matched the numbers of visual growth bands, data were analyzed using a multiple linear regression analysis with an alpha of 5%. All data analyses were done using Microsoft Excel and the Real Statistics data analysis tool pack.

Results

Calcium and strontium maps showed clear banding within vertebrae of all species, however, strontium bands were consistently more visible and detectable than calcium bands (Figure 1, 2). Strontium concentrations determined from the SXFM were often correlated with bands detected by light microscopy ($df = 4$, $F = 6.7$, $R^2 = 0.69$, $p = 0.08$), while calcium concentrations did not ($df = 4$, $F = 0.43$, $R^2 = 0.12$, $p = 0.55$; Figures 3-8). Strontium peak counts agreed with or were slightly under the counts of light microscopy bands, while calcium

peak counts were both under and over microscope band counts. In all cases, counts of strontium peaks corresponded more closely with banding counts determined by light microscopy than counts of the calcium peaks did, except in *S. albipunctata*, which has no visible growth bands through light microscopy, but detectable peaks of strontium (Figure 9).

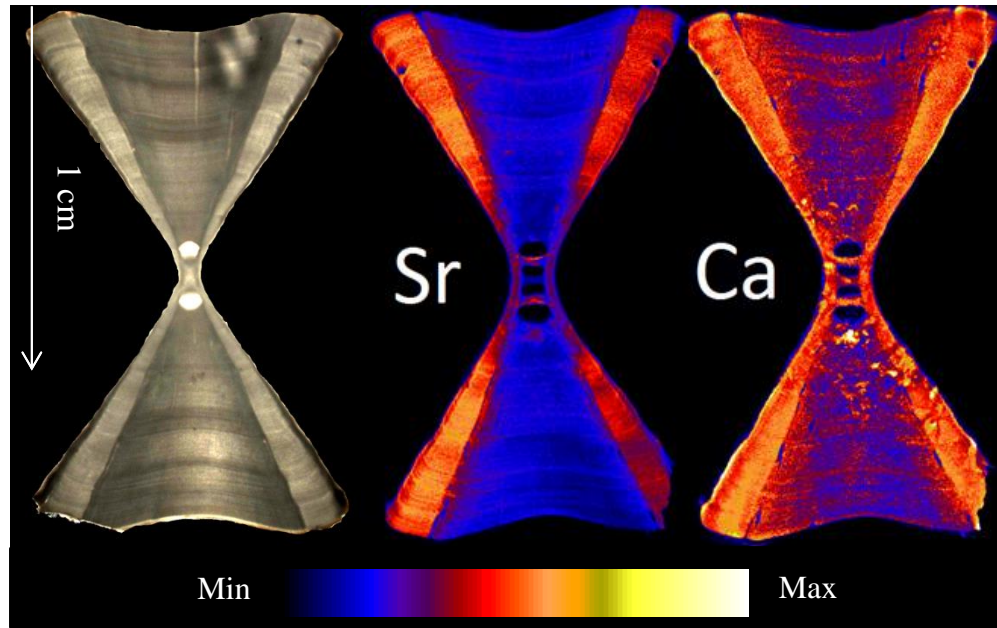


Figure 1. Images of light microscopy (LHS) and SXFM (centre, RHS) at different elemental spectra (in ppm) of unstained sagittal sections of a 1.7m female Smooth Hammerhead (*Sphyrna zygaena*) at 25 μ resolution. Banding studies usually focus on the *corpus calcarum*, the denser area on the outside of the vertebrae.

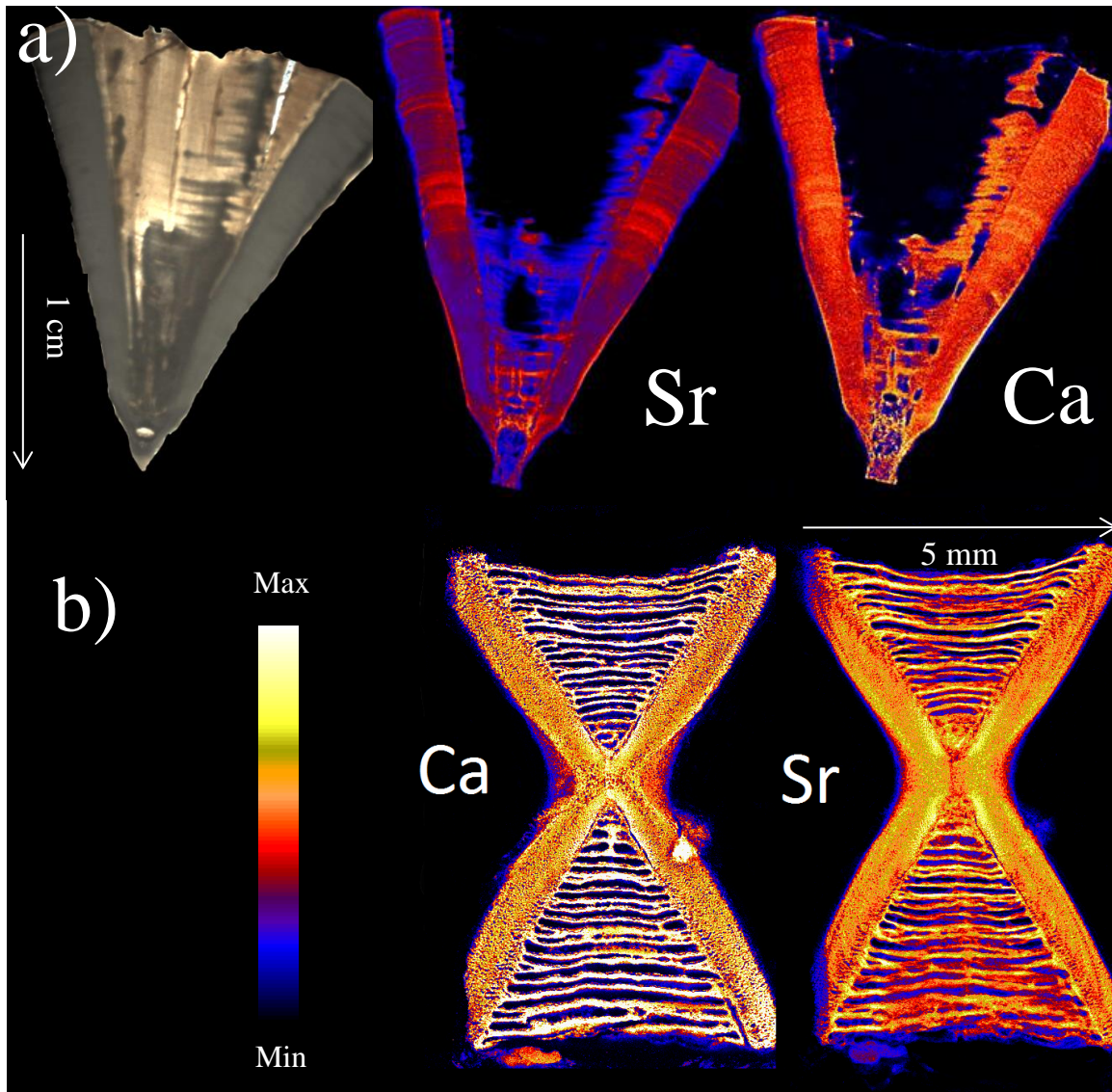


Figure 2. Images of light microscopy (top LHS) and SXFM (all others) at different elemental spectra (in ppm) of sectioned vertebrae of a) a 2.7m Great White (*Charcharodon carcharias*) vertebra and b) an 0.9m Eastern Angel Shark (*Squatina albipunctata*) at 25 and 15 micron resolution, respectively. Light microscopy of angel shark not shown due to lack of visible growth bands.

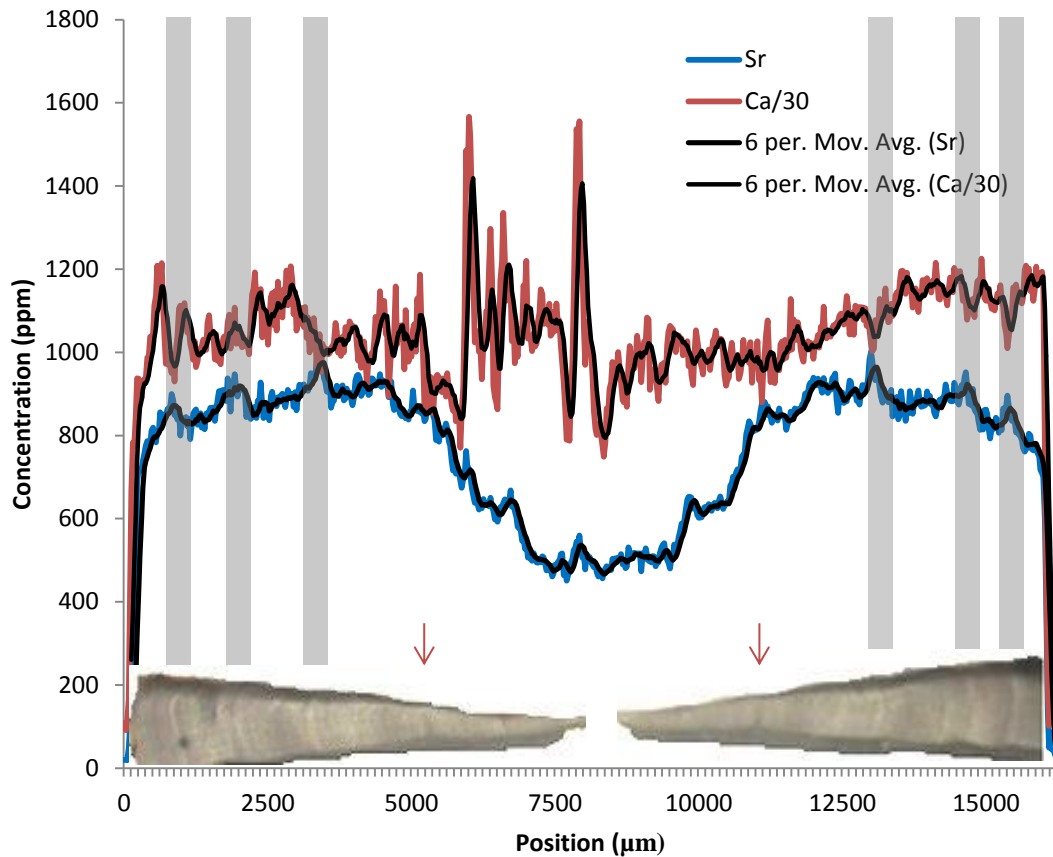


Figure 3. SXFM calcium and strontium concentrations of the *corpus calcarum* from the dorsal to ventral edge of the 1.7m Smooth Hammerhead (*Sphyrna zygaena*) shown in Fig 1. Calcium concentrations were reduced by a ratio of 30 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Birth marks are at roughly 5000 and 12000 μm (indicated with red arrows). Data are compared to a flattened horizontal microscope image of the *corpus calcarum* of the vertebra with marked 'traditional' age bands (grey bands for comparison with Sr and Ca). Note how strontium peaks are consistent on both sides of the vertebra, while calcium concentrations are not.

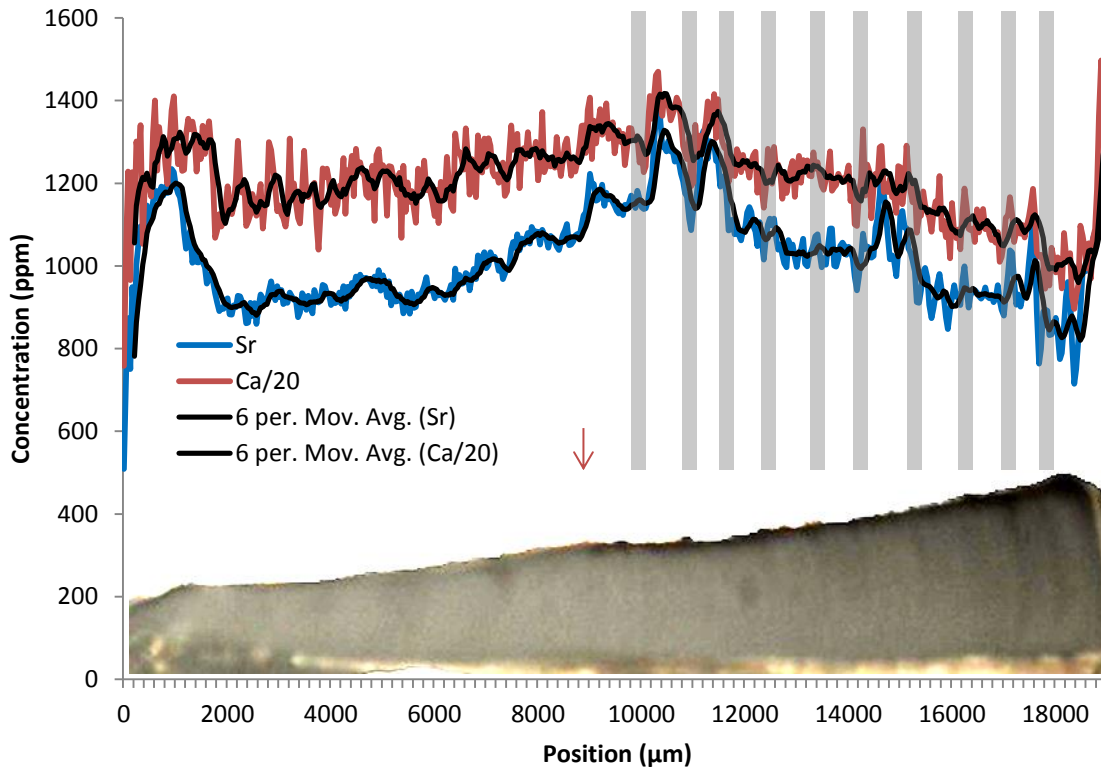


Figure 4. SXFM calcium and strontium concentrations of the *corpus calcarum* from the dorsal to central edge of a 2.7m female White Shark (*Charcharodon carcharias*). Calcium concentrations were reduced by a ratio of 20 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Birth mark is at 9000 μm (indicated with red arrow). Data were compared to a flattened horizontal microscope image of the corpus calcarum, with marked 'traditional' age bands (grey bands for comparison with Sr and Ca).

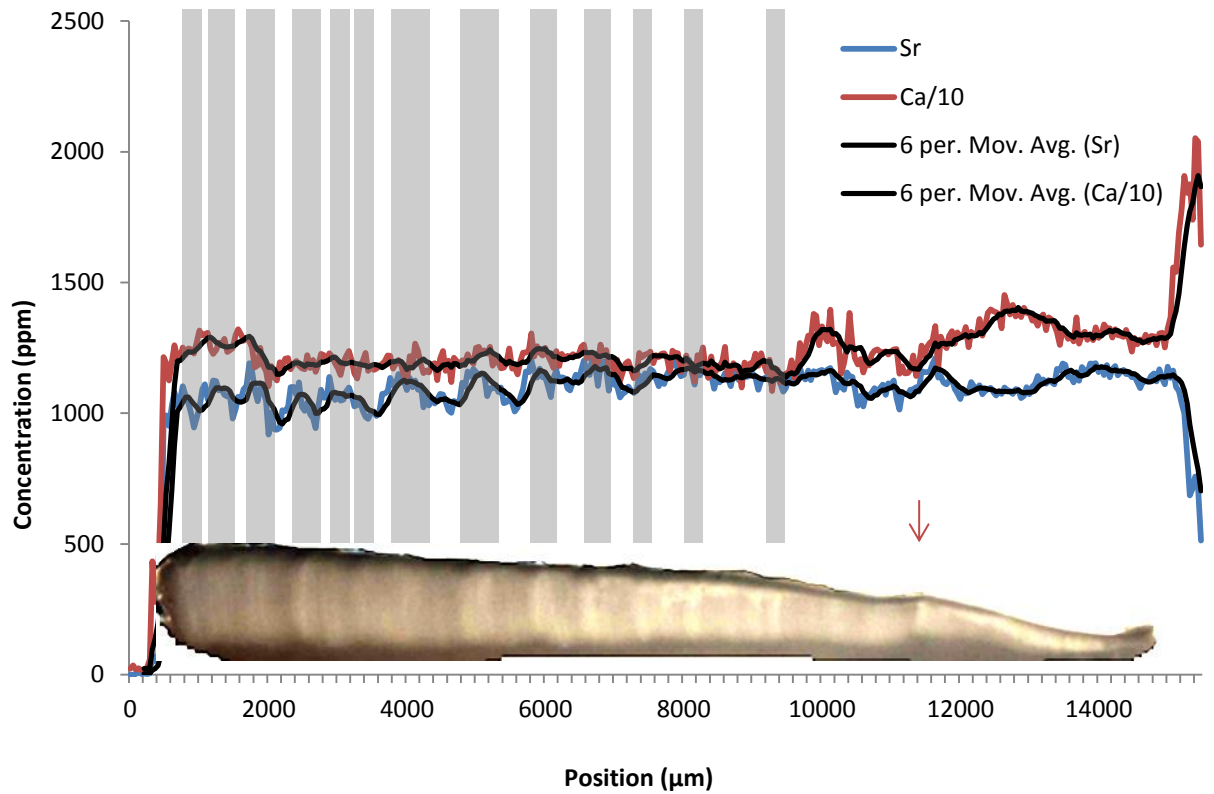


Figure 5. SXFM calcium and strontium concentrations of the *corpus calcarum* from the dorsal to ventral edge of a 2.4m female Spinner Shark (*Carcharhinus brevipinna*). Calcium concentrations were reduced by a ratio of 10 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Birth mark is roughly at 11500 μm (indicated with red arrow). Data are compared to a flattened microscope image of the corpus calcarum of the vertebra with marked 'traditional' age bands (grey bands for comparison with Sr and Ca).

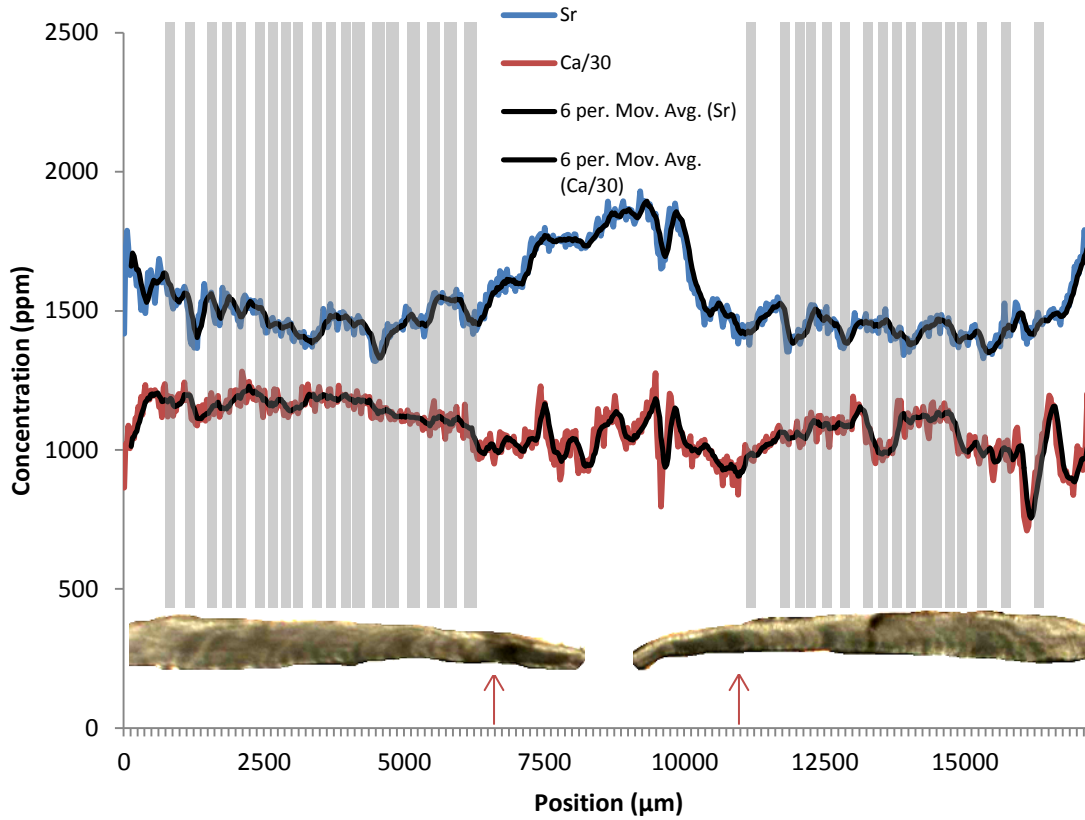


Figure 6. SXFM calcium and strontium concentrations of the *corpus calcarum* from the ventral to dorsal edge of a 1.15m female Port Jackson Shark (*Heterodontus portusjacksoni*). Calcium concentrations were reduced by a ratio of 30 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Birth marks are roughly at 7000 and 11000 μm (indicated with red arrow). Data are compared to a flattened microscope image of the corpus calcarum of the vertebra with marked 'traditional' age bands (grey bands for comparison with Sr and Ca).

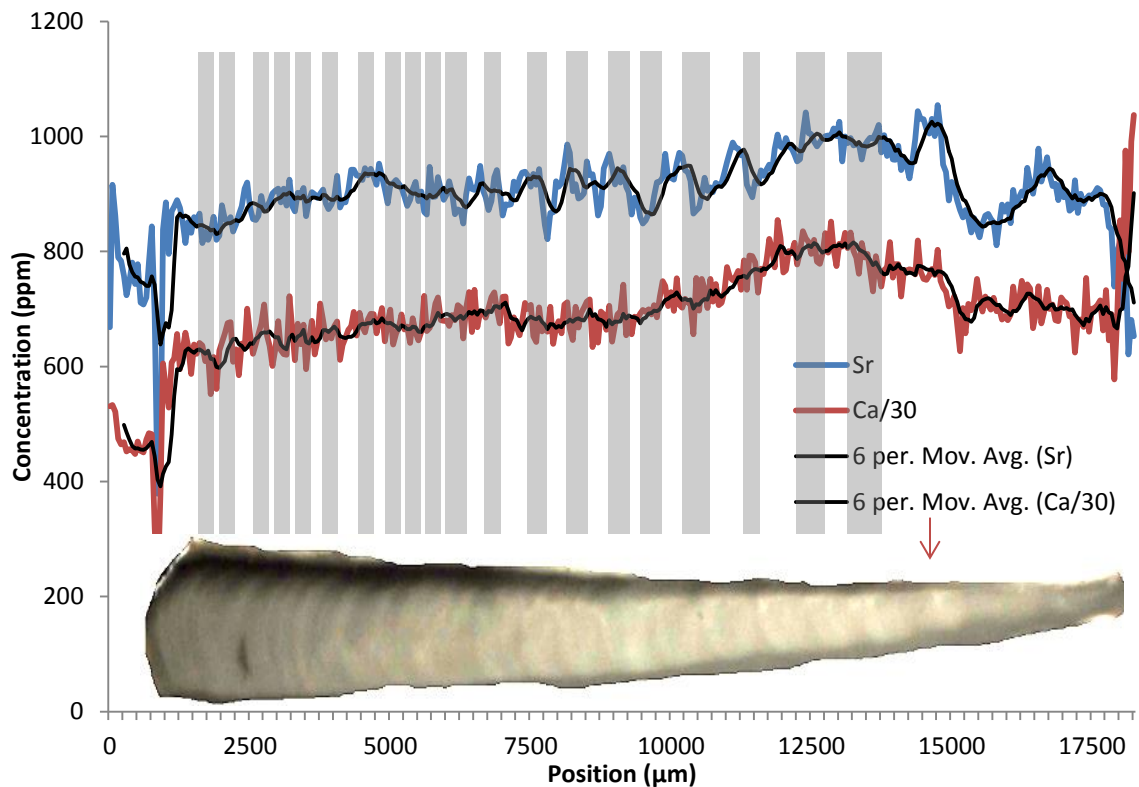


Figure 7. SXFM calcium and strontium concentrations of the *corpus calcarum* from the dorsal to ventral edge of a 2.8m female Dusky Shark (*Carcharhinus obscurus*). Calcium concentrations were reduced by a ratio of 20 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Birth mark is roughly at 14500 μm (indicated with red arrow). Data are compared to a flattened microscope image of the corpus calcarum of the vertebra with marked 'traditional' age bands (grey bands for comparison with Sr and Ca).

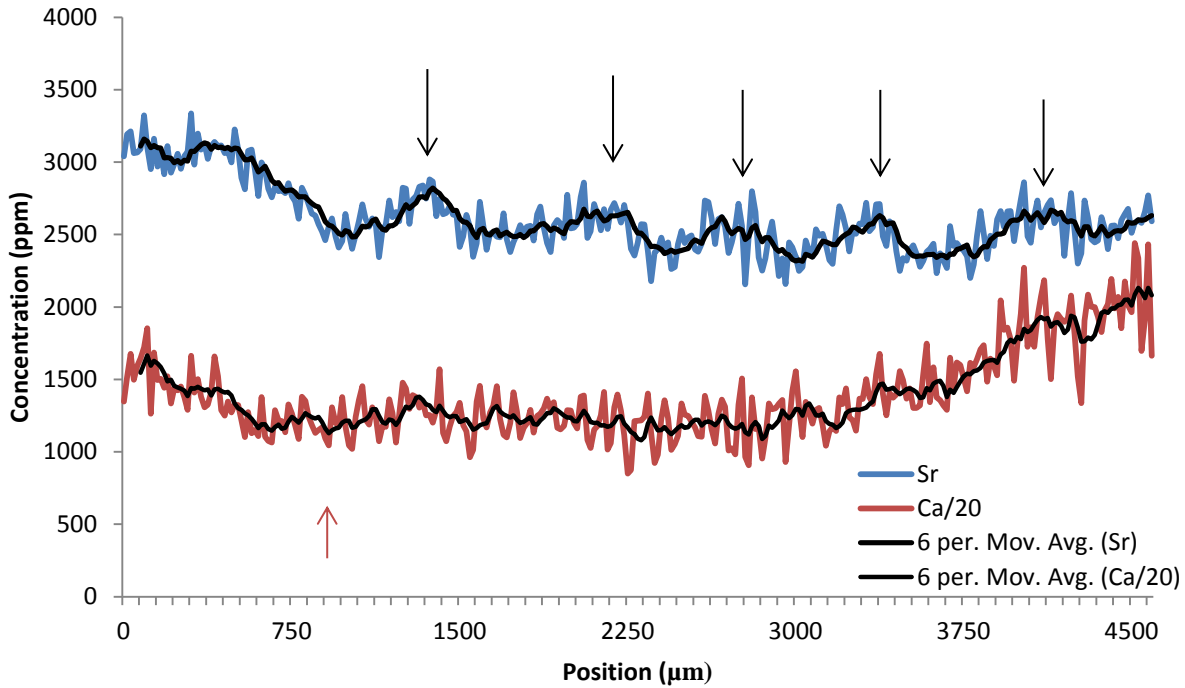


Figure 8. SXFM calcium and strontium concentrations of the *corpus calcarum* from the dorsal to ventral edge of an 839mm male Eastern Angel shark (*Squatina albipunctata*). Calcium concentrations were reduced by a ratio of 30 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Strontium peaks are marked with black arrows, birth mark is roughly at 1000 μm (indicated with red arrow). Image of light microscopy not shown as no bands were detectable in this study or in others (Baremore, Andrews *et al.* 2009; Natanson and Cailliet 1990).

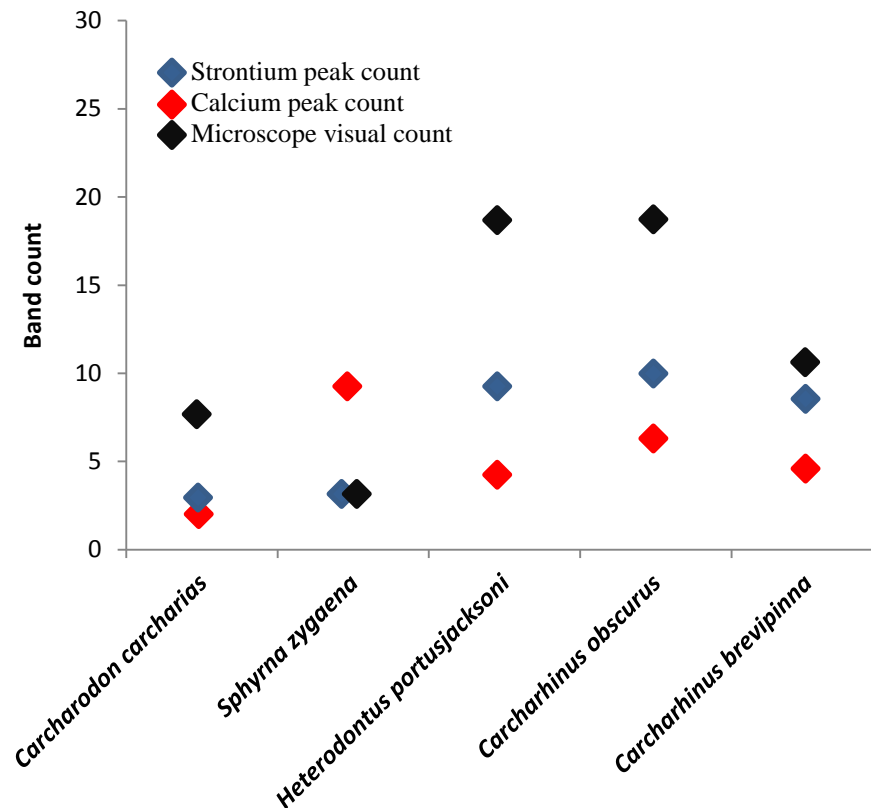


Figure 9. Counts of strontium and calcium peaks, compared to traditional microscope ageing length for (left to right): White Shark, Smooth Hammerhead, Port Jackson Shark, Dusky Shark and Spinner Shark.

Discussion

Results from this study indicate that shark vertebral growth banding may be more closely related to vertebral strontium deposition rather than calcium deposition as previously assumed in some species. Strontium and calcium peaks did not often correlate, and strontium peak numbers were more closely related to microscope growth band count than calcium peaks. Strontium peaks on the dorsal and ventral face of the vertebrae had symmetrical shapes and positioning (peaks were equidistant on dorsal and ventral sides), whereas calcium peaks were asymmetrical. Growth deposition is physiological, therefore symmetrical patterns are expected.

Strontium maps

Shark ageing can be greatly affected by observer bias (Goldman *et al.* 2012), and strontium maps can decrease measurement subjectivity in traditional ageing methods. In conjunction with traditional ageing methods, strontium mapping may increase the accuracy of measurements and/or allow ageing where it was previously unsuccessful (e.g. *Squatina* sp.), but only once species-specific seasonal movement patterns are understood and/or the technique has been validated with individuals of known age. Previous research on round stingrays (*Urobatis halleri*) also found that vertebral strontium concentrations correlated with growth bands (Hale *et al.* 2006), and the species in question also has seasonal movement patterns. The process of strontium deposition needs to be further investigated to determine whether it is more reliable for band analysis than methods that analyse calcium concentrations, and how/why strontium is deposited, particularly for benthic deep-water species. If strontium is a better banding indicator than calcium, it would explain why chemical stains historically used in shark ageing (alizarin red, silver nitrate) have varying results, since they are also indicators for calcium, strontium, magnesium and iron. Although Mn, Fe and Ba are readily mapped using SXFM, the measurement parameters used here do not provide sufficient sensitivity, as these elements are present only at extremely low concentrations in these specimens. The use of directed strontium reporters (chemicals that only react to strontium and not similar elements like calcium) may, therefore, produce better results. The effectiveness of strontium maps as an ageing tool could be validated as a technique by using age-validated samples for each species (either from tagging programs, bomb-radiocarbon dating specimens, or marking captive sharks with oxytetracycline), but may only be effective if species migrate in a known periodicity.

SXFM

SXFM allowed novel, high resolution, rapid analysis of the elemental composition of shark vertebrae. Nevertheless, future use of SXFM for analysis of shark vertebrae could be improved. A moving average (moving framework of six elemental results), used to reduce noise, reduced the effective spatial resolution to 6 times the sampling interval, or between 90 μm and 150 μm . The lowered resolution would explain why the oldest shark in this study did not display strontium peaks that corresponded to visual bands after nine years of age (Figure 7). Scanning with a finer sampling interval, longer dwell (time spent exposing the area) and a wider sampling band are all feasible using the SXFM, but those measurement parameters run counter to the broad survey of multiple species that we have preferred here. Using growth band counts to age older individuals is known to lead to age under-estimation due to the greater proximity of growth bands (Andrews *et al.* 2011; Hale *et al.* 2006), and so higher resolution scans – of order 1 μm – would be of immense interest. While variations in specimen thickness and surface topography can confuse the clear identification of banding, the measurements reported here are relatively free of such artefacts. We are developing further methods to reduce the impact of such measurement factors to facilitate future use of this approach.

Origins of strontium variation

Strontium is absorbed during otolith mineralization in bony fish, often replacing calcium (Schoenberg 1963), and otolith absorption rates are dependent on environmental concentrations (Farrell and Campana 1996). Strontium is preferentially absorbed relative to other trace elements present in the marine environment (Mg, Ba) (Campana 1999), whereas calcium is absorbed at a relatively constant rate regardless of environmental availability (Farrell and Campana 1996). Though the processes that govern the absorption of strontium in

fish are still debated, the hypothesis that best explains the various results on the subject is that preferential strontium deposition is linked to the rate of proteinacious matrix formation (Campana 1999). Incorporation of strontium into fish otoliths is not immediate: changes in ambient water strontium concentrations only have effects on mineralized concentrations after ten days, and sometimes up to sixty days after exposure (Yokouchi *et al.* 2011). Despite the apparent role of strontium in fish development, the physiological role of this element is still unknown (Hartl 2013). In elasmobranchs, the growth of vertebral cartilage may be governed by the periodic deposition of minerals adjacent to the perichondrium (Clement 1992).

Strontium modulates bone metabolism in mammals: higher strontium concentrations increase bone mass (Delannoy *et al.* 2002) or prevent bone loss (Marie *et al.* 2005). In humans the medical use of high-strontium-content derivatives significantly decreases vertebral and non-vertebral fracture risk (Roux *et al.* 2008; Seeman *et al.* 2010), offering promise as an anti-osteoporitic and anti-osteoarthritic. Recent studies have tried to determine how strontium affects mammalian bone growth, finding that nearly 50% of available strontium is incorporated into calcium hydroxyapatite or absorbed into collagen (Frankær *et al.* 2014). Strontium effects on mammalian cartilage are less well studied, but evidence suggests that increased strontium levels promote cartilage matrix formation (Henrotin *et al.* 2001). While mammalian and chondrichthyan bone/cartilage development are unlikely to be completely analogous, the mineral fraction of elasmobranch vertebrae is similar in composition to that of mammals (Porter *et al.* 2006). It is, therefore, possible that strontium also affects the resorption/formation of elasmobranch cartilage and vertebral bands in a similar fashion as in mammalian bones.

In this study, strontium peaks sometimes corresponded with thinner, opaque regions known as winter bands (Davenport and Stevens 1988). These areas precede areas of higher growth that have lower strontium concentrations. It is possible that the accumulation of strontium allows

increased bone development in subsequent months, resulting in thinner, high strontium ‘winter’ bands and thicker, low strontium ‘summer’ bands. The opaqueness of vertebral bands may also be due to a denser cartilage matrix, similar to those detected in mammals (Henrotin *et al.* 2001).

Previous research assumed that shark growth bands were caused by environmental variables such as temperature and salinity, resulting from changes in environmental conditions and/or metabolic rates (Branstetter and Stiles 1987). Spectrometry studies have shown that temperature has no effect on strontium concentrations in elasmobranch vertebrae though increased temperatures increase vertebral growth rates (Smith *et al.* 2013). Otolith strontium/calcium ratios in teleost fish are positively linked to environmental strontium concentrations (Kennedy *et al.* 1997), directly related to salinity in some species (Kawakami *et al.* 1998; Kraus and Secor 2004), but often unrelated to temperature (Clarke and Friedland 2004; Kawakami *et al.* 1998). Salinity varies seasonally (Delcroix and Hénin 1991) and increases with depth (Mantyla and Reid 1983), therefore vertebral growth bands may be partially caused by seasonal or depth salinity gradients rather than only changes in metabolic rates that have resulted from seasonal variations in temperature. This also raises the possibility of retroactively determining the salinity the shark was present in, just as it is possible to determine estuarine movements using teleost otoliths (Bath *et al.* 2000; Elsdon *et al.* 2008; Walther and Limburg 2012). Similar conclusions were obtained from analyses of mass spectrometry on shark vertebral composition, where lower strontium/barium ratios in *Carcharhinus leucas* were suggested to be related to movements into estuarine waters (Tillett *et al.* 2011). Smalltooth sawfish (*Pristis pectinata*) also present strontium variations in relation to changes in salinity (Scharer *et al.* 2012). In this study, *Squatina albipunctata* are thought to come inshore every year to pup or reproduce, which may explain the changes in strontium concentrations that we have observed. It should also be noted that while

correlations between strontium and visual growth bands for *Heterodontus portjacksoni* appear to be low at younger ages, they more closely match strontium peaks in the later years. While little is known about Port Jackson Shark (*H. portjacksoni*) movement patterns, they do appear to congregate for reproduction in estuaries in a yearly pattern once they are sexually mature (Powter and Gladstone 2008). It is possible that this post-maturity yearly migratory pattern may cause the increased correlation between visual bands in later years. *Carcharodon carcharias* movement patterns in south-east Australia are not well understood, but tagging of young (1.8-3.6 metres in total length) suggests that they undertake sporadic, large-scale movements (Bruce *et al.* 2006), which could agree with our strontium results. Future studies should conduct controlled experiments to determine the potential drivers of vertebral banding and their links with environmental variables, with a particular focus on the interactions between temperature, salinity, and strontium availability.

Movement patterns of elasmobranchs can change with ontogeny (Andrews *et al.* 2010), can be sex-linked (Domeier and Nasby-Lucas 2013), and may show a high degree of intraspecific variation (Bruce *et al.* 2006). Reliable ageing of species using strontium mapping, therefore, requires highly predictable migratory patterns that occur on the population level, at consistent intervals, and with known ontogenetic variations. *C. carcharias* on the east coast of Australia are known to migrate both north and south, with individualistic preferences (Bruce *et al.* 2006), while female *C. carcharias* found in the northeast Pacific have two-year migrations between aggregation areas, and male sharks found in the same area return to the same spot every year (Domeier and Nasby-Lucas 2007; Domeier and Nasby-Lucas 2013).

Consequently, strontium maps would not be useful for ageing *C. carcharias* found on the coasts of Australia. This logic may explain the disparity between visual band counts and strontium peak counts for the *C. carcharias* sample in this study. Conversely, due to their highly reliable movement patterns, strontium maps could be used to age *C. carcharias* from

the northeast Pacific if the observer was aware of the sex of the shark. Researchers with elasmobranch vertebrae sourced from populations with known population-level seasonal migrations that they are unable to age using traditional techniques could consider strontium mapping. If population-specific information was unavailable, such as it would be with a newly-discovered species, we recommend that strontium maps can only be used to infer the presence or absence of movement patterns across salinity gradients rather than to determine the age.

Our research shows that shark vertebral growth bands can be linked to changes in strontium levels rather than calcium concentrations in species that undergo seasonal migrations. The relationship between strontium and cartilage growth in sharks, along with links to strontium in the surrounding environment, should stimulate new research and debate on constitutive factors underlying seasonal deposition of cartilage in elasmobranchs. Mineralization in teleost otoliths (used in fish ageing) is currently a burgeoning research field (Elsdon *et al.* 2008), yet is largely unstudied in elasmobranchs. The presence of strontium bands in shark vertebrae also lays the foundation for new and alternative ageing methods that translate easily between species once appropriate validation studies have been conducted, thus making research directly comparable. More precise and better-understood shark ageing will lead to more accuracy in determining life history strategies and population dynamics and thereby more effective management of the shark populations and associated ecosystems that have been exploited by humans.

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Author Contributions

The author(s) have made the following declarations about their contributions: conceived and designed the experiments: VR VP DZ JW NH MdJ. Performed the experiments: VR VP DZ NH MdJ. Analysed the data: VR DZ NH DH MdJ. Contributed reagents/materials/analysis tools: DH MdJ. Wrote the paper: VR JW VP MdJ. First author VR.

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CHAPTER 5: Biology of angel sharks (*Squatina sp.*) and sawsharks (*Pristiophorus sp.*) caught in south-eastern Australian trawl fisheries

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Angel shark (*S. albigutta*) dissection conducted in the Cronulla Fisheries Research Centre of Excellence

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Abstract

Two species of angel shark (*Squatina australis*, *S. albipunctata*) and two species of sawshark (*Pristiophorus nudipinnis*, *P. cirratus*) are frequently caught in south-eastern Australia. Little is known about the biology of these elasmobranchs, despite being caught as secondary target species in large numbers. This study collected morphometric and reproductive data from sharks caught in shark control nets, commercial fishing trawlers, and research trawlers in south-eastern Australia. All four species had female-biased sexual size dimorphism, but growth curves between sexes did not differ. Male *S. australis* were fully mature at ~800mm total length, male *P. nudipinnis* at ~900mm, and male *P. cirratus* at ~800mm. Anterior pectoral margins could be used to determine the total length in all species. No morphometric measurement could reliably separate *Squatina* spp. or *Pristiophorus* spp., though *S. albipunctata* over 1000mm TL had larger eyes than *S. australis*.

Introduction

Charismatic species have traditionally received more research attention and funding than less attractive species (Clark and May 2002). While such an imbalance can have positive effects such as protecting large habitats (Carroll *et al.* 2001) or increasing awareness (Sergio *et al.* 2006), it still leaves the majority of species with unknown population statuses and biology. Focused research raises the possibility of species disappearing prior to investigation, and the problem is even more apparent for small, deep-ocean chondrichthyan species that may be subject to intensive fishing effort (Francis and Lyon 2012). Two chondrichthyan groups that belong to this category are angel sharks (*Squatina* spp.) and sawsharks (*Pristiophorus* spp.). Angel sharks are flat, ray-like ambush predators, found in the Indian, Pacific, and Atlantic oceans (Baremore 2010; Cailliet *et al.* 1992; Last and White 2008). Two species of angel sharks are endemic to south-eastern Australia, and found from Port Macquarie to Hobart:

Squatina australis (the Australian angel shark) and *S. albigunctata* (the eastern angel shark) (Last and Stevens 2009). Both species are caught commercially, yet information on their biology is lacking. Of the Australian angel sharks, only the ornate angel shark (*S. tergocellata*), which occurs in the Great Australian Bight, has been studied (Bridge *et al.* 1998). Species of angel sharks occurring in the eastern Pacific and Atlantic oceans have had a greater degree of scrutiny from the scientific community (Baremore *et al.* 2010; Capapé *et al.* 2005; Natanson and Cailliet 1986; Vaz and De Carvalho 2013), and some of these species have been classified as ‘endangered’ or ‘critically endangered’ (Hărășan and Petrescu-Mag 2008). While recent analyses of commercial and shark meshing program catches indicate that *S. australis* and *S. albigunctata* are fully fished and unlikely to fit the criteria of endangered (DPI 2015), their populations are depleted by ~60% from initial stocks. The *Squatina* genus is historically one of the most endangered group of sharks in the world (Polidoro *et al.* 2009), therefore the further study of the Australian species is warranted.

Sawsharks are small, elongate elasmobranchs characterised by their saw-like rostrum. Despite their global distribution and frequent occurrence as bycatch in many fisheries, their biology also remains largely unknown. For example, three of the five species of sawshark currently registered on the IUCN Red List have unknown population trends (IUCN 2015). Two species of sawsharks are endemic to south-eastern Australia: *Pristiophorus cirratus* (common or longnose sawshark) and *P. nudipinnis* (southern sawshark). Dietary analysis has shown that these two species have relatively high trophic positions and separate diets (Raoult *et al.* 2015). An examination of fisheries records in NSW shows that sawshark populations have declined in the last five decades (Raoult *et al.* unpublished), while an Australian Fisheries Management Authority report suggests that standardised catch rates are in a slight decline in the southern and eastern scale fish and shark fishery (Haddon 2012). Effective management guidelines are difficult to formulate without information on the basic biology of these species.

South-eastern Australian demersal trawl fisheries frequently catch angel sharks and sawsharks as bycatch, which is subsequently sold as a low-value fish product, with a total market value of ~ AUD \$700,000 per annum (DPI 2015). Due to the low value of these fish, their carcasses have the heads and guts removed at sea to increase the available space in cargo holds for higher-value products; a process called truncation. Truncation is common for most sharks caught in Australian fisheries. Subsequent analyses of sharks are difficult because proxy measurements for total lengths are not known. Misidentification within these two groups of species in commercial data is commonplace due to their similarity and co-occurrence, leading to management difficulties (DPI 2015). Identifying morphometric features that can easily separate the concurrent species would increase the accuracy of commercial data sets as currently only rostrum length can be reliably used to separate *P. cirratus* from *P. nudipinnis* (Last and Stevens 2009).

This study documents the biological characteristics of four species of angel sharks and sawsharks occurring in south-eastern Australia: *S. australis*, *S. albipunctata*, *P. cirratus*, and *P. nudipinnis*. Length and weight relationships were assessed. Maturity and reproductive stages were compared to total length (TL) to assess reproductive status in each sex. Data were used to determine morphometric features that could separate species and to reliably estimate total length from truncated specimens. Results from this study provide morphometric guidelines that allow easier separation of concurrent species, predictions of TL from truncated specimens, and life history parameters that could be used in future studies.

Materials and methods

Four shark species (*S. australis*, *S. albipunctata*, *P. cirratus*, *P. nudipinnis*) caught as a secondary target species from a number of fisheries, from December 2010 to October 2012,

were assessed in this study. Some *Squatina* spp. were caught by the New South Wales Shark Meshing (Bather Protection) Program (SMP), which has nets in place during the summer (September – April) on beaches from Newcastle to Wollongong in New South Wales (NSW), Australia. The majority of *Squatina* spp. were caught by the *Maybelle*, a commercial fishing trawler based in Sydney, NSW, Australia. The *Maybelle* fishes on sandy flats at a depth of 80m, and within 20 nautical miles from the coastline. *Pristiophorus* spp. and some *Squatina* spp. were caught by the Australian Maritime College's scientific research trawler *Bluefin*. Trawls were conducted on the eastern coast of Tasmania, Australia (40°18.101 S, 148°33.596 E), with a 35mm prawn net at a depth of ~30m. Only whole specimens were used in this study: direct contacts with commercial fishermen were made to ensure they did not truncate specimens.

Species were identified using guidelines from Last and Stevens (2009). Morphometric measurements were based on measurements proposed by Last and White (2008) and using terminology from Francis (2006). A total of 50 measurements were taken for each sawshark and 38 for each angel shark: these were primarily fin and total length measurements but included a number of anterior morphometric measurements (eye length, spiracle length, mouth width, etc.). Data were grouped by species, with the aim of estimating total lengths from other morphometric parameters.

Reproductive maturity for males was determined from the growth and calcification of claspers. The growth of elasmobranch claspers is related to the onset of maturity in sharks, and maturity is attained when clasper growth slows and claspers are fully calcified and rigid (Capapé *et al.* 2005; Capapé *et al.* 2002; Natanson and Cailliet 1986). Immature males had small, soft claspers with little or no calcification. The size range of females and the number of mature females were too low to estimate reproductive trends in females for all species (*S. australis*, *S. albigunctata*, *P. cirratus*, *P. nudipinnis*). In the females that were caught,

however, maturity was assessed through maximum oocyte diameter, and the width of the uteri (Frisk *et al.* 2001).

Data were analysed to determine the most accurate measurement that would represent total length from truncated samples. A regression analysis was conducted between each morphometric measurement (e.g. the 50 measurements for each species of sawshark) and total length, with the expectation that the highest r^2 relationship would reveal the type of measurement that is the most useful for predicting total length. Length-to-weight relationships for each species were calculated using the $W=a*TL^b$ equation routinely used in other studies ('W' being weight, 'a' a constant, 'TL' the total length, and 'b' another constant) (Kohler *et al.* 1996). Morphometric measurements were also compared between species within each group (*Squatina* spp. or *Pristiophorus* spp.) to assess whether a particular measurement on a truncated sample could be accurately used for species identification. Clasper inner length was plotted against total length to determine when clasper growth started, and when clasper length was indicative of mature individuals. All analyses were conducted using Microsoft Excel and the Real Statistics add-in.

Results

Twenty-five *S. albigunctata* were obtained from the New South Wales SMP and from a commercial fishing trawler (*Maybelle*) operating out of Sydney harbour at ~80m depth. Eighteen *S. australis* were obtained from the New South Wales SMP, a commercial fishing trawler (*Maybelle*) operating out of Sydney harbour at ~80m depth, and from the Australian Maritime College research vessel *Bluefin* in East Tasmania at ~30m depth. Thirty-eight *P. nudipinnis* and thirty-seven *P. cirratus* were obtained from the Australian Maritime College research vessel *Bluefin* in East Tasmania at ~30m depth.

Length-weight relationships

S. albipunctata sampling lacked small individuals, yet still allowed assessment of length-weight relationships ($y = 5E-08x^{3.7265}$, $R^2 = 0.914$, $n = 25$; fig 1). The heaviest individual sampled was over 17kg. *S. australis* were generally smaller than *S. albipunctata*, growing to a maximum of ~1000mm and just under 11kg (fig 1). Neonates, as identified by the presence of visible yolk sacs and umbilical cords, were 253-260mm in total length. Data followed an exponential curve ($2E-05x^{2.9077}$, $R^2 = 0.9957$, $n = 18$; fig 1).

P. cirratus grew to a maximum length of just over 1000mm and a maximum weight of 1.9kg (fig 2). The minimum recorded size was 460mm, though neonates are expected to be smaller. Length-weight relationships followed an exponential curve ($y = 0.0002x^{2.319}$, $R^2 = 0.8363$, $n = 37$; fig 2). *P. nudipinnis* grew slightly longer than *P. cirratus* to a maximum length of 1092mm, but were heavier at a maximum weight of 2.5kg (fig 2). Minimum size was 460mm, but again neonates are expected to be smaller. Length-weight relationships followed an exponential curve ($y = 4E-06x^{2.8933}$, $R^2 = 0.9622$, $n = 38$; fig 2).

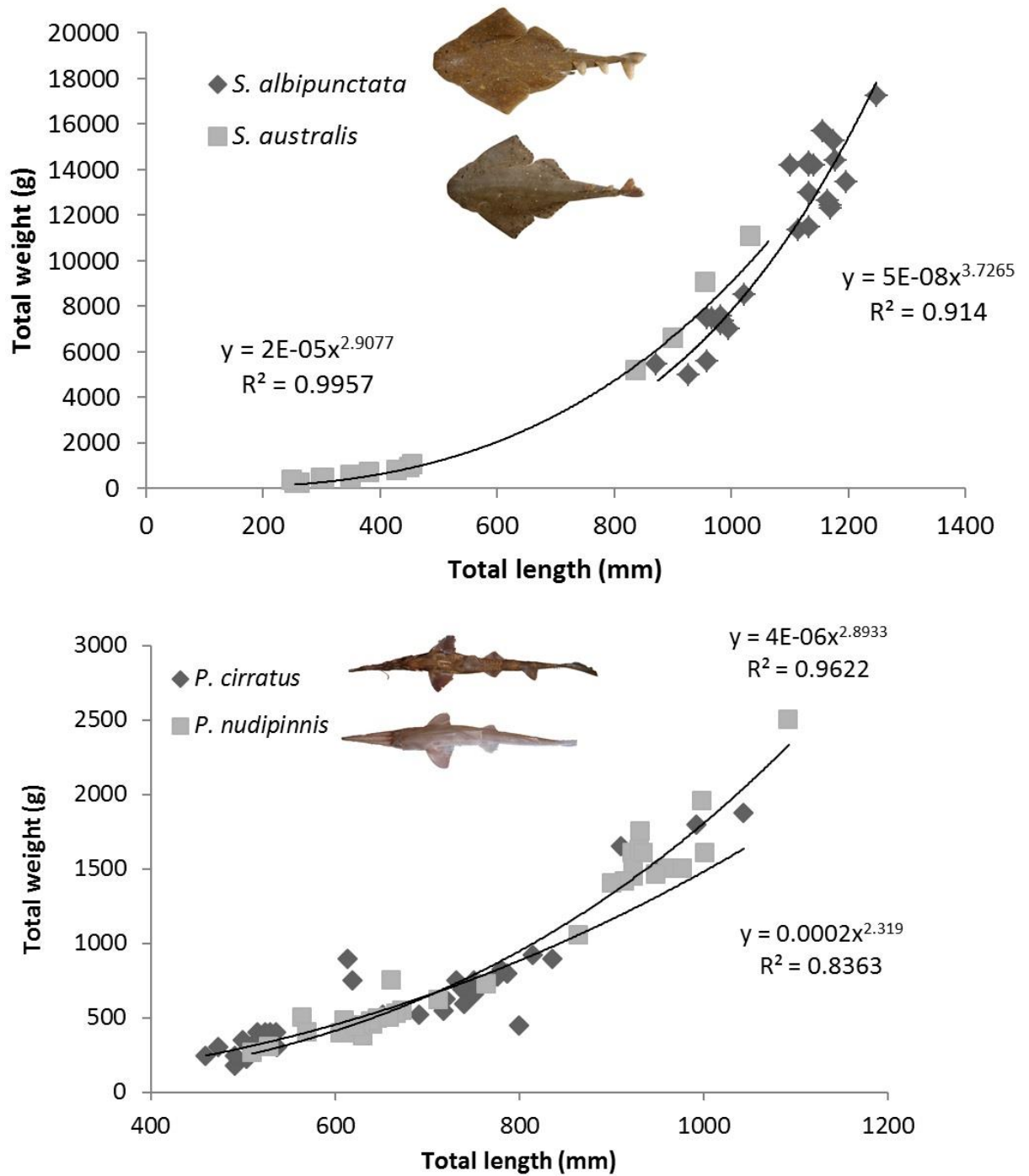


Figure 1: Length and weight relationships comparing *Squatina albigunctata* (n = 25; both sexes), *Squatina australis* (n = 18, both sexes), *Pristiophorus cirratus* (n = 37; both sexes) and *Pristiophorus nudipinnis* (n = 38, both sexes) Exponential trend lines added.

Morphometrics

Pectoral anterior margins, measured from origin to apex in a straight line, were the most accurate morphometric measurement for predicting total lengths in *S. australis*, *S. albipunctata*, *P. cirratus*, and *P. nudipinnis* ($R^2 > 0.86$ in all cases, up to 0.994; fig 2). Table 1 presents the formulae that were used to calculate total length from pectoral fin margins. Eye length was the best morphometric parameter for separating the two angel shark species, though data were only sufficient to determine this factor for adults (mean lengths significantly different, $t = 4.1$, $df = 30$, $p < 0.01$; fig 3). Sawshark rostrum length to total length ratio is already recorded as a species identification tool; all other morphometric measurements were too highly correlated to reliably separate species.

Table 1: Formulae for calculating TL from truncated specimens

Species name	Pectoral fin margin to total length conversion
<i>S. albipunctata</i>	$y = (x + 81.42) / 0.368$
<i>S. australis</i>	$y = (x + 3.515) / 0.288$
<i>P. nudipinnis</i>	$y = (x - 4.98) / 0.092$
<i>P. cirratus</i>	$y = (x + 6.68) / 0.131$

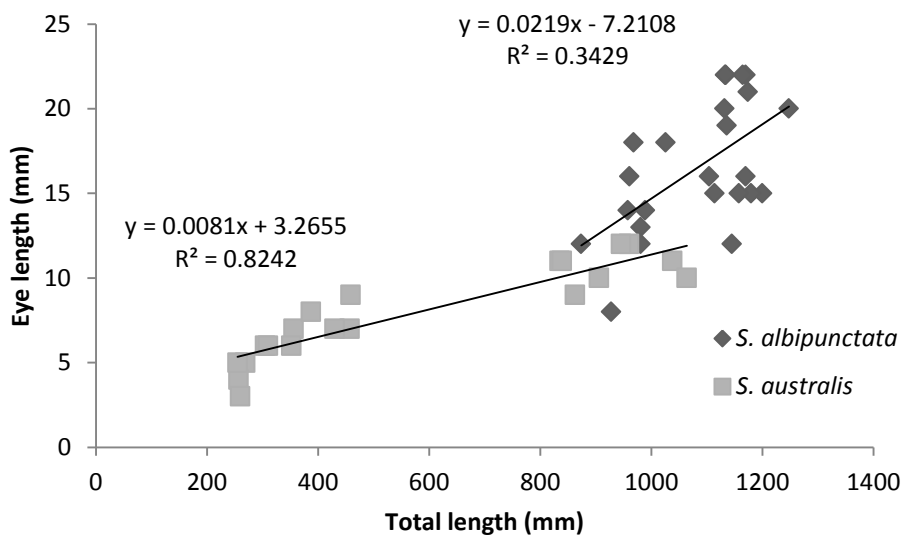
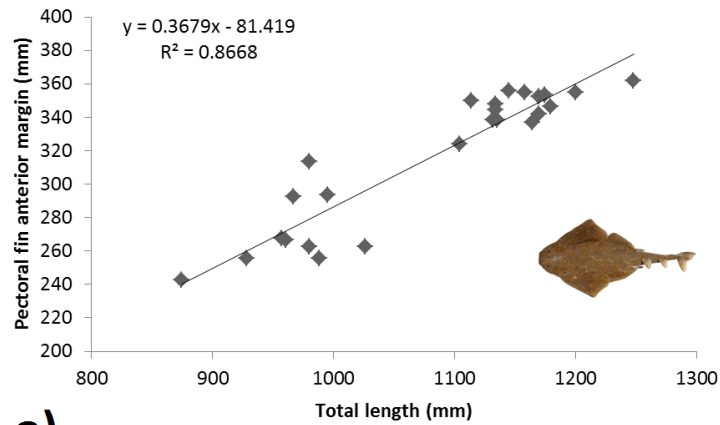
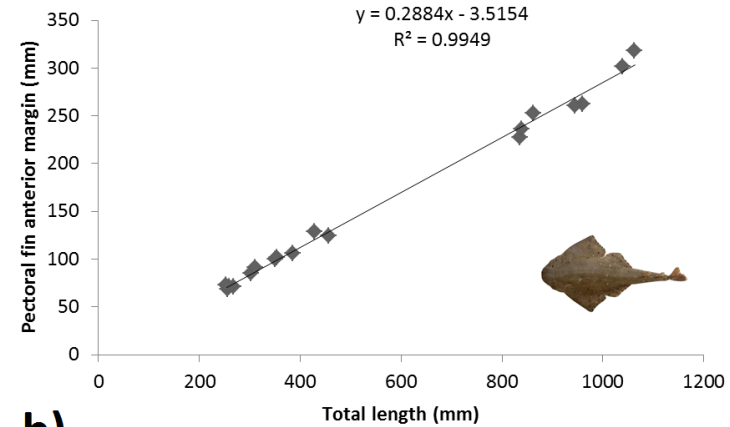


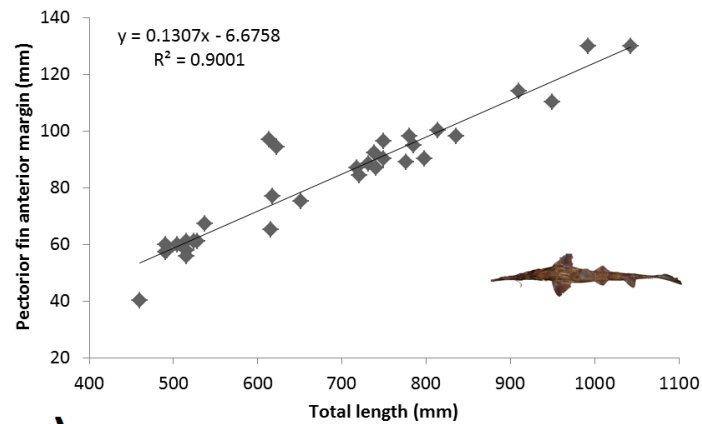
Figure 3: Relationship between total length and eye length for *S. australis* and *S. albipunctata*.



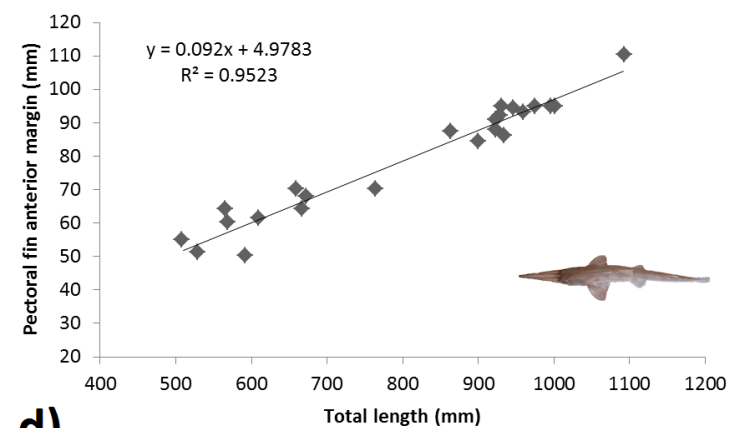
a)



b)



c)



d)

Figure 2: Relationship between total length and pectoral fin anterior margin of *Squatina albipunctata* (n = 25, a), *Squatina australis* (n = 18, b), *Pristiophorus cirratus* (n = 37, c), and *Pristiophorus nudipinnis* (n = 38, d). Linear trend lines added

Reproduction

Due to the lack of juveniles, relationships with clasper length could not be assessed for *S. albipunctata*. All male *S. albipunctata* sampled were mature and over 800mm TL. *S. australis* clasper length started to increase on individuals from 500mm TL, and all males over 800mm TL were mature and had a clasper length of over 120mm (fig 4). An increase in the length of claspers in *P. cirratus* occurred from 500mm and continued to increase in the larger individuals measured (fig 4). Individuals under 800mm in length were immature while all those over 900mm were mature. This study lacked mid-sized male *P. nudipinnis*, but clasper length increased after individuals reached 700mm TL (fig 4). All individuals over 900mm were mature while all those under 700mm were immature.

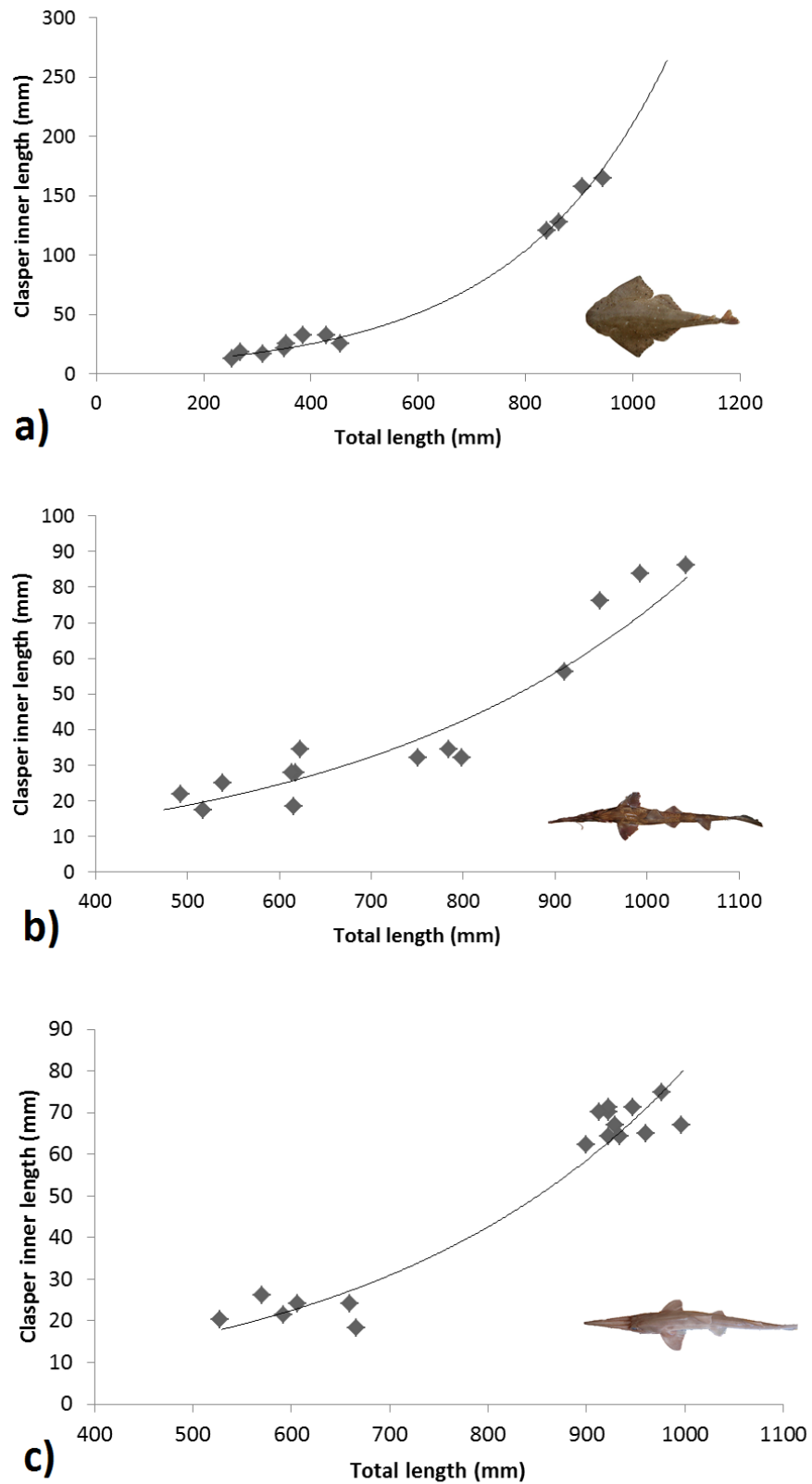


Fig 4: Total length and clasper inner length relationship for male *Squatina australis* (n = 12, a), male *Pristiophorus cirratus* (n = 14, b), and male *Pristiophorus nudipinnis* (n = 17, c). Exponential trend lines added.

Discussion

Biological characteristics of south-eastern Australian angel sharks *S. australis*, *S. albipunctata* and sawsharks *P. cirratus*, and *P. nudipinnis* were assessed. Females grew larger than males in all four species. Previous research trawling conducted by the *FRV Kapala* in the 1970 and 1990s also found female-biased total length ratios in the two angel shark species studied here, and the maximum sizes measured in this study correspond well with those results (DPI 2015). Angel sharks as an Order display female-biased total length sexual dimorphism (Bridge *et al.* 1998; Capapé *et al.* 2005; Capapé *et al.* 1990). Total lengths of *P. nudipinnis* and *P. cirratus* were similar, but *P. cirratus* were relatively lighter than similarly-sized *P. nudipinnis*. The weaker length-size relationship in *P. cirratus* can be explained by the relatively longer rostrum, which can add length to specimens with little weight (Nevatte 2015).

Pectoral anterior margins were highly correlated with total lengths (TL) in all four species. Pectoral anterior margins are the longest morphometric measurement in relation to TL that can be used in truncated angel sharks and sawsharks (aside from inter-dorsal fin distances), and may, therefore, have lower measurement error rates than other relationships. Pectoral fin margin measurements (the pectoral girdle and tip of the fin) are easier to define and are stiffer than other structures used in morphometrics, which often have boundaries that are soft and pliable (i.e., caudal fin widths) and are thus more difficult to measure. Due to the large difference in morphology between sawsharks and angel sharks (fusiform vs flat), it is possible that anterior margins are good predictors of total lengths for a large number of shark species. Fisheries managers should be able to determine TL in truncated specimens with relative confidence by using pectoral anterior margins.

Eye length in adult *Squatina* spp. differed between species. *S. albipunctata* had larger eyes, and unidentified specimens with eye lengths greater than 15mm are likely to be *S.*

albipunctata. It is possible that eye length can, therefore, be used to separate species easily though data were insufficient for juveniles. Eyes in larger elasmobranchs have higher spatial resolving power than smaller eyes (Litherland *et al.* 2009). *S. australis* and *S. albipunctata* have known depth segregation, with *S. albipunctata* occurring in deeper waters (Raoult *et al.* unpublished). Angel sharks are highly visual predators (Fouts and Nelson 1999), and consequently, the larger eyes of *S. albipunctata* may facilitate predation at greater depths than *S. australis* and add credence to predictions of depth segregation for these co-occurring species. Spatial segregation of size classes has been documented in other shark species (Borrell *et al.* 2011; Robbins and Booth 2012) and suspected in *S. tergocellata* (Bridge *et al.* 1998). Commercial trawlers caught only large (>800mm) *Squatina* spp. with a fish trawl net at 80m, while immature individuals were only caught by research trawls with a prawn net (smaller mesh) at ~30m depth. Smaller individuals may stay in shallower waters and larger ones may migrate to deeper waters as a result of the increased eye length in adults. While this is just one explanation, similar morphologies can result in trophic separation (Raoult *et al.* 2015), and therefore, these species may segregate as a result of diet preferences.

Embryos were present in one female *S. albipunctata*, with three young in each uterus. This female was caught in late autumn (April), and the embryos were 60mm, indicating they were not close to birth (minimum length estimated at the completion of gestation ~250mm, this study). As in some other *Squatina* studies (Bridge *et al.* 1998; Capapé *et al.* 1990), both ovaries were functional (producing oocytes) in *S. australis* and *S. albipunctata*. No egg cases were observed in this study, though the observed embryos were likely past the egg case stage for embryos under 60mm, as suggested by studies of *S. guggenheim* and *S. oculata* (Sunyem and Vooren 1997). Further research is needed to understand the gestation period and process in these sharks.

A crucial biological aspect that is missing from the species studied here is age. Studies have unsuccessfully attempted to determine the age of angel sharks using vertebrae (Baremore *et al.* 2009; Natanson and Cailliet 1990), but the vertebral structure of *Squatina* spp. is very different to other elasmobranchs. *Squatina* spp. have very distinct banding in the vertebral centra that are related to girth rather than age, and no visible banding in the *corpus calcareum*, which is generally used to age elasmobranchs (Ref). The only reliable age estimates of *Squatina* spp. are from tag-recapture studies on *S. californica*, which suggest the onset of maturity at ~5 years (Cailliet *et al.* 1992). A preliminary study that attempted to detect bands in the *corpus calcareum* of *S. australis* with a scanning x-ray fluorescence microscope found a result that correlated with the Cailliet *et al.* (1992) study of 5 bands/years for a near mature male (Raoult *et al.* unpublished). Accurate ageing of these sharks is necessary to predict the effects of commercial fisheries and to create accurate growth curves. Effort must be made to understand the development of shark vertebrae and to develop new ageing techniques.

The principle limitation of this study was the low number of study animals. Adequate reproductive analyses, particularly for females, were, therefore, difficult. Large adults and neonates were lacking for many of the study species. *S. albipunctata* length-weight relationships were the least reliable due to the absence of young individuals, and extrapolation of these curves may be inaccurate. Further research should aim to capture large females to determine reproductive periodicity and fecundity. Numbers of sub-adult *P. nudipinnis* were low, possibly because of depth segregation (seasonal effects were unlikely due to seasonal sampling patterns). High coefficients of determination (> 0.85) for all relationships may be a result of the small sample size: future studies should add measurements to this data set to verify whether it was an aberration.

This study demonstrates that simple morphometric measurements can be used for a variety of applications, both biological and management related. The two species of angel sharks found

in south-eastern Australia can be reliably separated according to their eye size, and TL can be inferred from anterior pectoral margins in these species with a high degree of accuracy.

Fisheries identification guides should, therefore, use these facts to facilitate the separation of these species. While the reproductive and size data presented here are sparse, they form the foundation for future studies assessing the biology of these little-known sharks.

Acknowledgements

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CHAPTER 6: Not all sawsharks are equal: species of co-existing sawsharks show plasticity in trophic consumption both within and between species

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The AMC *Bluefin*, responsible for sample collection in Tasmanian waters

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Abstract

Despite the global distribution of sawsharks, little is known about their diets or their role in the marine biosphere. As species in higher trophic positions are generally considered to be more at risk to perturbations such as fishing, understanding their role in the food chain will enable better conservation and management strategies for these species. Two sawshark species (*Pristiophorus cirratus*, *P. nudipinnis*) co-occur in waters off east Tasmania, Australia. This study determined the trophic positions of these sawsharks, and whether they avoided competing with each other through resource partitioning. Isotopic analysis of muscle tissue revealed that *P. cirratus* and *P. nudipinnis* had significantly different trophic levels, with *P. cirratus* likely to have a diet of primary consumers and *P. nudipinnis* likely to have a piscivorous diet. Due to their different isotopic signatures, it is also likely that the sawshark rostrum has multiple functions. Both species shifted to higher trophic levels during ontogeny. Maternal isotopic signatures were detectable in *P. cirratus* juveniles.

Keywords: *Pristiophoridae*, isotopes, diet, resource partitioning, niche divergence, *Pristidae*

Introduction

Species living in close proximity will tend to reduce competition through niche divergence or resource partitioning when faced with limited resources (Ross 1986; Schoener 1974). While terrestrial animals generally tend toward spatial niche separation (MacArthur 1965), trophic separation is commonly observed amongst organisms in the marine environment (Sale 1979). Research conducted in the area of resource partitioning often produces wide-ranging results, which range from the competitive exclusion of one species through the development of diverging sub-optimal resource use by that species (Schoener 1989), to competing species continuing to exist on a similar resource (Gabler and Amundsen 2010). Such conflicting

results on diet partitioning may be a product of the difficulty of accurately quantifying species' diets through short-term snapshots of stomach contents (Baker *et al.* 2014), or inappropriate levels of taxonomic resolution in the identification of prey (Barnes *et al.* 2011).

Stable isotopes are one marker that allows researchers to determine the trophic level (Vander Zanden *et al.* 1997), migratory patterns (Hansson *et al.* 1997), and short, medium and long-term diet shifts (Estrada *et al.* 2006; Hussey *et al.* 2011; Shiffman *et al.* 2012). Predators at high trophic levels enrich bioaccumulable markers present at lower trophic levels, enabling researchers to identify distinct chemical signatures present at each trophic level (Hobson *et al.* 2002). Unlike traditional gut content analyses, stable isotopes can provide average dietary information assimilated over time (Dalerum and Angerbjörn 2005), and are not affected by the digestibility of prey items, food expulsion during capture, or opportunistic feeding. Thus stable isotopes are a more reliable indicator of trophic level and long-term diet patterns than other more traditional methods, which are still necessary to correctly identify prey species.

Interspecific competition between closely related species sharing a common resource can be symmetric, where either species can fill a particular trophic level or niche (Munday 2004), or asymmetric, where one competitor is superior and a trade-off occurs in relation to the resource (Munday *et al.* 2001; Schoener 1983; Young 2004). Body and mouth size often play important roles in such competition because larger individuals with wider mouths can potentially feed on a greater range of prey sizes (Cohen *et al.* 1993). It is thus commonly assumed that closely-related species that are of similar size will have similar resource requirements. Where they co-occur, however, such species may have coevolved to use the divergent aspects of that resource over time (Connell 1980). In this case, we would hypothesise that the species that utilize prey from a higher trophic level would be gaining an advantage and would thus act as the superior competitor.

Sawsharks (*Pristiophoridae*) are elasmobranchs characterised by elongate bodies, ventral barbels, and saw-like rostrum (Last and Stevens 2009). To date, nine sawshark species have been identified, two of whom were classified in the last few years (Ebert and Cailliet 2011; Ebert and Wilms 2013). Two species co-occur in south-eastern Australian waters: *P. nudipinnis* and *P. cirratus*: the entire range of *P. nudipinnis* is within the wider range of *P. cirratus* (Last and Stevens 2009). Both species grow to similar lengths and weights, and can only be visually differentiated by slight changes in their rostral/body length ratios (higher for *P. cirratus*), the position of their rostral barbels (closer to the mouth for *P. nudipinnis*), and their colouration (spotted yellow/brown *P. cirratus*, uniform brown *P. nudipinnis*) (Last and Stevens 2009). Both species occur on the continental shelf at depths between 50 and 500 meters, and predominantly inhabit sandy benthic environments (Raoult *et al.*, unpublished data).

Little is known about the diets or ecology of sawsharks. Research on the critically endangered sawfish (*Pristis microdon*) found that the saw-like rostrum is used to disable schools of fish in the water column (Wueringer *et al.* 2012). Sawfish rostral teeth are permanent and much thicker and smoother than the finer, more jagged sawshark teeth that are frequently replaced (Slaughter and Springer 1968). While the two taxa have evolved rostral saws independently (Aschliman *et al.* 2012), it is highly probable that the rostrum may serve a different use between sawfish and sawsharks. Alternate uses for the rostrum could include defence from predators, or foraging through the sediment.

Three-hundred tons of *P. cirratus* and *P. nudipinnis* valued at over \$2 million AUD are caught annually in south-eastern Australian commercial fisheries. The majority of this catch is bycatch via gill nets and fish trawling, and few other commercial fishing methods (i.e. demersal longlining) capture these fish reliably (Raoult *et al.*, unpublished data). Australian government authorities (e.g. the New South Wales Department of Primary Industries) have

flagged *P. nudipinnis* and *P. cirratus* for target research due to the large number of sharks caught yearly and the dearth of information on their life-history traits.

We hypothesised that where *P. cirratus* and *P. nudipinnis* co-occurred, the two species would have divergent prey resources at different trophic levels to reduce dietary overlap and thus competition despite their similar morphology. Moreover, we hypothesised that if trophic separation occurred, such dietary divergence would occur between the two species throughout ontogeny. This study used muscle-tissue isotope analysis to determine the trophic levels of juvenile and adult *P. cirratus* and *P. nudipinnus* from the north-eastern coast of Tasmania, Australia, to ascertain their average trophic level. Using this trophic data, it may be possible to infer diet composition and, therefore, the use of the sawshark rostrum during feeding. The ontogenetic profiles of these species were compared against those from other ontogenetic studies worldwide to document whether patterns of isotopic plasticity were prevalent in sharks. A greater understanding of the trophic levels of *P. cirratus* and *P. nudipinnus* will increase our knowledge of the ecology of the community, and thus, reduce uncertainty in management and conservation efforts for these understudied species.

Materials and Methods

P. cirratus and *P. nudipinnis* were collected as bycatch from repeated research trawls on board the Australian Maritime College vessel *FTV Bluefin* in July, August and December 2011-2012 along the north-eastern coastline of Tasmania, Australia (40°18.101 S, 148°33.596 E; figure 1). These trawls were aimed at catching the majority of the benthic community at a depth of ~30m while using a 35mm mesh prawn net and at a depth of ~80m when using a 70mm mesh demersal fish net. All trawls were conducted for 30 minutes at a speed of ~3.1kts.

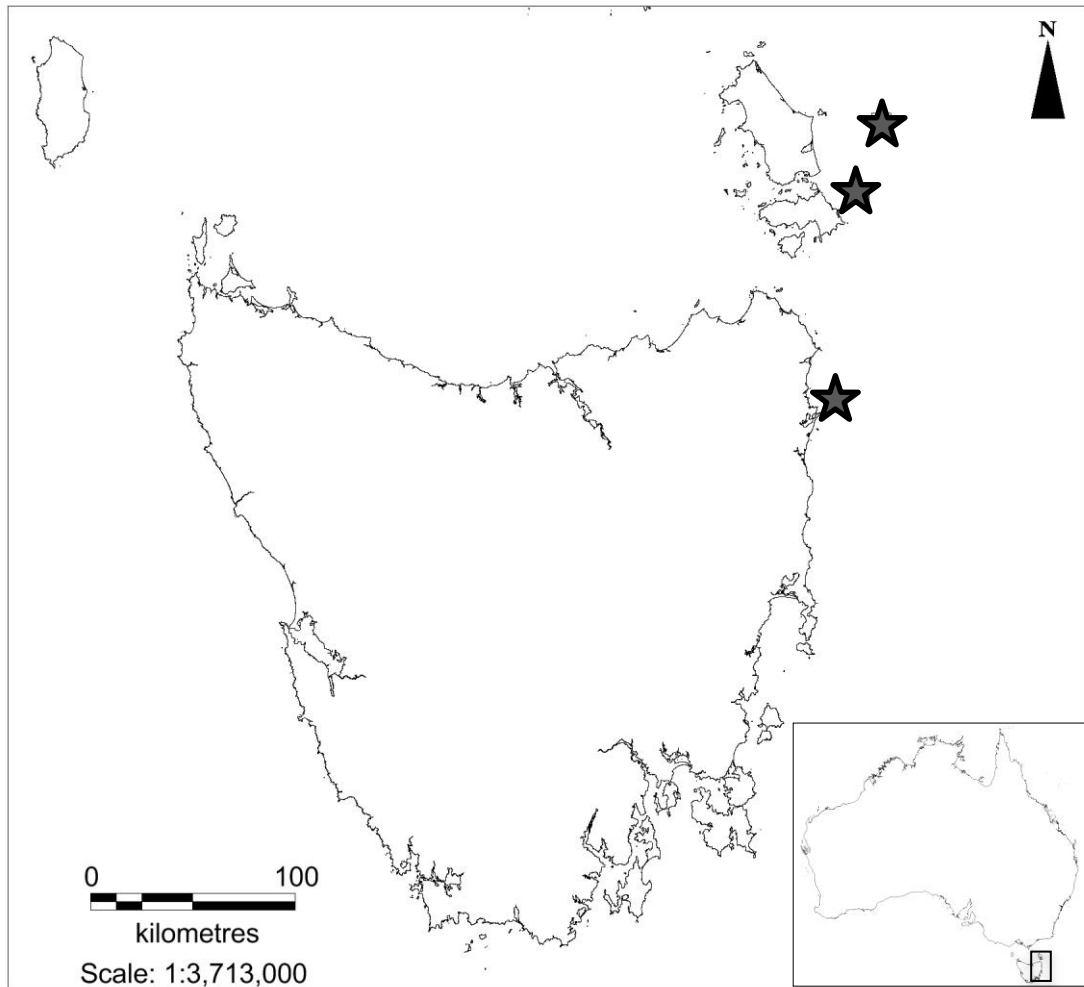


Figure 1: Map of Tasmania and research trawl sites (marked with blue stars)

Pristiophorus nudipinnis and *P. cirratus* caught in trawls were measured (total length, rostral length, mouth width, fins dimensions, in mm) and weighed (g). Each animal was then dissected immediately. The muscle in marine elasmobranchs represents, on average, a reliable isotopic signature for diet over the past months to years (Kim *et al.* 2012; MacNeil *et al.* 2006; Madigan *et al.* 2012). Muscle tissue samples ($\sim 3\text{cm}^3$) were therefore taken from the area dorsal of the cervical vertebra for isotope analyses (Hussey *et al.* 2012; Post *et al.* 2007). Care was taken to ensure samples were taken from the same position on each animal. Muscle samples were stored in 5ml screw-cap tubes and frozen at -20°C for storage until analysis as

recommended by Kim and Koch (2012). Twenty-one *P. cirratus* muscle samples were collected from different individuals, and 24 *P. nudipinnis* samples.

Muscle samples were dried at 60°C for 36 hours, ground to a fine powder using a mortar and pestle and 1-2 mg was transferred to tin capsules, as per Bessey and Vanderklift (2014). Samples were then analysed for carbon (^{13}C : ^{12}C) and nitrogen (^{15}N : ^{14}N) stable isotopes using a Europa EA GSL elemental analyser coupled to a Hydra 2022 mass spectrometer (Sercon Ltd, UK) at Griffith University (Queensland, Australia). Precision for this spectrometer is expected to be within 0.2 for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios. The ratios of $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$) and $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$) were expressed as the relative per thousand (‰) difference between the sample and standard (Pee Dee belemnite for carbon and atmospheric nitrogen for nitrogen).

To assess whether the isotope ratios (and thus diet) differed significantly between *P. nudipinnis* and *P. cirratus*, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values were analysed with a two sample T-test. Linear regressions were then used to determine the relationship between length and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for both species. Individuals were further split into juvenile and adults (*P. nudipinnis* > 800mm TL were adults (n = 10); *P. cirratus* > 600mm TL were adults (n = 14)) and the relationship between length (and thus maturity) and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determined. Mouth width is known to be related to prey choice (Karpouzi and Stergiou 2003; Kwak *et al.* 2004), consequently, mouth width was compared to $\delta^{15}\text{N}$ to determine whether the size difference of the two species may have an effect on prey choice using a generalized linear model (GLM). Studies that compared juvenile and adult shark isotopic ratios were compiled and contrasted from this study: only studies that examined sharks from juvenile to adulthood were used.

Results

Different trophic levels were observed for *P. cirratus* and *P. nudipinnis*. Mean isotope ratios were significantly different between the two species for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($df=44$, $t=2.02$, $p < 0.01$ in both cases, Fig. 2). *P. cirratus* mean isotope ratios were 13.5 ± 0.07 $\delta^{15}\text{N}$ and -17.5 ± 0.05 $\delta^{13}\text{C}$, while *P. nudipinnis* mean isotope ratios were 14.7 ± 0.09 $\delta^{15}\text{N}$ and -16.8 ± 0.07 $\delta^{13}\text{C}$. Trophic shifts occurred during ontogeny in both *P. cirratus* and *P. nudipinnis*: $\delta^{15}\text{N}$ was significantly positively correlated with length (*P. cirratus*: $df=1$, $F=16.39$, $p < 0.01$, $R^2=0.46$; *P. nudipinnis*: $df=1$, $F=7.76$, $p=0.01$, $R^2=0.25$, respectively, Fig. 3). *P. cirratus* $\delta^{13}\text{C}$ was significantly correlated with length ($df=1$, $R^2=0.43$, $F=14.37$, $p < 0.01$, Fig. 4). This change in $\delta^{13}\text{C}$ during ontogeny was not detectable in *P. nudipinnis* ($df=1$, $R^2=0.03$, $F=0.95$, $p > 0.05$, Fig. 4). There was no significant interaction between species and mouth width on $\delta^{15}\text{N}$ values (GLM, $F=0.04$, $p > 0.05$, Fig. 5).

$\delta^{15}\text{N}$ values were negatively correlated with length in juvenile *P. cirratus* ($df=1$, $R^2=0.73$, $F=11.31$, $p < 0.05$). $\delta^{15}\text{N}$ values of juvenile *P. nudipinnis* and adult stages of *P. cirratus* and *P. nudipinnis* did not change during ontogeny, as they were not correlated with length ($df=1$, $R^2=0.19$, $F=2.56$, $p > 0.05$; $df=1$, $R^2=0.10$, $F=1.52$, $p > 0.05$; $df=1$, $R^2=0.39$, $F=3.9$, $p > 0.05$, respectively). While some studies suggest that lipid extraction from muscle tissue is necessary for some elasmobranch species (Hussey *et al.* 2012), C:N ratios in these sharks had a mean of 2.65, indicating low lipid levels below the recommended threshold where lipid extractions or corrections are necessary (C:N > 3.5; (Post *et al.* 2007)).

It is unclear whether sharks always exhibit maternal isotopic signatures, and whether size is always related to prey choice. Furthermore, to determine likely prey items and trophic level, the results from this study were compared with those of Davenport and Bax (2002), which were also obtained in south-eastern Australia: *P. cirratus* $\delta^{15}\text{N}$ values were in the upper range

of tertiary consumers in the area, while *P. nudipinnis* $\delta^{15}\text{N}$ values were above tertiary consumers (Table 1). Eight studies documented shark $\delta^{15}\text{N}$ isotope ratios from juveniles to adults (Table 2). Only two studies found maternal $\delta^{15}\text{N}$ signatures (only one *Carcharodon carcharias* study detected such signatures). Not all studies found significant $\delta^{15}\text{N}$ /size ratios, and some populations/individuals within the same species had different $\delta^{15}\text{N}$ /size slopes.

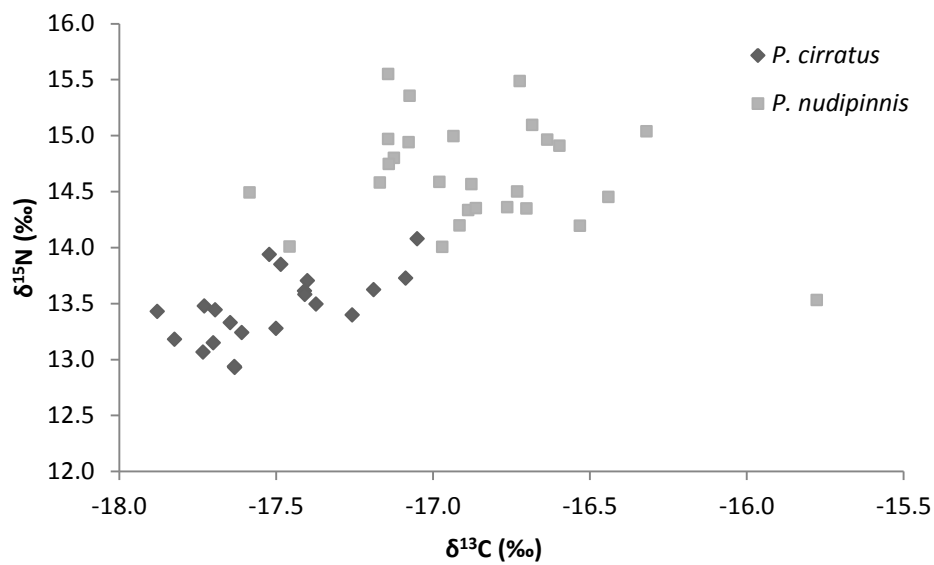


Figure 2: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for individual *P. cirratus* and *P. nudipinnis*.

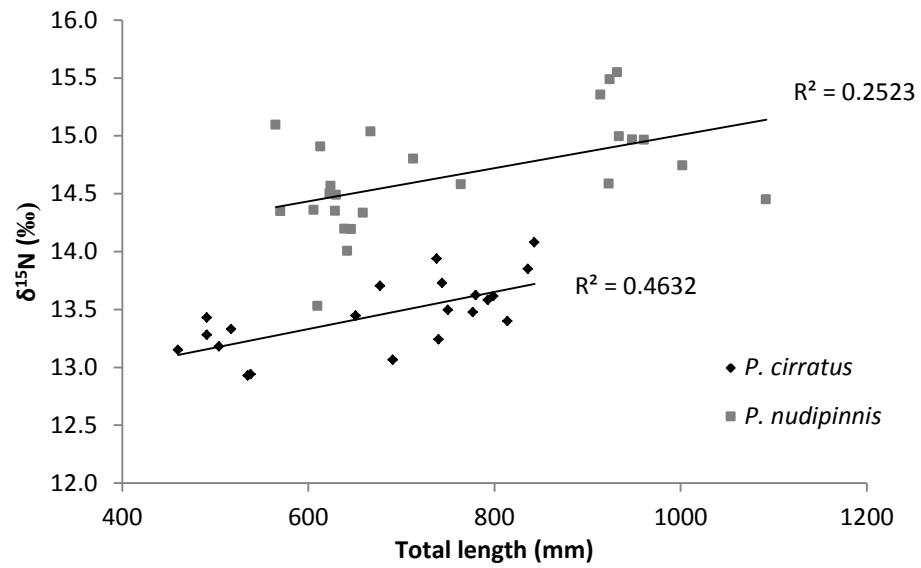


Figure 3: $\delta^{15}\text{N}$ in relation to total length for *P. cirratus* and *P. nudipinnis*. Trend lines show significant correlations between length and isotopic delta for each species.

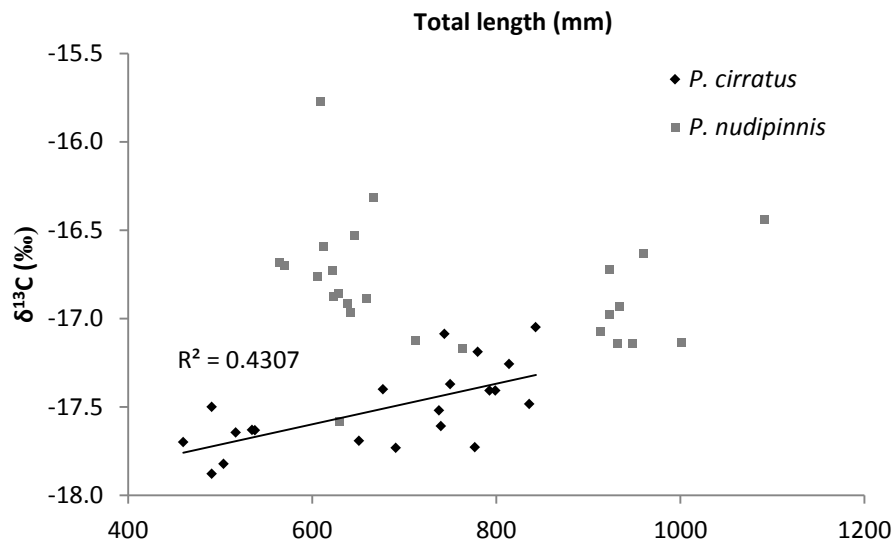


Figure 4: $\delta^{13}\text{C}$ in relation to total length for *P. cirratus* and *P. nudipinnis*. Trend lines show significant correlations.

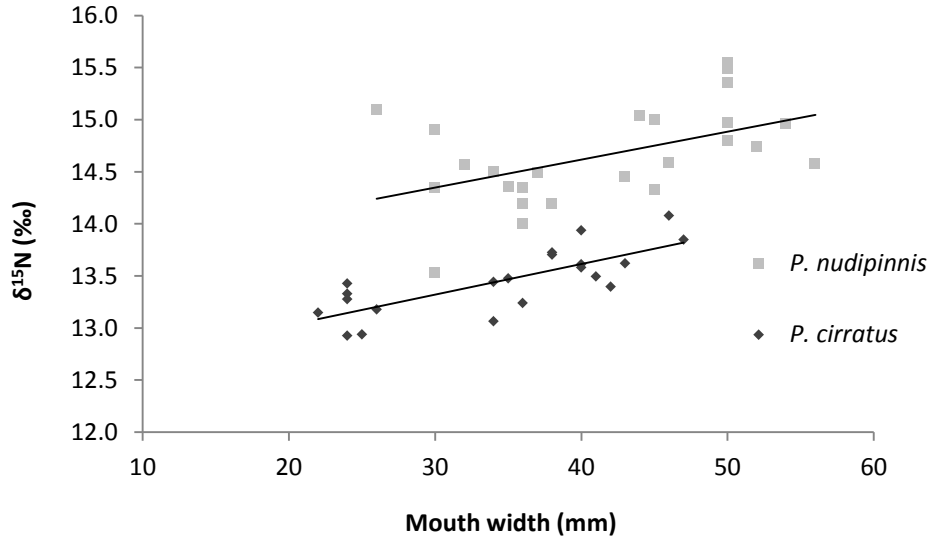


Figure 5: Mouth width/ $\delta^{15}\text{N}$ relationship for *P. cirratus* and *P. nudipinnis*.

Table 1: Comparison of soft tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges from species caught in south-eastern Australia (taken from Davenport and Bax (2002)), and the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *P. nudipinnis* and *P. cirratus* from this study.

Consumer	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Tertiary consumers (e.g. Pink Ling, <i>Genypterus blacodes</i>)	12.2 – 13.8	(-14.6) - (-17.2)
Secondary consumers (e.g. Elephant Fish, <i>Callorhynchus milii</i>)	10.3 – 13.1	(-16.8) - (-19.1)
Primary consumers (e.g. Ophiuroids)	5.3 – 9.4	(-16.7) - (-18.1)
<i>P. cirratus</i> (Common Sawshark/Longnose Sawshark)	12.9 – 14.1	(-17.1) – (-17.9)
<i>P. nudipinnis</i> (Southern Sawshark)	13.5 – 15.5	(-15.8) – (-17.1)

Table 2: Comparison of tissue $\delta^{15}\text{N}$ (‰) for studies that recorded isotopic levels through ontogeny.

Study	Species	$\delta^{15}\text{N}$ (‰) maternal signature yes/no	Significant $\delta^{15}\text{N}$ (‰)/size relationship yes/no	Slope of $\delta^{15}\text{N}$ (‰)/size relationship
Current study	<i>P. nudipinnis</i> (southern sawshark), <i>P. cirratus</i> (common sawshark)	No/Yes	Yes	Linear/linear
Borrell <i>et al.</i> (2011)	<i>Rhincodon typus</i> (whale shark)	No	Yes	logarithmic
Borrell <i>et al.</i> (2010)	<i>Rhincodon typus</i> (whale shark), <i>Sphyrna lewini</i> (scalloped hammerhead)	No/No	Yes/Yes	Logarithmic/logarithmic
Abrantes and Barnett (2011)	<i>Notorynchus cepedianus</i> (broadnose sevengill)	No	Yes/No (depending on site)	Linear
Estrada <i>et al.</i> (2006)	<i>Carcharodon carcharias</i> (white shark)	Yes	Yes	Three step
Fisk <i>et al.</i> (2002)	<i>Somniosus microcephalus</i> (Greenland shark)	No (only 2 juveniles)	Yes	Linear
Speed <i>et al.</i> (2012)	<i>Carcharhinus melonopterus</i> (blacktip reef shark), <i>C. amblyrhynchos</i> (grey reef shark)	No	No	N/A
Malpica-Cruz <i>et al.</i> (2013)	<i>Isurus oxyrinchus</i> (shortfin mako), <i>Carcharodon carcharias</i> (white shark)	No	Yes	Sigmoid/Logarithmic
Kim <i>et al.</i> (2012)	<i>Carcharodon carcharias</i> (white shark)	No	Yes/No (depending on individuals)	Logarithmic/linear (depending on individuals)
Campana <i>et al.</i> (2010)	<i>Carcharhinus leucas</i> (bull shark), <i>Galeocerdo cuvier</i> (tiger shark)	Yes	Yes/Maybe	Inversed parabolic/Possibly inversed parabolic
Hussey <i>et al.</i> (2012)	<i>Carcharodon carcharias</i> (white shark)	No	Yes	Sigmoid or logarithmic
Werry <i>et al.</i> (2011)	<i>Carcharhinus leucas</i> (bull shark)	No (perhaps in $\delta^{13}\text{C}$?)	Yes	Logarithmic
Papastamatiou <i>et al.</i> (2010)	<i>Carcharhinus melanopterus</i> (blacktip reef shark)	No	No/Yes (dependent on region)	Linear

Discussion

P. cirratus and *P. nudipinnis* caught in Tasmania exhibited significant inter and intraspecific trophic plasticity. A comparison between isotope levels of *P. nudipinnis* and previous research suggests that their diet was predominantly piscivorous and were potentially quaternary consumers. Isotope values of *P. cirratus* are consistent with those of secondary/tertiary consumers. Ontogenetic shifts in diet were apparent between juveniles and adults in both species, and in all but three of the ontogeny studies from other species of sharks. This is the first study to determine the trophic level of sawsharks.

The difference in isotope levels between *P. cirratus* and *P. nudipinnis* suggests that, when co-occurring, they appear to avoid direct competition through resource partitioning at different trophic levels. Mean $\delta^{13}\text{C}$ values were significantly different between *P. cirratus* and *P. nudipinnis*, but this it is unlikely to be indicative of separate diet sources and more likely a consequence of the higher trophic diet of *P. nudipinnis* (difference of 1‰), which is causing higher carbon/nitrogen fractionation at higher trophic levels (Zanden and Rasmussen 2001). A study by Kinney *et al.* (2011) detected resource partitioning in various juvenile shark species in shark nursery areas at comparable trophic levels, therefore, this may be a widespread occurrence with co-occurring elasmobranchs.

In competitive environments, larger animals have access to a greater size range of prey, and *P. nudipinnis* grows to lengths 200mm longer than *P. cirratus*. The maximum size of each species size is unlikely to have a significant effect on prey choice; however, as there was no significant interaction between species and mouth width on isotope ratios. The main morphological differences between these two species, aside from total length and colour patterns, are the width and thickness of the rostrum. The driver for resource partitioning

between these two species of sawshark is, therefore, more likely to be behaviourally-driven rather than morphological.

The function of the rostral saw in sawsharks is unknown, although they are routinely hypothesised to be used in a similar fashion as sawfish (*Pristid* spp.) for prey capture (Slaughter and Springer 1968; Wueringer *et al.* 2009). This study suggests that the trophic level of *P. cirratus* is similar to species that prey on benthic primary consumers; consequently, *P. cirratus* may use their rostrums to sift through the substrate in the search of prey. The isotope levels of *P. nudipinnis* suggest a more piscivorous diet, and raise the possibility that they feed in a similar manner as some *Pristidae* species (Wueringer *et al.* 2012). Further research into diet, feeding modes, and rostrum use is necessary to elucidate the specifics of feeding in these species.

Both species of sawshark were hypothesised to show similar traits because they share the same development pattern (aplacental viviparity), and the few studies on sawshark phylogeny suggest they are a highly related monophyletic group (Dosay-Akbulut 2006). The negative isotopic correlation of juvenile *P. cirratus* was indicative of maternal isotope signatures. This pattern was not visible in *P. nudipinnis* samples, possibly due to the lack of very young individuals in this data set. Other shark species have similar maternal isotopic signatures or fractionation in juveniles (Campana *et al.* 2010; Vaudo *et al.* 2010), however only two studies that analysed isotopic signatures throughout development detected such patterns (Campana *et al.* 2010; Estrada *et al.* 2006). It is possible that the maternal signatures disappear quickly after birth and that the lack of newborns in data sets under-estimates the number of shark species with maternal isotopic signatures.

Adult isotope levels that were not correlated with length suggest that a dietary shift occurs quickly during the sub-adult phase in *P. cirratus* and *P. nudipinnis*. As similar ontogenetic

patterns have been observed in other species of sharks (Kim *et al.* 2012), it is possible that different sawshark trophic ecotypes exist in other areas, or that in areas where the two species do not co-occur it is possible that the remaining species will fill the available trophic niche. The majority of studies that examined isotope ratios during ontogeny found similar length-correlated ratios (Fig. 2), though the degree of variation during ontogeny varied inter and intra-specifically. This suggests that ontogenic shift in diet may be partially linked to species-specific traits. Geographically distinct populations of certain species also have different patterns of trophic shift (Abrantes and Barnett 2011), suggesting that an ontogenetic shift in diet may also be related to prey availability and/or environmental conditions. The spread of isotopic deltas per length (in this study as well as others listed above) suggests a high degree of individual feeding preference within species (Kim *et al.* 2012). Studies that examine trophic plasticity through ontogeny must, therefore, do so on small scales and at high resolutions, otherwise, results will be diluted due to noise from inter and intra-specific variability. Furthermore, these conclusions suggest that changes in temporal and geographical variables greatly affect trophic studies that use stable isotopes, and further research should aim to quantify the level of these effects.

Compliance with ethical standards

Trawling for research purposes was permitted in accordance with ethical guidelines number A0011023, UTAS ACEC. All procedures performed were in accordance with the ethical standards of the University of Tasmania.

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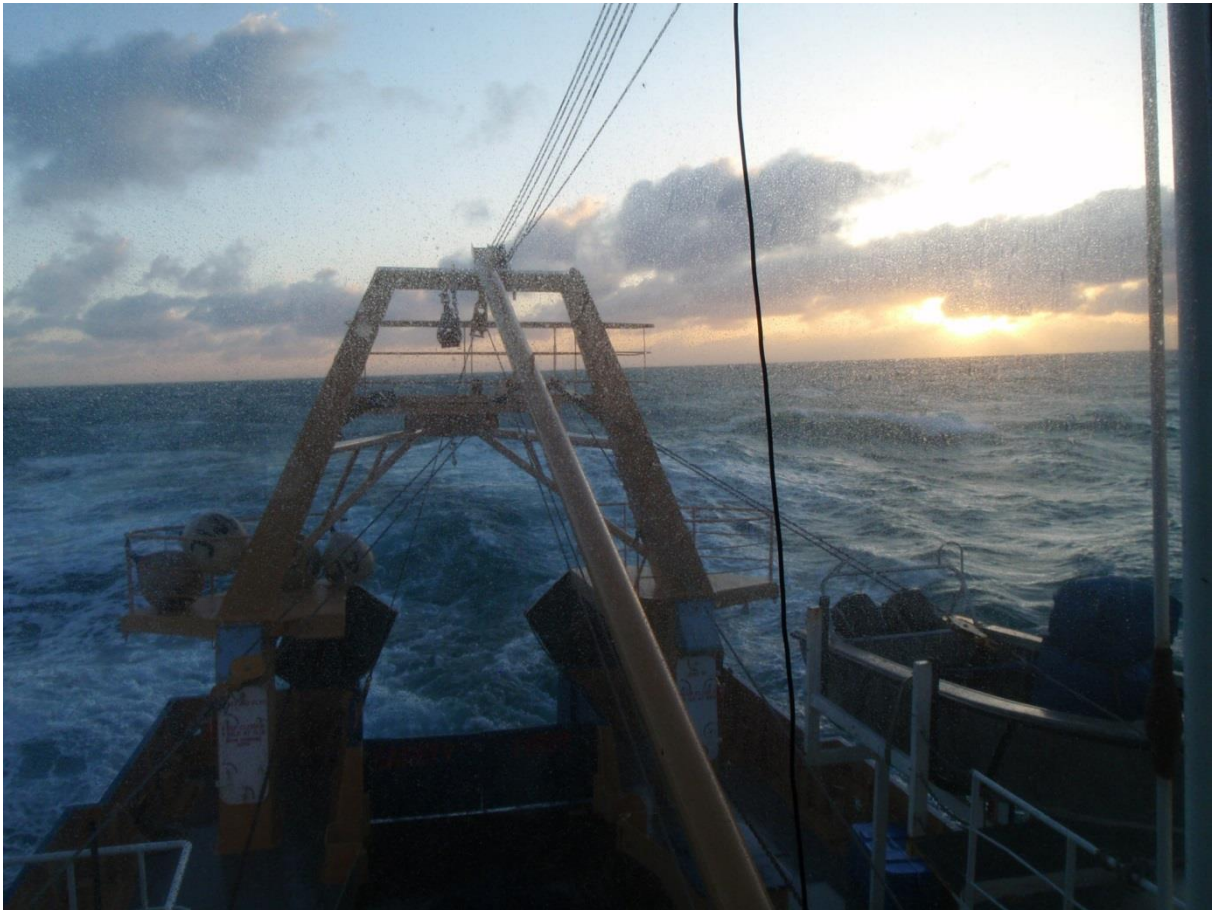
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CHAPTER 7: General discussion and conclusions



A bumpy sunset on the Bass Strait, taken from the wheelhouse of the *AMC Bluefin*

Management implications

Sustainability

Current fishing levels of sawsharks and angel sharks in south-eastern Australia appear to be sustainable, although fisheries management based on catch per unit effort is not always reliable and should be combined with biological assessments (i.e. length-frequency distributions, data on recruitment etc.) (Harley *et al.* 2001). Furthermore, marked declines since the 1950s suggest that current populations of *Squatina* spp. and *Pristiophorus* spp. are at significantly lower levels ($\leq 40\%$) than they were only a few decades ago. All four species could be considered to fall under the classification ‘sustainably overfished’ and urgently require further monitoring.

The major issue with regards to management of sawsharks and angel sharks in the area is whether the objective is to return populations to pre-1950 levels, or to maintain current fishing rates and populations. Shifting baselines are a growing issue with commercial fisheries around the world, whereby recent generations have skewed perspectives on what qualifies a large catch that does not reflect those of previous generations (Baum and Myers 2004; Knowlton and Jackson 2008; Sáenz-Arroyo *et al.* 2005). Due to declines of sawsharks and angel sharks of around 90% in the last 50 years, it is possible that baselines will or have shifted for these sharks, and that current ‘large’ catches are much smaller than 50 years ago. Fisheries managers must recognize that populations are significantly smaller than previous decades when considering the management of these sharks.

Angel sharks

Three independent data sets at different resolutions for angel sharks (NSW Shark Mesh (bather protection) Program, Australian Fisheries Management Authority data, New South Wales Fisheries data) are in agreement that fishing of *Squatina* spp. in the south-eastern

Australia over the last ~20 years appears sustainable on a significantly reduced population. Although CPUE from a single fishery is not always reliable as a sole determinant of the sustainability of catches (Harley *et al.* 2001), it is unlikely that these three independent data sets and sampling methodologies would display a similar catch patterns if populations were not stable. The length-frequency shift from larger to smaller animals of *S. albipunctata* recorded by the *FRV Kapala* also demonstrates that while fishing has impacted populations, mature individuals (mean length shift of ~10cm) are still prevalent. Moreover, reproductive data suggest that mean catch sizes (if *Kapala* data can be extrapolated to commercial trawlers) are still adults/sub-adults, thus relative reproductive ability of the populations remains similar to before commercial fisheries entered the area.

Sawsharks

Populations of sawshark in south-eastern Australia do not appear to be in further decline. Unlike *Squatina* spp., sawshark CPUE of the *FRV Kapala* between the 1970s and 1990s did not decline by 90%, though they did decline significantly by two-thirds between that time period. Catch per unit effort in Commonwealth and New South Wales waters has been relatively stable for the last 20 years at roughly one third the original catch levels. Significant declines in CPUE were detected in some areas in the last 20 years, though due to the relatively short study period it is possible that these declines are seasonal shifts. Preliminary ageing data suggest that sawsharks are relatively short-lived and fast-growing, and thus may be able to sustain higher levels of fishing than angel sharks. A recent review by the Australian Fishing Management Authority (AFMA) also suggests that an extra ~13.5% of the total catch of sawsharks are discarded, which represents fifty tonnes per annum. Due to the large number of sawsharks caught in the area (>350 tonnes per annum), along with the duration and intensity of the fishery, it is recommended that these populations are closely monitored for further declines, and that the Total Allowable Catch be adjusted accordingly.

Catch data

A prevalent issue that arose when analysing the various data sets was the low reliability of species-specific catch identification. This lack of reliable species-specific data makes a clear assessment of stocks difficult. Although both Commonwealth and New South Wales fisheries have recently (2009) upgraded to recording species-specific catches, there are still a large number of records marked as ‘unidentified’ (both from commercial fishermen records and fisheries observers) within this process. Furthermore, logbooks frequently recorded only a single species repeatedly in areas where multiple species were likely to co-occur. This made it impossible to use more recent data with any degree of confidence for species-specific applications. If improvements are not made, there is little benefit of continuing species-specific analysis given the increased workload on fisheries staff and the added difficulties of analysing data. Causes of misidentification or simplistic logging in this area are two-fold: (1) there is a difficulty of separating the species within the genus, and (2) complications arising from catching large numbers of both *Squatina* spp. and/or *Pristiophorus* spp.. that may discourage fishermen from accurate sorting. Attributing the term ‘unidentified species’ was prevalent in both NSW state and Commonwealth logbook reporting: fishermen should undergo a training session to correctly identify species, and the use of ‘unidentified species’ should no longer be condoned.

To combat the first issue, clear identification guidelines could be given to both fisheries observers and fishermen. To separate *Squatina australis* from *S. albipunctata*, a viable technique was to look at the pectoral fins: *S. australis* has distinct white saw-tooth edges on the posterior pectoral margin (figure 1) whereas *S. albipunctata* does not. Dorsal markings/colour patterns are not recommended for identification guidelines because the colour seems quite variable and *Squatina* spp. produce large amounts of mucus post-mortem,

which can camouflage any colour patterns. Species of sawsharks are relatively straightforward to tell apart when whole (compare the barbel position relative to the mouth and the shape of the rostrum), but difficult to distinguish between species when their head and viscera have been removed at sea. Colouration varies greatly, and while *P. cirratus* are the only species in the area that have distinct patterns along the body, those patterns can vary in intensity from very distinct to almost non-existent (figure 2). Thus, while identification post-decapitation is of distinctly patterned *P. cirratus* is straightforward, species with little or no visible colouration, or other species of angel sharks, are unable to be reliably identified (or *P. cirratus* individuals with no visible colouration). Morphometrics were unfortunately not able to reliably separate species post-decapitation (Chapter 5).



Figure 1: Juveniles (top) and adult (bottom) *Squatina australis* with 'saw-tooth' edges on pectoral fins that separate *S. australis* from *S. albiguttata*.



Figure 2: *Pristiophorus nudipinnis* (top) and *P. cirratus* (bottom). Notice the broad, triangular rostrum in *P. nudipinnis* and the long, straight rostrums of *P. cirratus*. Also, notice the colour variation between two similarly-sized *P. cirratus*.

Causes of misidentification also arise from the issue of time taken to identify large catches of sharks to species level onboard commercial vessels, especially when there is little benefit to the fisher to do so. Commercial trawlers can catch over 300kg of any of these sharks in a trip (Chapters 2, 3), which can represent nearly 700 sawsharks or over 50 angel sharks: it is uneconomical for fishermen to actively separate and record the two species, particularly when identification can be difficult. Thus, the most realistic way to monitor species-specific catch composition is through an observer program. With improved identification guides, observers will be less likely to group species as unidentified. Having trained fisheries observers that accurately and independently record catches onboard fisheries vessels, while costly, can

increase the reliability of catch data, and allow extrapolation to the entire fishery. The lack of reliable observer data made analysing these data very difficult, and also made species separation within data sets impossible.

Shark vertebral ageing

The ageing work presented in this thesis demonstrated that strontium was a better banding indicator than calcium when assessing bands to estimate the age of sharks. Although the sample size in this study was relatively small, the diversity of the groups studied indicates that strontification may occur in all elasmobranchs. Other studies have outlined the need to develop widely-applicable ageing techniques (Cailliet *et al.* 2006), and the use of strontium as an age determinant may be a way forward. Furthermore, in some species, vertebral bands seem to be related to somatic growth rather than to age (Chidlow *et al.* 2007; Huveneers *et al.* 2013), and understanding strontium deposition may help elucidate the drivers of vertebral band deposition. Once strontium band deposition is understood it would be possible to infer age even from species of sharks with non-yearly vertebral band deposition by understanding the temporal drivers behind band deposition. Finally, the use of strontium concentrations as a basis for ageing can remove observer bias from shark ageing because banding can be analysed analytically, using statistical packages to analyse data rather than rely on observers.

Reliable ageing of sharks and rays is difficult, and there are costs and benefits associated with all current methods. To determine strontium concentrations it was necessary to use a Scanning X-ray Fluorescence Microscope. This equipment is prohibitively expensive (requires access to a synchrotron), rare (only one such microscope in the southern hemisphere), and difficult to access (long waiting times). It is thus not an ideal technique for the routine ageing of sharks. X-ray fluorescence microscopes are more common, but are generally limited by slow imaging

speed and small observable areas (Fahrni 2007). Mass spectrometry of various sorts has already been used with sharks and teleosts (Sripadi *et al.* 2009; Tillett *et al.* 2011; Werry *et al.* 2011), is affordable and can be found in most research-centred universities. However, this method is limited in its range (limited resolution), must generally scan in lines, and is prone to contamination (Tillett *et al.* 2011). As demonstrated by this study strontium bands are rarely perpendicular to vertebral growth, consequently, it is improbable that mass spectrometry of shark vertebrae will reliably detect strontium bands and/or that it would be prohibitively expensive to scan large areas of the vertebrae. A chemical indicator for strontium is unlikely to be available due to the chemical similarities between strontium and calcium. Currently, it seems that there are no easily accessible, affordable imaging systems that reliably detect elemental bands in shark vertebrae.

Other more widely-available physical imaging techniques were trialled for *Squatina* spp. and *Pristiophorus* spp. prior to using the SXFM. Magnetic Resonance Imaging (MRI) was used but often resulted in aberrations that made analysis impossible. Micro Computed Tomography (MicroCT) was successful with *Charcharhinid* spp. (Geraghty *et al.* 2012), but did not appear to detect age bands in *Squatina* spp. or *Pristiophorus* spp. (Raoult, unpublished data). After extensive testing and perfecting the preparation method, results from the SXFM were successfully replicated using a microCT (Appendix II).

MicroCTs are relatively common due to their use for medical experimentation with rodents (Holdsworth and Thornton 2002). While not yet widely available or accessible, the technology is frequently upgraded, miniaturised, and becoming less expensive (Ritman 2011). Due to the possibility of detecting banding even in sharks that are known to be difficult to age, such as angel sharks, there is potential for microCT to be a universal ageing tool for elasmobranchs. Furthermore, vertebrae are not damaged as a result of microCT analysis and can be reused in other applications, such as isotopic analysis (Kim *et al.* 2012). The greatest

advantage of microCT over traditional methods is that it removes observer bias as results can be analysed analytically.

Biology of angel sharks and sawsharks

Angel sharks

Growth curves for *S. australis* and *S. albipunctata* had similar traits to those found in other *Squatina* spp. (Bridge *et al.* 1998; Shelmerdine and Cliff 2006). Like other angel shark species, growth curves were similar for males and females in *S. albipunctata* and *S. australis*, though *S. albipunctata* grew to larger sizes. Depth segregation was apparent between the two species, possibly partially a result of the larger eyes of adult *S. albipunctata* (Lisney and Colin 2007). Due to similar mouth morphology and total size, it is unlikely that the two species have widely differing diets, and preliminary gut content analysis suggests that both species eat a variety of benthic teleosts and cephalopods (Raoult, unpublished data). This means that overfishing of these sharks may have trophic effects on benthic teleosts and cephalopods that are of commercial value (i.e. squid and flounders).

Sawsharks

Muscle isotopic deltas indicated that sawsharks, especially *P. nudipinnis*, are top predators in the deeper waters of south-eastern Australia, and are at a higher trophic level than tertiary consumers in the area (Chapter 6). As predicted, the two co-occurring species may avoid direct competition through resource partitioning. If feeding behaviour is similar to that of *Pristidae* (Wueringer *et al.* 2009), differences in rostral morphology between *P. cirratus* and *P. nudipinnis* may be partially responsible for the trophic separation (wider, sturdier rostrum in *P. nudipinnis*). This research has highlighted how little is known about this genus, and

further research should aim to understand their behaviour, movement patterns, and interactions within their ecosystems.

Limitations

A lack of a large number of samples from a wide range of sizes for each species was clearly a limitation of this research. Despite low sample sizes, there were sufficient individuals from all species to develop reliable growth curves. The principle issue with the sample set as it was presented was the general lack of new-born individuals and mature, gravid females that hindered the study of reproductive parameters of the different species.

Sample collection from a range of sites, depths, and times was also difficult due to reliance on multiple commercial fishermen operating in different areas and the sporadic nature of the Shark Mesh (bather protection) Program, which also resulted in selective sampling locations. Sample collection was further hindered by attempts to age *Squatina* spp. and *Pristiophorus* spp., which required investing time and effort familiarising with various imaging techniques, applying for funds/access, preparing samples, and analysing results. Catch data could rarely be analysed on a species-specific basis due to the low reliability of species identification, and therefore, individual species' population statuses could not be determined.

Future directions

Further research is needed on developing adequate methods to age sharks. The diversity of vertebral structures in elasmobranchs appears large, which makes using a single method of ageing difficult at present. Unique vertebral characteristics that differed from the 'normal' vertebrae in Carcharhinids were apparent in each family studied here (i.e. multiple tissue layers in *Squatinidae*, double tissue layer in *Pristiophoridae*. Further studies on vertebral

evolution in sharks would be beneficial to the field of ageing through a greater understanding of vertebral development, which would allow researchers to more reliably estimate age, and perhaps with simpler, cheaper techniques. Furthermore, shark ageing techniques are based on fish otolith ageing, as fishes present growth bands similar to those found in shark vertebrae (Campana 2001). Due to the advancement of chemical analysis techniques, fish otoliths have become a chemical record of life histories rather than just an ageing tool (Campana 2005; Elsdon *et al.* 2008; Kalish 1989). Applying techniques such as the SXFM to fish otoliths may reveal further elemental information that could be applied to life-histories and movement patterns.

Studies are needed to understand the population structure of angel sharks and sawsharks in south-eastern Australia. While this body of work increases the knowledge of angel sharks and sawsharks, it also underlines how little is known about these species in comparison with other sharks and rays. Future studies should aim to determine the reproductive frequency, movement patterns, behaviour, and age. All of the species seem to occur in relatively large ranges, yet angel sharks are known to have significant genetic divergence over small areas (Gaida 1997): population genetics analyses could help determine whether these sharks migrate, but also whether genetically distinct populations occur. Genetic studies would also help determine whether hybridisation occurs in sawsharks and angel sharks as in other co-occurring shark species of the same genus (Morgan *et al.* 2012). Sawshark barbels are a unique morphological feature among elasmobranchs, and their function and purpose should be investigated. Due to the depth at which these sharks occur, it is unlikely that satellite tracking could function. Acoustic monitoring arrays are well developed along south-eastern Australia, and the technique has been used on sharks with a large degree of success (Bruce and Bradford 2013; Vianna *et al.* 2014). Acoustic tagging of angel sharks and sawsharks should enable researchers to understand whether they undertake seasonal migrations as this

study suggests, and whether sawsharks, in particular, spend large amounts of time in deeper waters. Finally, NSW and Commonwealth fisheries should continue monitoring these four species to ensure that they remain sustainable for years to come.

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APPENDIX I: Localized zinc distribution in shark vertebrae

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Controlling the scans at the SXFM in the Australian Synchrotron (Nicholas Howell in background)

Abstract

Shark vertebrae have been used to determine age for over three decades, yet little is understood about their development. This study used a Scanning X-ray Fluorescence Microscope to observe zinc distribution within vertebrae of eleven shark species. Zinc was mostly localised within the *intermedialis* and was only present in the *corpus calcarum* in one species. Intermedial banding in zinc corresponded to strontium bands. In some species, zinc concentrations were higher pre-birth mark, indicating a prenatal use of zinc and possible uptake via uterine fluid.

Keywords: elements, synchrotron, particle accelerator, elasmobranch, growth, enzymes

Introduction

The recent explosion of commercial fishing practices has led to global declines in fish stocks and significant shifts in marine communities (Myers and Worm 2003; Ward and Myers 2005). Management efforts have increased proportionally to these declines but are still marred by a lack of biological information necessary to predict species recovery. Sharks and elasmobranchs have been under increased threat and are less comprehensively managed than teleost fish stocks (Stevens *et al.* 2000). Increases in correct management strategies should be developed in light of the critical role these large predators play in ecological stability (Ferretti *et al.* 2010; Heithaus *et al.* 2008; Myers *et al.* 2007).

Determining age at maturity and longevity are two critical components of effective fisheries management (Musick and Bonfil 2005; Musick *et al.* 2000). In sharks, age is determined by counting bands on the corpus calcarum of the vertebrae (Cailliet *et al.* 1983). Although shark vertebrae have been analysed using this method for over thirty years, information on shark

vertebral development is still lacking. This knowledge gap has led to a number of preparation techniques that aim to increase the accuracy of age band counting, with a subsequent call for more consistent methodologies (Cailliet *et al.* 2006). A greater understanding of vertebral development would allow for simpler, more directed ageing techniques that could be more widely applied with more accurate results.

Shark vertebrae have mainly been used for the purpose of ageing, but recently they have been used to track chemical variables throughout an animal's life. For example, tissues taken from successive vertebral bands in great white sharks (*Carcharodon carcharias*) have allowed researchers to track isotope ratios and diet shifts during ontogeny (Estrada *et al.* 2006). Heavy metals within shark tissues can be used in a similar fashion to track changes in trophic levels, patterns of diet and pollutants the individual may have absorbed (Domi *et al.* 2005).

Zinc is a metal commonly found in the marine environment that is involved in enzyme formation. It accumulates in marine organisms and is heavily concentrated within eyes (Hogstrand and Wood 1996). Zinc is an environmental pollutant released as a by-product of human activities. Concentrations of zinc in marine environments increase with depth and are related to silica levels (Bruland 1980). In fish, zinc uptake is linked to diet and bone development (Li and Robinson 1996). Although the uptake of zinc has been extensively studied to optimize growth rates in cultured fish (Burridge *et al.* 2010; Canli and Atli 2003) most studies on elasmobranchs considered zinc solely as an environmental contaminant (Marcovecchio *et al.* 1991; Stevens and Brown 1974). No studies have assessed fine-scale zinc distribution or variations in zinc concentration through the life history of elasmobranchs. This study determined the distribution of zinc within shark vertebrae as a first assessment of the role of zinc on vertebral development.

Materials and Methods

Samples were caught in the New South Wales Shark Mesh Program and from fishing trawlers based in Sydney and Launceston (NSW and Tasmania, respectively). Species included *Charcarodon carcharias*, *Sphyrna zygaena*, *Heterodontus portusjacksoni*, *Carcharhinus tilstoni*, *Carcharhinus brachyurus*, *Carcharhinus brevipinna*, *Pristiophorus nudipinnis*, *Squatina albipunctata*. Animals were at varied stages of maturity and age. Cervical vertebrae were retrieved from the samples and roughly cleaned of tissue with a sharp knife. Each vertebra was then kept frozen until ready for sectioning. To determine whether there were links between prenatal yolk and zinc concentrations, a sample of yolk was taken from one of the mature specimens tested (*S. albipunctata*).

Each sample was manually cleaned with a sterile scalpel before being sectioned. No chemical agents were used to avoid possible contamination (e.g. sodium hypochlorite, ethanol).

Samples were sectioned using an Isomet circular saw with a diamond-edged blade. Sections were cut dorso-ventrally through the centre of the vertebrae. Cuts were roughly 0.6mm in thickness dependent on the calcification (more calcified specimens could be cut thinner). Each cut was immediately placed on Kapton film and covered with Kapton adhesive tape. This created an airtight seal that would prevent dehydration of the samples that can cause severe tissue warping. Samples were then placed between two microscope slides to keep them flat during transport to the Australian Synchrotron in Melbourne.

Prepared samples were attached to the Scanning X-ray Fluorescent Microscope's polycarbonate frames using clear double-sided tape, with roughly 15-20 samples per frame. Smaller samples that would require greater resolution were scanned at 15 micron resolution, (e.g. the sawsharks and angel sharks) while larger samples were scanned at 25 microns (e.g. the whaler sharks). Ideal resolution would be higher, but scanning resolution is time and area

dependent. Scanning time for each frame varied depending on the samples and total area covered, and was between 16 and 22 hours. Images were then processed and viewed using Geopixe. Graphs used to analytically determine zinc distribution were taken with data from the corpus calcarum, from the centre to the outer edge.

Results

For the majority of species, zinc was highly localised within the vertebrae. There were negligible zinc levels on the *corpus calcareum*, with the exception of the Port Jackson shark (*Heterodontus portjacksoni*) that displayed zinc banding (figure 1). Concentrations of zinc appeared to be highest pre-birth before varying with age. No zinc was present in the sampled egg-yolk.

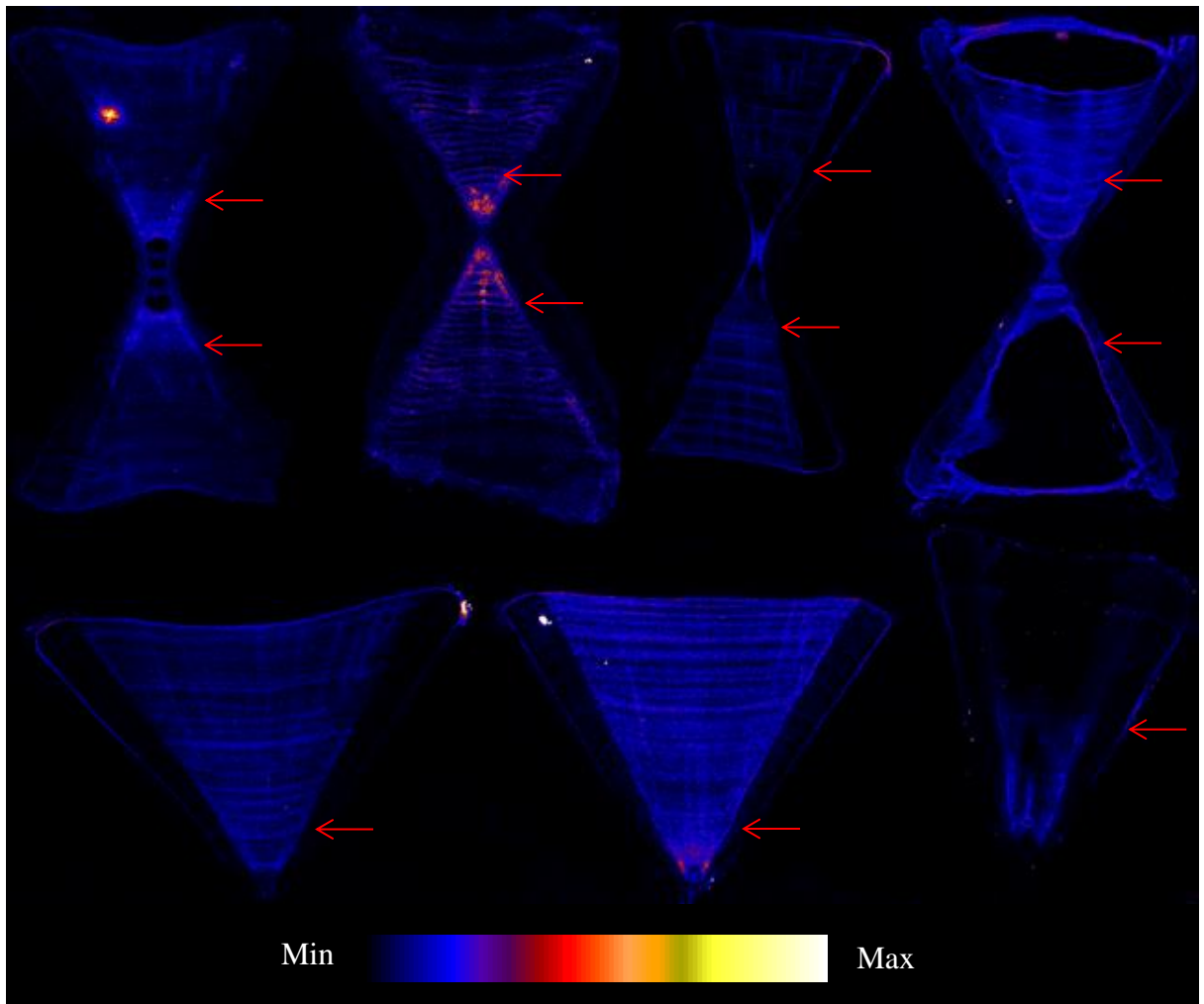


Figure 1: Scanning X-Ray Fluorescence Microscope (SXFM) image of shark vertebrae in the zinc spectrum (size not to scale) for (from left to right, top to bottom) smooth hammerhead (*Sphyrna zygaena*), eastern angel (*Squatina albipunctata*), blacktip (*Carcharhinus tilstoni*), port Jackson (*Heterodontus portjacksoni*), spinner (*Carcharhinus brevipinna*), dusky (*Carcharhinus obscurus*), and great white sharks (*Carcharodon carcharias*). Lighter colours signify higher concentrations of zinc. Zinc concentrations are highest in the *intermedialis* in all species, the highest concentrations often occurring pre-birth. Birth marks indicated with red arrows.

Discussion

This study found that many shark species have non-homogenous zinc concentrations in their vertebrae. Five of the eight species tested had high pre-birth zinc concentrations. Zinc was primarily located within the *intermedialis* and the outer surface of the vertebrae. Zinc bands were visible that correspond to visual age bands in the *intermedialis* (except for sawsharks and angel sharks, in which the *intermedialis* bands are not correlated with age).

Zinc distribution was highly localised in all the species tested. Zinc concentrations were higher in the *intermedialis* than the *corpus calcarum*. The assumption that vertebral growth is correlated between the *intermedialis* and the *corpus calcarum* implies that different coordinated growth processes are occurring in shark vertebrae, and these processes require further investigation. Zinc concentrations in the *intermedialis* correlated with historical visual age banding patterns. This implies that zinc concentrations post-birth are related to growth processes.

Five of the species had higher pre-birth zinc concentrations than post-birth in the *intermedialis*. Some species of shark have been known to display maternal signatures (Campana *et al.* 2010; Estrada *et al.* 2006), with varied development types. This study agrees with their conclusions. As both aplacental (*Squatina albiguttata*) and placental (*Sphyrna tiburo*) species had higher pre-birth concentrations, maternal signatures are independent of development type. In this study, zinc was not detectable in maternal yolk sacks, however, a greater number of samples would need to be analysed to ensure the validity of this result. It is probably that pre-birth zinc is absorbed from the uterine fluid, which should have zinc concentrations similar to the environment.

Future studies should perform controlled experiments to determine why some species have comparatively higher pre-birth zinc concentrations in their *intermedialis*. The marked difference between *intermedialis* and *corpus calcarum* zinc concentrations also suggests that differential processes are involved in vertebral deposition between these two structures, and these processes require further examination.

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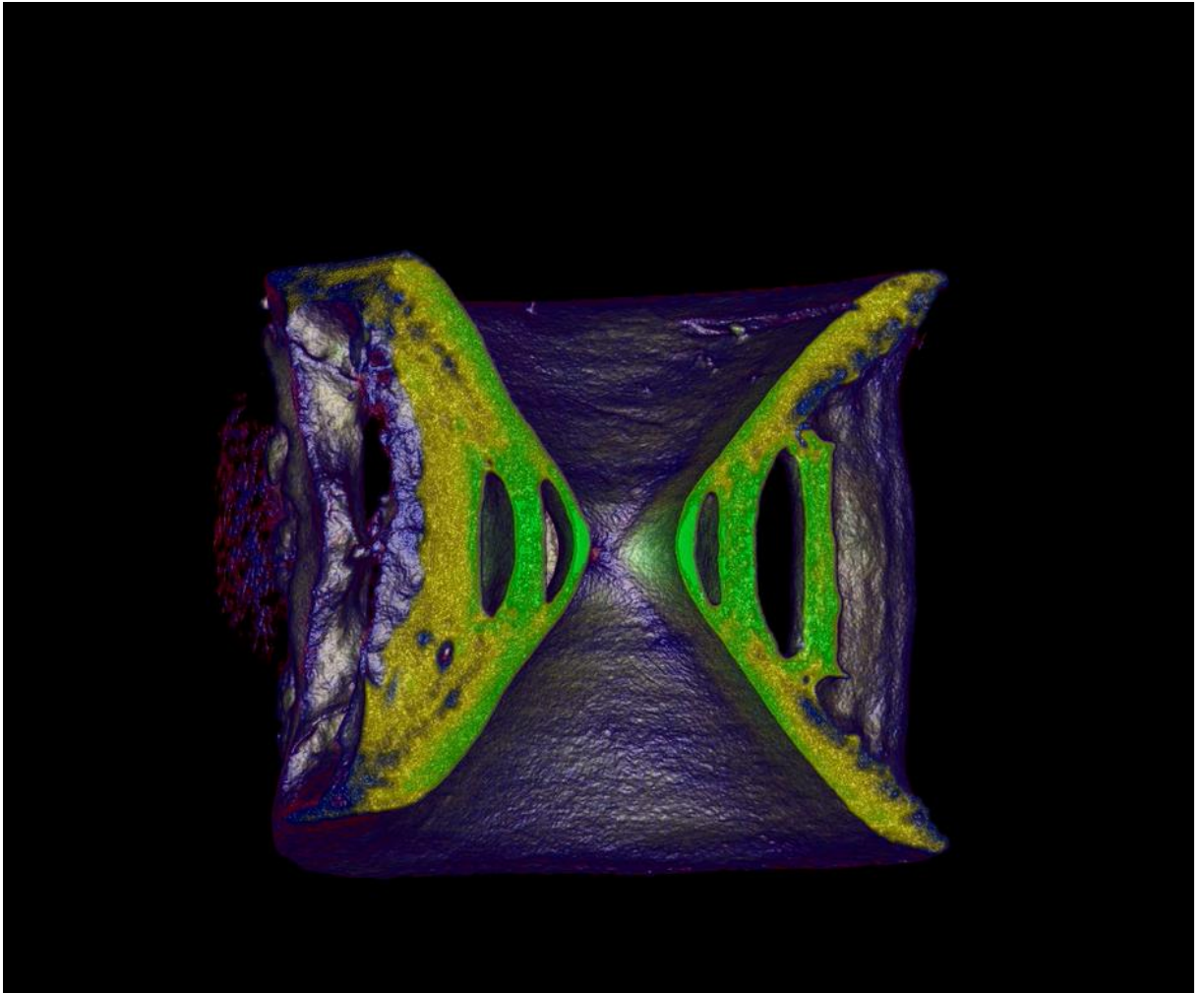
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Appendix II: MicroCT as a universal elasmobranch ageing tool

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MicroCT image of sawshark (*P. nudipinnis*) sectioned cervical vertebrae, taken at AINSE Lucas Heights facility by David Zahra

Abstract

Accurate elasmobranch ageing is necessary in order to correctly estimate the recovery rates of wild-caught populations. The success rates of the available ageing techniques vary greatly, and some experts in the field have suggested that a more consistent, widely applicable technique is necessary to enable direct comparisons between different studies. This study tried to replicate results of elemental studies using the more widely-available microCT. Similar bands were detected as those using an SXFM in species that were traditionally not thought to display growth bands (*Squatina australis*, *Squatina albipunctata*, *Pristiophorus nudipinnis*, *Pristiophorus cirratus*). This, in conjunction with the past use of microCT for *Charcharhinid* species, suggests that microCT may be the elasmobranch ageing technique for the future.

Introduction

Increased levels of commercial fishing have led to significant pressure on oceanic ecosystems. Commercial efforts targeting large predatory fish by industrial fishing techniques has lowered populations to less than 10% of their historic numbers (Worm and Branch 2012) and has prompted worldwide concern regarding the status of these stocks. Sharks are especially vulnerable due to their low reproductive rates and delayed maturity (Baum and Worm 2009; Ward-Paige *et al.* 2010) and have highlighted the need for sustainable exploitation by appropriate management of associated fisheries (Ferretti *et al.* 2010). Their role as apex predators in many marine ecosystems (Myers *et al.* 2007) and charismatic nature has meant that considerable resources have been allocated to their management and conservation. However, basic information about life history parameters such as reproduction, diet and age structure are often lacking, making an assessment of shark stocks difficult and potentially leading to fishery mismanagement.

Age determination of shark species is difficult, highly variable and species specific. Inaccurate estimates lead to poor predictions of population growth, and subsequent overfishing (Campana 2001). Typically, shark ageing has been based on a modification of fish otolith ageing (Cailliet *et al.* 2006). Vertebral bands in sharks have been counted, similar to the rings in a tree, for yearly growth patterns (Cailliet *et al.* 2006), under the assumption that banding patterns result from changes in calcification rates proportional to seasonal fluctuations in rates of growth (Cailliet *et al.* 1983). The visibility and nature of this banding pattern is determined by the vertebral anatomy. Larger pelagic species characteristically have circular shaped vertebrae and higher levels of calcification, making the banding pattern prominent and relatively easily discernible. However, the smaller benthic species tend to have lower, more variable levels of calcification and irregular shaped vertebrae resulting in banding patterns being much more difficult to interpret and often not visible at all.

The technique of vertebral band counting is not a universal or robust method for age determination and the mechanisms leading to the formation of these bands is not yet fully understood. Therefore, published shark ageing methods vary significantly because different preparatory methods must be tested to determine the most effective technique for each new species (Goldman *et al.* 2012). Different ageing methods and subjective differences in interpretation make comparative studies problematic (Francis, Campana and Jones 2007). The problematic application to smaller benthic species is potentially leading to mismanagement of this fishery in particular, leading to over, or under, utilisation.

To this day, much of this analysis is conducted on sagittally cut vertebral sections. The physical manipulations involved are not suitable for small, low calcified species and are a potentially high source of error affecting the counting process. Micro-computed X-ray tomography (micro CT) has been demonstrated as a valid alternative to sectioning vertebrae, allowing the sample to be ‘virtually’ sectioned along any plane (Geraghty *et al.* 2012). After

dissection, very little manipulation of the sample is required before the acquisition. Micro CT is, therefore, a potentially suitable technique for vertebral band analysis of relatively small and soft samples.

Four benthic species of shark that are regularly fished on the east coast of Australia, for which no age profiles have been successfully determined, were investigated by producing micro CT 3D reconstructions of dissected vertebrae. Two species angel shark, the Australian (*Squatina australis*) and Eastern (*S.albipunctata*) angel sharks, and two species of sawhark, the southern (*Pristiophorus nudipinnis*) and common (*P.cirratus*) sawsharks, were collected from various commercial and scientific fishing vessels along the south eastern coast of Australia from Newcastle to Tasmania.

Methods

Sample preparation – Dry: Vertebrae samples were removed from saline solution and left to dry on an absorbent mat for 1hr prior to scanning. The sample was then placed in a plastic bag to prevent equipment contamination and mounted on the carbon fibre scanner bed and secured with painter's masking tape (strong adhesion but low residue upon removal). Sample acquisition was tested in both directions.

X-ray computed tomography (CT) Imaging: CT images were acquired using a pre-clinical Siemens Inveon Multi-modality PET/CT (Siemens, Knoxville TN) with version 1.5 of the Inveon Acquisition Workplace software. The CT component is equipped with the standard SRI cone-beam x-ray source (80kV_p max voltage, 500uA max current), and the standard Dalsa CCD camera options. All CT scans of the *Squatina* spp. and *Pristiophorus* spp. performed under high magnification and no binning i.e. bin = 1. Using the maximum x-ray tube voltage and current the maximum exposure time (9000ms) was required to obtain sufficient information and minimise noise. This resulted in very long scan times; in excess of

one hour for the target image acquisition, along with another hour for the dark / light calibration prior to each scan. CT Centre-offset calibrations were performed prior to each day's scanning due to the high-resolution requirements being demanded of the system. This helped to minimise artefacts due to gantry rotation. The camera was oriented in "rat" mode with detector dimensions of x-pixels in the axial plane and y-pixels in the trans-axial. Source to detector distance was x, source to centre distance was y, which provided a magnification of z and a maximum field of view. Since we were acquiring images with no binning we adjusted the CCD field of view - using the CT pre-scan scout images as a guide - to the smallest area possible in the trans-axial dimension to minimise the data footprint. This made the visualisation of the resulting reconstructed images much more manageable. In order to provide a visual indication of the smallest resolvable object a MicroCT-Barpattern-Phantom (resin) developed by QRM GmbH, Germany was scanned using the same CT acquisition parameters.

Sample preparation – Sections: refer to Chapter 4

Synchrotron X-ray Fluorescence Microscopy (SXFm): Refer to Chapter 4

Sample preparation – Wet (halves): The vertebrae halves which were sliced for the SXFM scans were scanned in the CT again. This presents the situation where the features near the face which was cut should be very similar to what was observed in the associated sections scanned in the SXFM. The halves were placed in syringe tubes of small enough diameter such that they were not able to easily move around. The syringe tubes were filled with phosphate buffer solution (PBS) with both ends being plugged to prevent leaking. The syringe tube was placed horizontally along the axial plane of the carbon fibre scanner bed and secured with painter's masking tape for imaging.

CT reconstruction: all CT acquisitions were reconstructed with no downsampling, but otherwise using the system default settings (2DFBP [FDK], bilinear interpolation, Shepp-Logan filter).

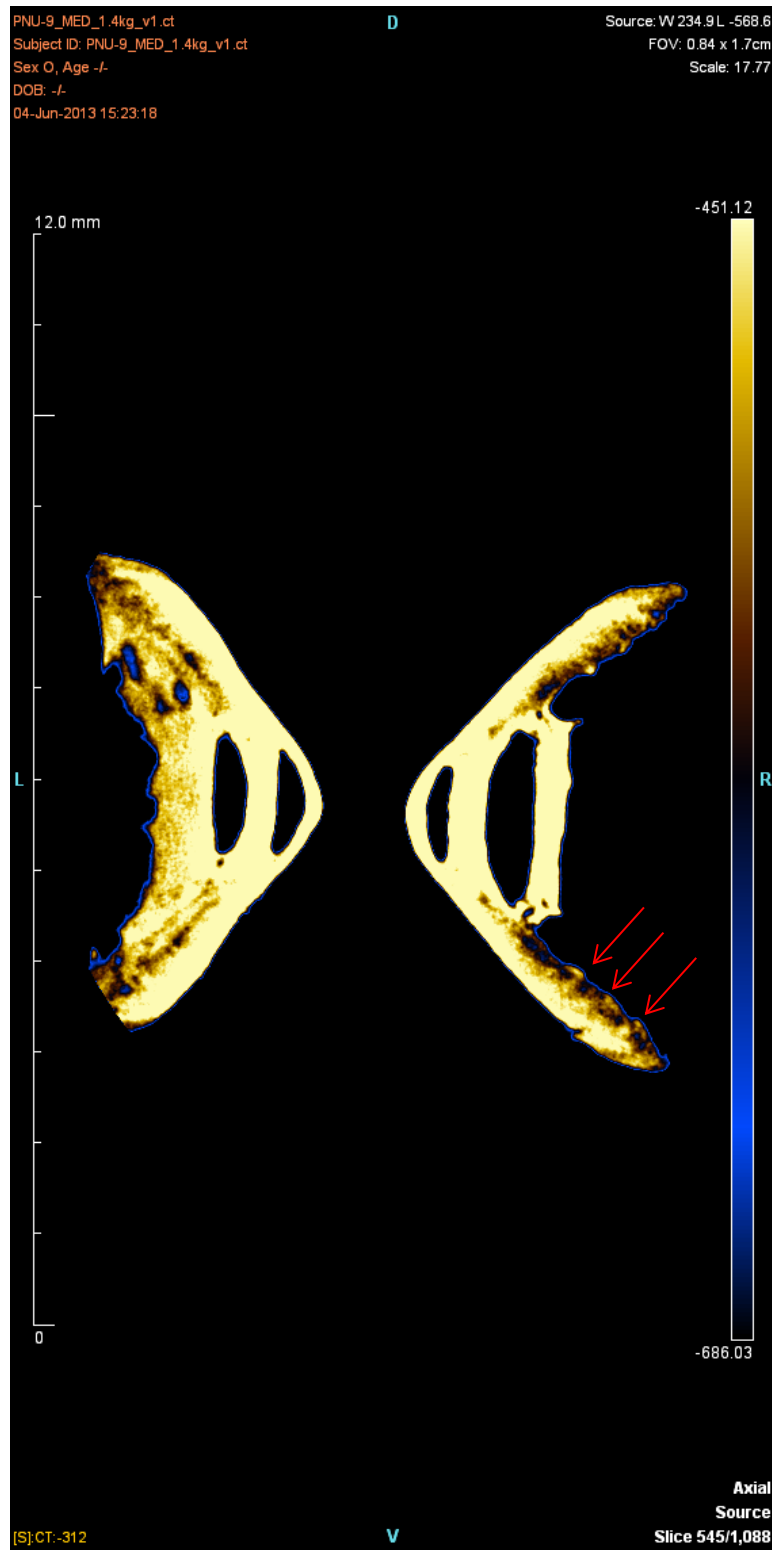


Figure 1: MicroCT scan of a *Pristiophorus nudipinnis* vertebra aligned perpendicularly to the vertebral plane and through the centre of the vertebra. Possible age bands indicated with red arrows.

Discussion

In previous work on *Charcharodon carcharias* vertebrae (O'Connor 2011) and publications by Geraghty *et al.* (2012) on spinner shark *Carcharhinus brevipinna*, microCT has been shown as an alternative method of determining the age of sharks using their vertebrae in a non-destructive fashion. For this study the focus was to observe the age bands some more problematic species; the *Squatina australis*, *S. albipunctata* and *Pristiophorus nudipinnis*, *P. cirratus*. In order to first show a visual indication of the smallest resolvable object, the microCT bar phantom was scanned. The smallest distinguishable bar and dot patterns are ~50µm in size (figure 1). The resulting images from the CT showed that the banding was not as distinct as for *Charcharhinid* spp. that have been used as models for shark vertebral ageing (see Geraghty *et al.* 2012). There were a few issues observed with this technique, which were also potentially preventing the observation of age bands. One notable issue, especially with the sawshark samples due to their soft gelatinous internal structure, was motion artefacts as a result of the dehydration shrinking during scanning. Although not as obvious in the *Squatina* spp. samples, this slight motion may have been enough to blur out the small structures. Note that prior to this study no observations of growth bands in these species had been made. For the angel shark samples the dehydration shrinking also resulted in more severe cracking of the vertebral samples due to more complicated variations in the structure as compared to the *Pristiophorus* spp. samples, while also causing uneven shrinking and deformation of the *corpus calcareum* (the outside of the vertebrae) thus hiding the band structure to a greater extent than the sawshark samples. It was determined that this technique was perhaps not the most suitable to visualize the information we were after, however with the hint of some form of banding we had enough justification to apply for access to SXFM beamlines.

The bulk of the SXFM results and their findings are already described in our previous work (Raoult *et al.* 2016), so this will only be a short recount of how it was used to verify the CT

data. The *Squatina* spp. (shown in chapter 4) and *Pristiophorus* spp. vertebral sections do have banding that may be annual, however the form this banding takes is somewhat different to what has been observed in the larger pelagic species. Where observations of these pelagic sharks tend to be concentric rings, the bands on the *Squatina* spp. and *Pristiophorus* spp. appear to have a more layered structure. Although clear using this technique you will note that the contrast between these bands is comparatively not particularly high, this would be one reason why they are so difficult to identify using traditional techniques. The synchrotron scan had been performed at a resolution of 25um, which is better than what we are able to achieve using the CT (~50um, figure 1), however with a little modification to our sample preparation we may be able to reproduce this with the much more accessible CT while also verifying the observations with the corresponding SXFM scans.

Given the success of the synchrotron imaging data, and now having a clear idea of the true structure of the bands, an additional effort was undertaken to achieve similar results from the CT. As was identified previously in the standard *ex vivo* CT results, there were two major issues observed: cracking and shrinking of the vertebrae samples resulting in damage/loss of fine structure, and motion artefacts. Both of these issues being due to dehydration during the long scan times required to achieve the resolution to visualise the vertebral banding of these species. Allowing the sample to dry for a period prior to scanning helped to minimise the motion artefact but, there was still the damage to the fine structure caused by shrinking from water loss. This is consistent with various other sample preparation techniques in that an ideal treatment for one component of the morphology is often detrimental to the other as summarised by Dean *et al.* (2008). Due to the non-destructive nature of CT we could combat this by developing a hydrated CT acquisition method. Since the CT Hounsfield Unit value of water is much lower than that of the mineralised vertebral samples, the impact of the water or any connective soft tissue would have a negligible impact on the scan. Due to their constant

hydration, there was little observed dehydration of the vertebrae, and the movement artefacts previously observed were no longer present. Although the resolution was not as high as that obtained by the SXFM images, the bands were easier to identify than those obtained by the standard ex vivo CT method, and when compared to the associated SXFM sample image are of the same shape and positioning. The delicate but still relatively large size of these samples makes them unsuitable for cryo-micro CT as we observe sample degradation and obscuring of banding as a result of freezing. Performing the acquisitions at room temp in isotonic buffer allowed for ex vivo micro CT sample analysis with minimal sample degradation. The maintenance of in vivo vertebral anatomy during the ex vivo procedure provided the contrast required to visualize the vertebral banding and requires no modification to the existing sample environment of the micro CT.

Conclusion

Raoult *et al.* (2016) demonstrated previously that vertebral growth banding is present in these species by using a high-resolution synchrotron radiation X-ray fluorescent microscopy technique, where previously it had been described as absent. While synchrotron-based x-ray microscopy is unrivaled for its high resolution, contrast and large available scanning areas, the limited availability of beam time and expense make it unviable for the acquisition of data sets with the sample sizes required for ecological assessment and population management of elasmobranchs. This study demonstrates the utility of synchrotron techniques in guiding the application and development of more accessible lab-based techniques.

The above technique allows the analysis of vertebral banding in four species of small benthic shark using relatively cheap lab based micro CT and requires very little modification to the sample environment of the instrument. We also demonstrate some considerations that must be taken into account when performing ex vivo micro CT analysis of semi-soft biological tissues.

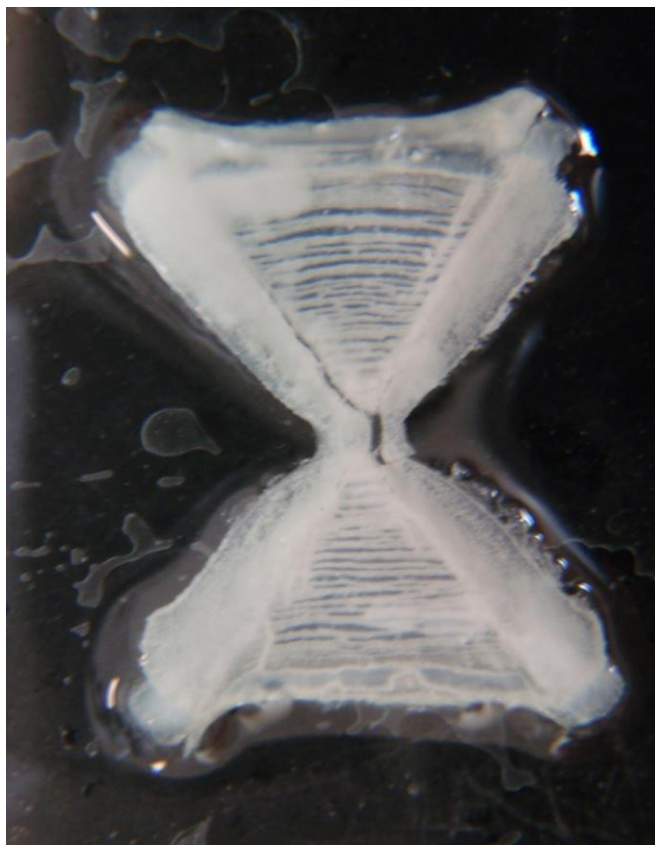
This development provides a viable method for obtaining statistically significant age data on these species hopefully leading better to better understanding of their population dynamics and appropriate management of the associated fishery.

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APPENDIX III: Preliminary age analysis of angel sharks and sawsharks



Section of *Squatina australis* vertebrae. Notice the distinct bands in the centrum that are related to girth but not age, no apparent banding in the *corpus calcareum*, and the low levels of calcification.

Introduction

Traditional ageing methods are ineffective on angel sharks and sawsharks (Natanson and Cailliet, 1990; Baremore *et al.*, 2009). In addition, vertebral preparation in sawsharks is made more difficult due to their small size, lack of calcification, and a central vertebral hole that means sectioned halves can separate (personal observation, also see figure 1). Previous research suggests that measuring strontium banding can be used as a proxy for age bands in many shark species or as a proxy for movements across salinity gradients (Raoult *et al.* 2016). Ageing was attempted using a scanning x-ray fluorescence microscope (Paterson *et al.*, 2011) as part of a larger study (Raoult *et al.* 2016). Three sawsharks (two *P. cirratus* and one *P. nudipinnis*) and five angel sharks (one *S. albipunctata* and four *S. australis*) had vertebrae sectioned and examined using this equipment. Results were visually observed in the strontium spectrum, and strontium concentrations in the *corpus calcarum* were graphed.

Results

Growth bands were detectable on the *corpus calcarum* in the strontium spectrum in both species (figure 1). These correspond to traditional visual age bands, and may be indicative of age. Sawshark vertebral structure was unique, with a hole in the centre of the vertebra, and two large tissue layers in the intermedialis. These tissue layers were consistent in both newborn and larger individuals. Layering was visible on the *corpus calcarum* of both angel shark species and appeared to occur along the entire span of the vertebra.

Ageing data suggest that all four species are relatively fast-growing. The two largest *Squatina australis* had four detectable strontium peaks in a 946mm total length male (figure 2) and five peaks in an 839mm total length male (Chapter 4), indicating ages of four and five years,

respectively. The largest sawshark tested, a 932mm mature female *P. nudipinnis*, had 3 detectable peaks, indicating an age of at least 3 years (figure 3).

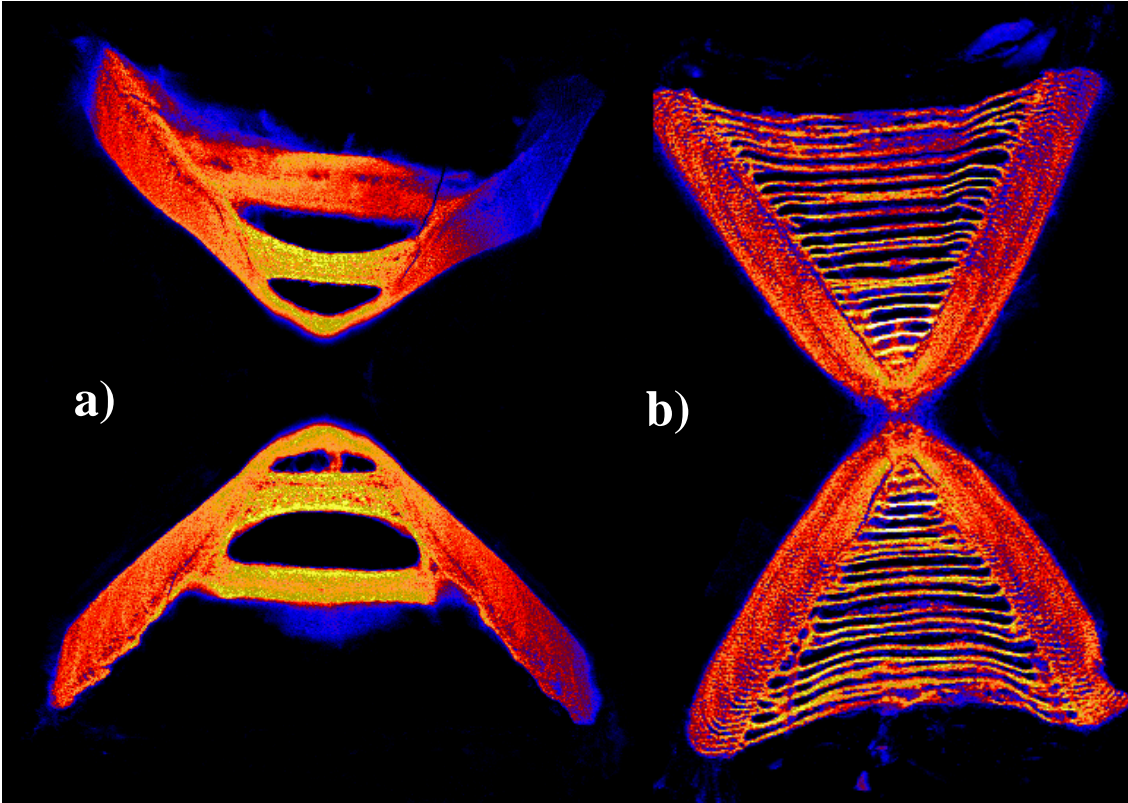


Figure 1: SXFM image in the strontium spectrum of dorso-ventral vertebral sections of a 932mm female *Pristiophorus nudipinnis* (a), and a 946mm male *Squatina australis* (b).

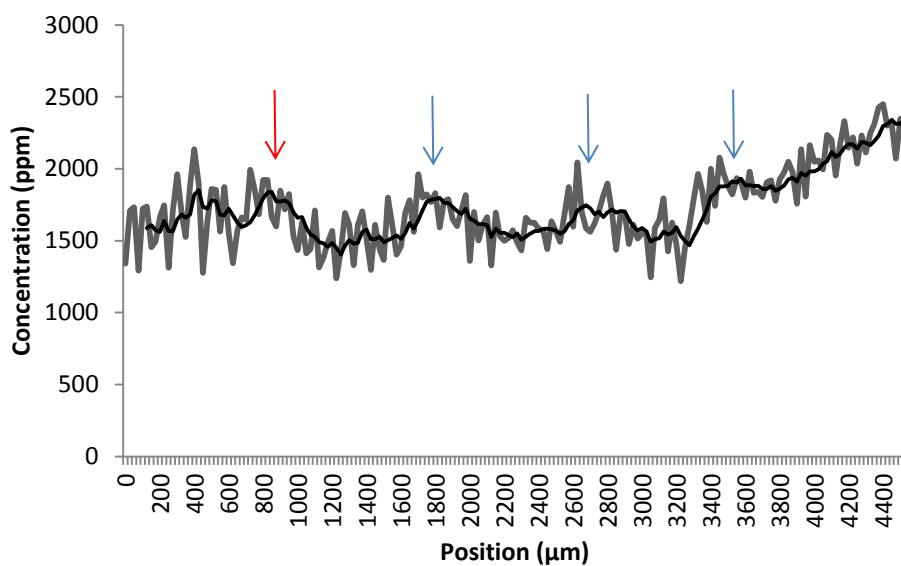


Figure 2: SXFM strontium concentrations on the dorsal edge of the *corpus calcarum* of a 946mm male *Squatina australis*. Calcium concentrations were divided by 60 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Concentration peaks and birth marks indicated by red and blue arrows, respectively.

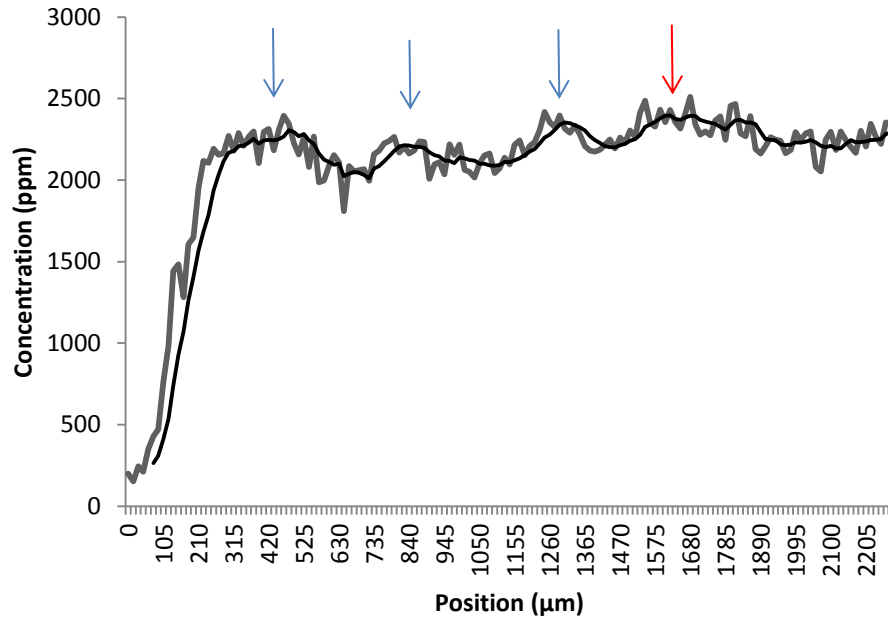


Figure 3: SXFM strontium concentrations in the *corpus calcarum* for a 932mm female *Pristiophorus nudipinnis*. Calcium concentrations were divided by 75 to make them comparable to strontium concentrations. 6 point moving averages were added to reduce noise. Concentration peaks and birth marks are indicated by blue and red arrows, respectively.

Discussion

It appears that growth until maturity is relatively rapid for both *Pristiophorus* spp. and *Squatina* spp. compared to other elasmobranch species, which often mature after 10 years of age, and sometimes as late as 20 years (Frisk *et al.* 2001). While age at maturity may be relatively young for these genera, previous tag-recapture studies have shown that *Squatina californica* can live to 27 years of age (Cailliet *et al.* 1992), which implies that *S. australis* and *S. albiguttata* individuals may be able to reproduce for at least 20 years. Elemental analyses may be the key to effectively determining the ages of these genera.

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Not all sawsharks are equal: species of co-existing sawsharks show plasticity in trophic consumption both within and between species

Vincent Raoult, Troy F. Gaston, and Jane E. Williamson

Abstract: Despite the global distribution of sawsharks, little is known about their diets or their role in the marine biosphere. As species in higher trophic positions are generally considered to be more at risk to perturbations such as fishing, understanding their role in the food chain will enable better conservation and management strategies for these species. Two sawshark species (*Pristiophorus cirratus*, *Pristiophorus nudipinnis*) co-occur in waters off east Tasmania, Australia. This study determined the trophic positions of these sawsharks and whether they avoided competing with each other through resource partitioning. Isotopic analysis of muscle tissue revealed that *P. cirratus* and *P. nudipinnis* had significantly different trophic levels, with *P. cirratus* likely to have a diet of primary consumers and *P. nudipinnis* likely to have a piscivorous diet. Owing to their different isotopic signatures, it is also likely that the sawshark rostrum has multiple functions. Both species shifted to higher trophic levels during ontogeny. Maternal isotopic signatures were detectable in *P. cirratus* juveniles.

Résumé : Malgré la répartition planétaire des requins-scies, leurs régimes alimentaires et le rôle qu'ils jouent dans la biosphère marine demeurent méconnus. Comme les espèces de position trophique supérieure sont généralement considérées comme étant plus vulnérables aux perturbations comme la pêche, la compréhension de leur rôle dans la chaîne alimentaire permettra d'améliorer les stratégies de conservation et de gestion visant ces espèces. Deux espèces de requins-scies (*Pristiophorus cirratus*, *Pristiophorus nudipinnis*) coexistent dans les eaux au large de la Tasmanie orientale (Australie). L'étude a permis de déterminer les positions trophiques de ces requins-scies et d'établir s'ils évitaient de se faire concurrence grâce au partage différentiel des ressources. L'analyse isotopique de tissus musculaires a révélé que *P. cirratus* et *P. nudipinnis* occupent des niveaux trophiques significativement différents, *P. cirratus* étant susceptibles d'avoir un régime alimentaire constitué de consommateurs primaires et *P. nudipinnis* étant susceptible d'avoir une alimentation piscivore. Étant donné ces signatures isotopiques distinctes, il est également probable que les rostres des requins-scies aient plus d'une fonction. Les deux espèces passaient à des niveaux trophiques plus élevés durant l'ontogenèse. Des signatures isotopiques maternelles étaient décelables chez les *P. cirratus* juvéniles. [Traduit par la Rédaction]

Introduction

Species living in close proximity will tend to reduce competition through niche divergence or resource partitioning when faced with limited resources (Ross 1986; Schoener 1974). While terrestrial animals generally tend toward spatial niche separation (MacArthur 1965), trophic separation is commonly observed among organisms in the marine environment (Sale 1979). Research conducted in the area of resource partitioning often produces wide-ranging results, from competitive exclusion of one species through the development of diverging suboptimal resource use by that species (Schoener 1989) to competing species continuing to exist on a similar resource (Gabler and Amundsen 2010). Such conflicting results on diet partitioning may be a product of the difficulty of accurately quantifying species' diets through short-term snapshots of stomach contents (Baker et al. 2014) or inappropriate levels of taxonomic resolution in identification of prey (Barnes et al. 2011).

Stable isotopes are one marker that allows researchers to determine trophic level (Vander Zanden et al. 1997), migratory patterns (Hansson et al. 1997), and short-, medium-, and long-term diet shifts (Estrada et al. 2006; Hussey et al. 2011; Shiffman et al. 2012). Predators at high trophic levels enrich bioaccumulable markers

present at lower trophic levels, enabling researchers to identify distinct chemical signatures present at each trophic level (Hobson et al. 2002). Unlike traditional gut-content analyses, stable isotopes can provide average dietary information assimilated over time (Dalerum and Angerbjörn 2005) and are not affected by the digestibility of prey items, food expulsion during capture, or opportunistic feeding. Thus, stable isotopes are a more reliable indicator of trophic level and long-term diet patterns than other more traditional methods, which are still necessary to correctly identify prey species.

Interspecific competition between closely related species sharing a common resource can be symmetric, where either species can fill a particular trophic level or niche (Munday 2004), or asymmetric, where one competitor is superior and a trade-off occurs in relation to the resource (Munday et al. 2001; Schoener 1983; Young 2004). Body and mouth size often play important roles in such competition because larger individuals with wider mouths can potentially feed on a greater range of prey sizes (Cohen et al. 1993). It is thus commonly assumed that closely related species that are of similar size will have similar resource requirements. Where they co-occur, however, such species may have co-evolved to use the divergent aspects of that resource over time (Connell 1980). In this case, we would hypothesize that the species that utilize prey

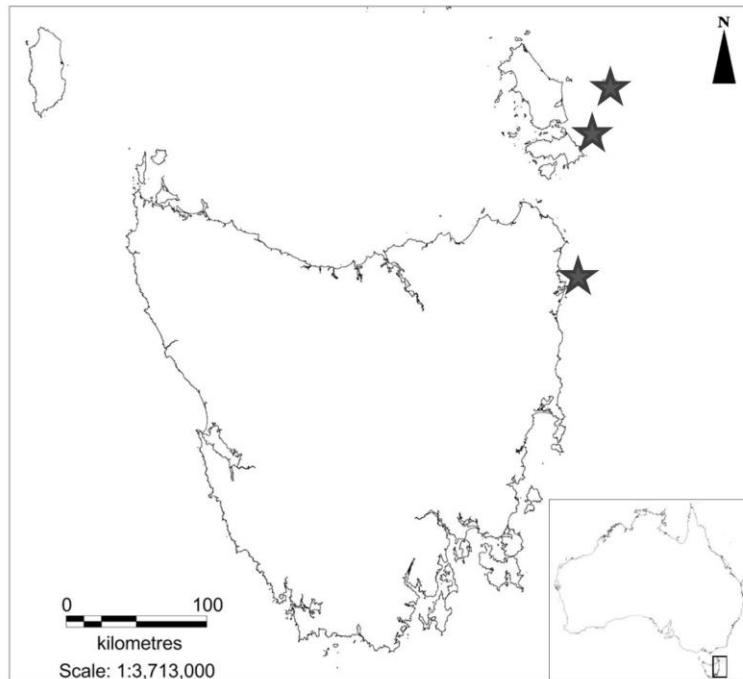
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Fig. 1. Map of Tasmania and research trawl sites (marked with stars).

from a higher trophic level would be gaining an advantage and would thus act as the superior competitor.

Sawsharks (Pristiophoridae) are elasmobranchs characterized by elongate bodies, ventral barbels, and saw-like rostrum (Last and Stevens 2009). To date, nine sawshark species have been identified, two of whom were classified in the last few years (Ebert and Cailliet 2011; Ebert and Wilms 2013). Two species co-occur in southeastern Australian waters: *Pristiophorus nudipinnis* and *Pristiophorus cirratus*; the entire range of *P. nudipinnis* is within the wider range of *P. cirratus* (Last and Stevens 2009). Both species grow to similar lengths and masses and can only be visually differentiated by slight changes in their rostral/body length ratios (higher for *P. cirratus*), the position of their rostral barbels (closer to the mouth for *P. nudipinnis*), and their colouration (spotted yellow or brown for *P. cirratus*, uniform brown for *P. nudipinnis*; Last and Stevens 2009). Both species occur on the continental shelf at depths between 50 and 500 m and predominantly inhabit sandy benthic environments (V. Raoult, V. Peddemors, K. Rowling, and J.E. Williamson, unpublished data).

Little is known about the diets or ecology of sawsharks. Research on the critically endangered largemouth sawfish (*Pristis microdon*) found that the saw-like rostrum is used to disable schools of fish in the water column (Wueringer et al. 2012). Sawfish rostral teeth are permanent and much thicker and smoother than the finer, more jagged sawshark teeth that are frequently replaced (Slaughter and Springer 1968). While the two taxa have evolved rostral saws independently (Aschliman et al. 2012), it is highly probable that the rostrum may serve a different use between sawfish and sawsharks. Alternate uses for the rostrum could include defence from predators or foraging through sediment.

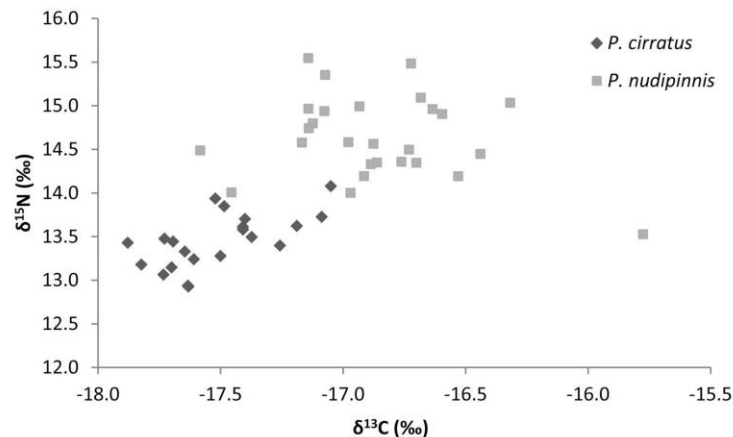
Three hundred tonnes of *P. cirratus* and *P. nudipinnis* valued at over AU\$2 million are caught annually in southeastern Australian commercial fisheries. The majority of this catch is bycatch via gill nets and fish trawling and few other commercial fishing methods

(i.e., demersal longlining) capture these fish reliably (V. Raoult, V. Peddemors, K. Rowling, and J.E. Williamson, unpublished data). Australian government authorities (e.g., the New South Wales Department of Primary Industries) have flagged *P. nudipinnis* and *P. cirratus* for target research owing to the large number of sharks caught yearly and the dearth of information on their life-history traits.

We hypothesized that where *P. cirratus* and *P. nudipinnis* co-occurred, the two species would have divergent prey resources at different trophic levels to reduce dietary overlap and thus competition despite their similar morphology. Moreover, we hypothesized that if trophic separation occurred, such dietary divergence would occur between the two species throughout ontogeny. This study used muscle-tissue isotope analysis to determine the trophic levels of juvenile and adult *P. cirratus* and *P. nudipinnis* from the northeastern coast of Tasmania, Australia, to ascertain their average trophic level. Using this trophic data, it may be possible to infer diet composition and therefore the use of the sawshark rostrum during feeding. The ontogenetic profiles of these species were compared against those from other ontogenetic studies worldwide to document whether patterns of isotopic plasticity were prevalent in sharks. A greater understanding of the trophic levels of *P. cirratus* and *P. nudipinnis* will increase our knowledge of the ecology of the community and thus reduce uncertainty in management and conservation efforts for these understudied species.

Materials and methods

Pristiophorus cirratus and *P. nudipinnis* were collected as bycatch from repeated research trawls on board the Australian Maritime College vessel *FTV Bluefin* in July, August, and December 2011–2012 along the northeastern coastline of Tasmania, Australia (40°18.101 S, 148°33.596 E; Fig. 1). These trawls were aimed at catching the majority of the benthic community. Two trawl methods were

Fig. 2. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for individual *P. cirratus* and *P. nudipinnis*.

used: one at a depth of ~30 m while using a 35 mm mesh prawn net and another at a depth of ~80 m while using a 70 mm mesh demersal fish net. All trawls were conducted for 30 min at a speed of ~3.1 knots (1 knot = 1.852 km·h⁻¹).

Pristiophorus nudipinnis and *P. cirratus* caught in trawls were measured (total length, rostral length, mouth width, fins dimensions, in mm) and weighed (g). Each animal was then dissected immediately. The muscle in marine elasmobranchs represents, on average, a reliable isotopic signature for diet over the past months to years (Kim et al. 2012a; MacNeil et al. 2006; Madigan et al. 2012). Muscle tissue samples (~3 cm³) were therefore taken from the area dorsal of the cervical vertebra for isotope analyses (Hussey et al. 2012b; Post et al. 2007). Care was taken to ensure samples were taken from the same position on each animal. Muscle samples were stored in 5 mL screw-cap tubes and frozen at -20 °C for storage until analysis as recommended by Kim and Koch (2012). Twenty-one *P. cirratus* muscle samples were collected from different individuals along with 24 *P. nudipinnis* samples.

Muscle samples were dried at 60 °C for 36 h, ground to a fine powder using a mortar and pestle, and 1–2 mg was transferred into tin capsules, as per Bessey and Vanderklift (2014). Samples were then analysed for carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N) stable isotopes using a Europa EA GSL elemental analyser coupled to a Hydra 2022 mass spectrometer (Sercon Ltd., UK) at Griffith University (Queensland, Australia). Precision for this spectrometer is expected to be within 0.2 for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios. The ratios of ¹³C/¹²C ($\delta^{13}\text{C}$) and ¹⁵N/¹⁴N ($\delta^{15}\text{N}$) were expressed as the relative per thousand (‰) difference between the sample and standard (Pee Dee Belemnite for carbon and atmospheric nitrogen for nitrogen).

To assess whether the isotope ratios (and thus diet) differed significantly between *P. nudipinnis* and *P. cirratus*, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values were analysed with a two-sample *t* test. Linear regressions were then used to determine the relationship between length and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for both species. Individuals were further split into juveniles and adults (*P. nudipinnis* > 800 mm total length (TL) were adults (*n* = 10); *P. cirratus* > 600 mm TL were adults (*n* = 14)), and the relationship between length (and thus maturity) and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were determined. Mouth width is known to be related to prey choice (Karpouzi and Stergiou 2003; Kwak et al. 2004); consequently mouth width was compared with $\delta^{15}\text{N}$ to determine whether the mouth size difference of the two species may have an effect on prey choice using a generalized linear model (GLM). Studies that compared juvenile and adult shark isotopic ratios were compiled and contrasted with this study; only studies that examined sharks from juvenile to adulthood were used.

Results

Different trophic levels were observed for *P. cirratus* and *P. nudipinnis*. Mean isotope ratios were significantly different between the two species for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (*df* = 44, *t* = 2.02, *p* < 0.01 in both cases; Fig. 2). Trophic shifts occurred during ontogeny in both *P. cirratus* and *P. nudipinnis*; $\delta^{15}\text{N}$ was significantly positively correlated with length (*P. cirratus*: *df* = 1, *F* = 16.39, *p* < 0.01, *R*² = 0.46; *P. nudipinnis*: *df* = 1, *F* = 7.76, *p* = 0.01, *R*² = 0.25; Fig. 3). *Pristiophorus cirratus* $\delta^{13}\text{C}$ was significantly correlated with length (*df* = 1, *R*² = 0.43, *F* = 14.37, *p* < 0.01; Fig. 4). This change in $\delta^{13}\text{C}$ during ontogeny was not detectable in *P. nudipinnis* (*df* = 1, *R*² = 0.03, *F* = 0.95, *p* > 0.05; Fig. 4). There was no significant interaction between species and mouth width on $\delta^{15}\text{N}$ values (GLM, *F* = 0.04, *p* > 0.05; Fig. 5).

$\delta^{15}\text{N}$ values were negatively correlated with length in juvenile *P. cirratus* (*df* = 1, *R*² = 0.73, *F* = 11.31, *p* < 0.05). $\delta^{15}\text{N}$ values of juvenile *P. nudipinnis* and adult stages of *P. cirratus* and *P. nudipinnis* did not change during ontogeny, as they were not correlated with length (*df* = 1, *R*² = 0.19, *F* = 2.56, *p* > 0.05; *df* = 1, *R*² = 0.10, *F* = 1.52, *p* > 0.05; *df* = 1, *R*² = 0.39, *F* = 3.9, *p* > 0.05; respectively). While some studies suggest that lipid extraction from muscle tissue is necessary for some elasmobranch species (Hussey et al. 2012b), C:N ratios in these sharks had a mean of 2.65, indicating low lipid levels below the recommended threshold where lipid extractions or corrections are necessary (C:N > 3.5; Post et al. 2007).

It is unclear whether sharks always exhibit maternal isotopic signatures irrespective of the style of embryonic development and whether size is always related to prey choice. Furthermore, to determine likely prey items and trophic level, the results from this study were compared with those of Davenport and Bax (2002), which were also obtained in southeastern Australia; *P. cirratus* $\delta^{15}\text{N}$ values were in the upper range of tertiary consumers in the area, while *P. nudipinnis* $\delta^{15}\text{N}$ values were above tertiary consumers (Table 1). Twelve studies documented shark $\delta^{15}\text{N}$ isotope ratios from juveniles to adults (Table 2). Only two studies found maternal $\delta^{15}\text{N}$ signatures (only one *Carcharodon carcharias* study detected such signatures). Not all studies found significant $\delta^{15}\text{N}$ /size ratios, and some populations or individuals within the same species had different $\delta^{15}\text{N}$ /size slopes.

Discussion

Pristiophorus cirratus and *P. nudipinnis* caught in Tasmania exhibited significant inter- and intraspecific trophic plasticity. A comparison between isotope levels of *P. nudipinnis* and previous

Fig. 3. $\delta^{15}\text{N}$ in relation to total length for *P. cirratus* and *P. nudipinnis*. Trend lines show significant ($p < 0.01$) correlations between length and isotopic delta for each species.

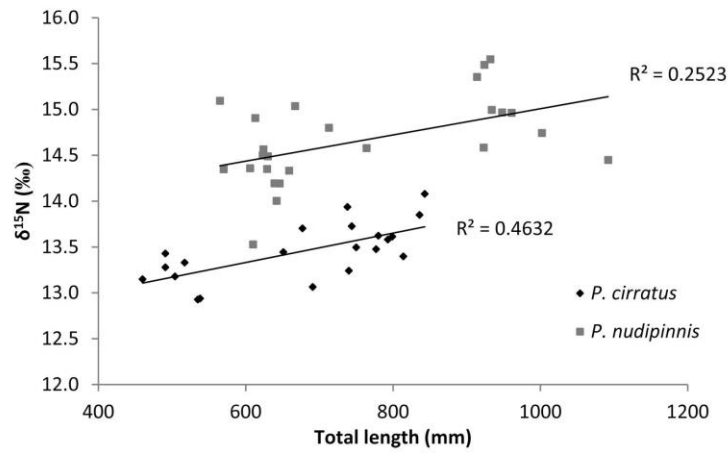


Fig. 4. $\delta^{13}\text{C}$ in relation to total length for *P. cirratus* and *P. nudipinnis*. Trend line shows significant ($p < 0.01$) correlation for *P. cirratus*.

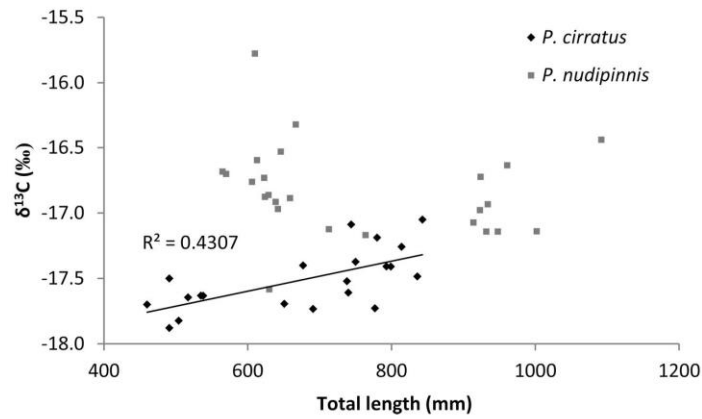


Fig. 5. Mouth width/ $\delta^{15}\text{N}$ relationship for *P. cirratus* and *P. nudipinnis*.

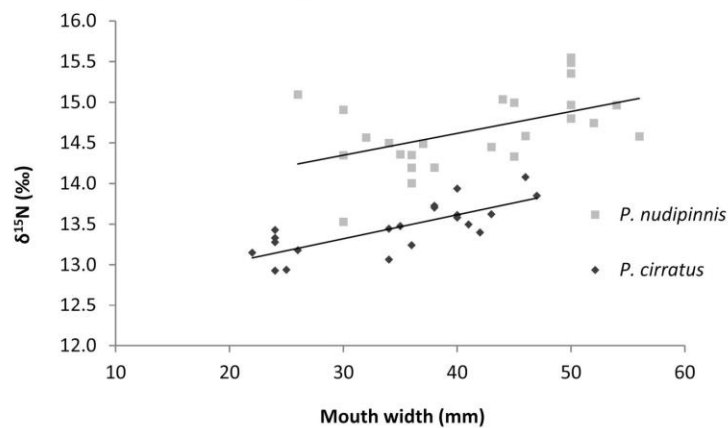


Table 1. Comparison of soft tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges from species caught in southeastern Australia (taken from Davenport and Bax (2002)) and the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Pristiophorus nudipinnis* and *Pristiophorus cirratus* from this study.

Consumer	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Tertiary consumers (e.g., pink ling, <i>Genypterus blacodes</i>)	12.2–13.8	(–14.6) – (–17.2)
Secondary consumers (e.g., elephant fish, <i>Callorhynchus milii</i>)	10.3–13.1	(–16.8) – (–19.1)
Primary consumers (e.g., ophiuroids)	5.3–9.4	(–16.7) – (–18.1)
<i>P. cirratus</i> (common (or longnose) sawshark)	12.9–14.1	(–17.1) – (–17.9)
<i>P. nudipinnis</i> (southern sawshark)	13.5–15.5	(–15.8) – (–17.1)

Table 2. Comparison of tissue $\delta^{15}\text{N}$ (‰) for studies that recorded isotopic levels through ontogeny.

Study	Species	$\delta^{15}\text{N}$ maternal signature (yes or no)	Significant $\delta^{15}\text{N}$ /size relationship (yes or no)	Slope of $\delta^{15}\text{N}$ /size relationship
Current study	<i>Pristiophorus nudipinnis</i> (southern sawshark); <i>Pristiophorus cirratus</i> (common sawshark)	No; yes	Yes	Linear; linear
Borrell et al. 2011	<i>Rhincodon typus</i> (whale shark)	No	Yes	Logarithmic
Borrell et al. 2010	<i>Rhincodon typus</i> (whale shark); <i>Sphyrna lewini</i> (scalloped hammerhead)	No; no	Yes; yes	Logarithmic; logarithmic
Abrantes and Barnett 2011	<i>Notorynchus cepedianus</i> (broadnose sevengill shark)	No	Yes or no (depending on site)	Linear
Estrada et al. 2006	<i>Carcharodon carcharias</i> (great white shark)	Yes	Yes	Three-step
Fisk et al. 2002	<i>Somniosus microcephalus</i> (Greenland shark)	No (only two juveniles)	Yes	Linear
Speed et al. 2012	<i>Carcharhinus melanopterus</i> (blacktip reef shark); <i>Carcharhinus amblyrhynchos</i> (grey reef shark)	No	No	NA
Malpica-Cruz et al. 2013	<i>Isurus oxyrinchus</i> (shortfin mako); <i>Carcharodon carcharias</i> (great white shark)	No	Yes	Sigmoid; logarithmic
Kim et al. 2012b	<i>Carcharodon carcharias</i> (great white shark)	No	Yes or no (depending on individual)	Logarithmic or linear (depending on individuals)
Campana et al. 2010	<i>Carcharhinus leucas</i> (bull shark); <i>Galeocerdo cuvier</i> (tiger shark)	Yes	Yes; maybe	Inversed parabolic; possibly inversed parabolic
Hussey et al. 2012a	<i>Carcharodon carcharias</i> (great white shark)	No	Yes	Sigmoid or logarithmic
Werry et al. 2011	<i>Carcharhinus leucas</i> (bull shark)	No (perhaps in $\delta^{13}\text{C}$?)	Yes	Logarithmic
Papastamatiou et al. 2010	<i>Carcharhinus melanopterus</i> (blacktip reef shark)	No	Yes or no (dependent on region)	Linear

research suggests that their diet was predominantly piscivorous and were potentially quaternary consumers. Isotope values of *P. cirratus* are consistent with those of secondary and tertiary consumers. Ontogenetic shifts in diet were apparent between juveniles and adults in both species and in all but three of the ontogeny studies from other species of sharks. This is the first study to determine the trophic level of sawsharks.

The difference in isotope levels between *P. cirratus* and *P. nudipinnis* suggests that when co-occurring, they appear to avoid direct competition through resource partitioning at different trophic levels. Mean $\delta^{13}\text{C}$ values were significantly different between *P. cirratus* and *P. nudipinnis*, but this is unlikely to be indicative of separate diet sources and more likely a consequence of the higher trophic diet of *P. nudipinnis* (difference of 1‰), which causes higher carbon–nitrogen fractionation at higher trophic levels (Vander Zanden and Rasmussen 2001). A study by Kinney et al. (2011) detected resource partitioning in various juvenile shark species in shark nursery areas at comparable trophic levels; therefore, this may be a widespread occurrence with co-occurring elasmobranchs.

In competitive environments, larger animals have access to a greater size range of prey, and *P. nudipinnis* grow to lengths

200 mm longer than *P. cirratus*. The maximum size of each species size is unlikely to have a significant effect on prey choice, however, as there was no significant interaction between species and mouth width on isotope ratios. The main morphological differences between these two species, aside from total length and colour patterns, are the width and thickness of the rostrum (Last and Stevens 2009). The driver for resource partitioning between these two species of sawshark is therefore more likely to be behaviourally driven rather than morphological.

The function of the rostral saw in sawsharks is unknown, although they are routinely hypothesized to be used in a similar fashion as sawfish (*Pristid* spp.) for prey capture (Slaughter and Springer 1968; Wueringer et al. 2009). This study suggests that the trophic level of *P. cirratus* is similar to species that prey on benthic primary consumers; consequently, *P. cirratus* may use their rostrums to sift through substrate in the search of prey. The isotope levels of *P. nudipinnis* suggest a more piscivorous diet and raise the possibility that they feed in a similar manner as some Pristidae species (Wueringer et al. 2012). Further research into diet, feeding modes, and rostrum use is necessary to elucidate the specifics of feeding in these species.

Both species of sawshark were hypothesized to show similar traits because they share the same development pattern (aplacental viviparity), and the few studies on sawshark phylogeny suggest they are a highly related monophyletic group (Dosay-Akbulut 2006). The negative isotopic correlation of juvenile *P. cirratus* was indicative of maternal isotope signatures. This pattern was not visible in *P. nudipinnis* samples, possibly owing to the lack of very young individuals in this data set. Other shark species have similar maternal isotopic signatures or fractionation in juveniles (Campana et al. 2010; Vaudo et al. 2010); however, only two studies that analysed isotopic signatures throughout development detected such patterns (Campana et al. 2010; Estrada et al. 2006). Maternal signatures are lost quickly after birth (Olin et al. 2011), and the lack of newborns in data sets underestimates the number of shark species with maternal isotopic signatures. Future research should examine the drivers and diversity of maternal isotopic signatures in elasmobranchs.

Adult isotope levels that were not correlated with length suggest that a dietary shift occurs quickly during the subadult phase in *P. cirratus* and *P. nudipinnis*. As similar ontogenetic patterns have been observed in other species of sharks (Kim et al. 2012b), it is possible that different sawshark trophic ecotypes exist in other areas or that in areas where the two species do not co-occur it is possible that the remaining species will fill the available trophic niche. The majority of studies that examined isotope ratios during ontogeny found similar length-correlated ratios (Fig. 2), though the degree of variation during ontogeny varied inter- and intra-specifically. This suggests that ontogenetic shift in diet may be partially linked to species-specific traits. Geographically distinct populations of certain species also have different patterns of trophic shift (Abrantes and Barnett 2011), suggesting that an ontogenetic shift in diet may also be related to prey availability and (or) environmental conditions. The spread of isotopic deltas per length (in this study as well as others listed above) suggests a high degree of individual feeding preference within species (Kim et al. 2012b). Studies that examine trophic plasticity through ontogeny must therefore do so on small scales and at high resolutions, otherwise results will be diluted because of noise from inter- and intraspecific variability. Furthermore, these conclusions suggest that changes in temporal and geographical variables greatly affect trophic studies that use stable isotopes, and further research should aim to quantify the level of these effects.

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Biology of angel sharks (*Squatina* sp.) and sawsharks (*Pristiophorus* sp.) caught in south-eastern Australian trawl fisheries and the New South Wales shark-meshing (bather-protection) program

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Abstract. Two species of angel shark (*Squatina australis*, *S. albiguttata*) and two species of sawshark (*Pristiophorus nudipinnis*, *P. cirratus*) are frequently caught in south-eastern Australia. Little is known of the biology of these elasmobranchs, despite being caught as secondary target species in large numbers. The present study collected morphometric and reproductive data from sharks caught in shark-control nets, commercial fishing trawlers and research trawlers in south-eastern Australia. All four species had female-biased sexual size dimorphism, but growth curves between sexes did not differ. Male *S. australis* individuals were fully mature at ~800-mm total length, male *P. nudipinnis* at ~900 mm, and male *P. cirratus* at ~800 mm. Anterior pectoral margins could be used to determine total length in all species. No morphometric measurement could reliably separate *Squatina* spp. or *Pristiophorus* spp., although *S. albiguttata* over 1000-mm total length had larger eyes than did *S. australis*.

Additional keywords: elasmobranchs, management, reproductive biology.

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Introduction

Charismatic species have traditionally received more research attention and funding than less attractive species (Clark and May 2002). Although such an imbalance can have positive effects such as protecting large habitats (Carroll *et al.* 2001) or increasing awareness (Sergio *et al.* 2006), it still leaves the majority of species with unknown population statuses and biology. Focussed research raises the possibility of species disappearing before investigation, and the problem is even more apparent for small, deep-ocean chondrichthyan species that may be subject to intensive fishing effort (Francis and Lyon 2012). Two chondrichthyan groups that belong to this category are angel sharks (*Squatina* spp.) and sawsharks (*Pristiophorus* spp.).

Angel sharks are flat, ray-like ambush predators, found in the Indian, Pacific and Atlantic oceans (Cailliet *et al.* 1992; Last and White 2008; Baremore 2010). Two species of angel shark, namely, *Squatina australis* (the Australian angel shark) and *S. albiguttata* (the eastern angel shark), are endemic to south-eastern Australia, from Port Macquarie to Hobart (Last and Stevens 2009). Both species are caught commercially, yet information on their biology is lacking. Of the Australian angel sharks, only the ornate angel shark (*S. tergocellata*), which occurs in the Great Australian Bight, has been studied (Bridge

et al. 1998). Species of angel shark occurring in the eastern Pacific and Atlantic oceans have had a greater degree of scrutiny from the scientific community (Natanson and Cailliet 1986; Capapé *et al.* 2005; Baremore *et al.* 2010; Vaz and De Carvalho 2013), and some of these species have been classified as 'endangered' or 'critically endangered' (Hârşan and Petrescu-Mag 2008). Although recent analyses of catches from commercial and shark-meshing program have indicated that *S. australis* and *S. albiguttata* are fully fished and unlikely to fit the criteria of endangered (NSW DPI 2015), their populations are depleted by ~60% from initial stocks. The *Squatina* genus is historically one of the most endangered group of sharks in the world (Polidoro *et al.* 2009); therefore, further study of the Australian species is warranted.

Sawsharks are small, elongate elasmobranchs characterised by their saw-like rostrum. Despite their global distribution and frequent occurrence as by-catch in many fisheries, their biology also remains largely unknown. For example, three of the five species of sawshark currently registered in the IUCN Red List have unknown population trends (The IUCN Red List of Threatened Species, see <http://www.iucnredlist.org/>). Two species of sawsharks are endemic to south-eastern Australia, namely, *Pristiophorus cirratus* (common or longnose sawshark)

and *P. nudipinnis* (southern sawshark). Dietary analysis has shown that these two species have relatively high trophic positions and separate diets (Raoult *et al.* 2015). An examination of fisheries records in NSW shows that sawshark populations have declined in the past five decades (V. Raoult, K. Rowling, V. Peddemors, J. E. Williamson, unpubl. data), whereas an Australian Fisheries Management Authority report suggests that standardised catch rates are in a slight decline in the southern and eastern scalefish and shark fishery (Haddon 2012). Effective management guidelines are difficult to formulate without information on the basic biology of these species.

South-eastern Australian demersal trawl fisheries frequently catch angel sharks and sawsharks as by-catch, and these are subsequently sold as a low-value fish product, with a total market value of ~AU\$700 000 per annum (NSW DPI 2015). Owing to the low value of these fish, their carcasses have the heads and guts removed at sea to increase the available space in cargo holds for higher-value products, a process called truncation. Truncation is common for most sharks caught in Australian fisheries. Subsequent analyses of sharks are difficult because proxy measurements for total lengths are not known. Misidentification within these two groups of species in commercial data is commonplace because of their similarity and co-occurrence, leading to management difficulties (NSW DPI 2015). Identifying morphometric features that can easily separate the concurrent species would increase the accuracy of commercial datasets, because, currently, only rostrum length can be reliably used to separate *P. cirratus* from *P. nudipinnis* (Last and Stevens 2009).

The present study documents the biological characteristics of the following four species of angel sharks and sawsharks occurring in south-eastern Australia: *S. australis*, *S. albipunctata*, *P. cirratus* and *P. nudipinnis*. Length and weight relationships were assessed. Maturity and reproductive stages were compared to total length (TL) to assess reproductive status in each sex. Data were used to determine morphometric features that could separate species and to reliably estimate the total length from truncated specimens. Results from the present study provide morphometric guidelines that allow easier separation of concurrent species, predictions of TL from truncated specimens, and life-history parameters that could be used in future studies.

Materials and methods

Four shark species (*S. australis*, *S. albipunctata*, *P. cirratus*, *P. nudipinnis*) caught as a secondary target species from several fisheries, from December 2010 to October 2012, were assessed in the present study. Some *Squatina* spp. were caught by the New South Wales shark-meshing (bather-protection) program (SMP), which has nets in place during the summer (September–April) on beaches from Newcastle to Wollongong in New South Wales (NSW), Australia. The majority of *Squatina* spp. were caught by the *Maybelle*, a commercial fishing trawler based in Sydney, NSW, Australia. The *Maybelle* fishes on sandy flats at a depth of 80 m, and within 20 nautical miles (~37 km) from the coastline. *Pristiophorus* spp. and some *Squatina* spp. were caught by the scientific research trawler *Bluefin* of the Australian Maritime College. Trawls were conducted on the eastern coast of Tasmania, Australia (40°18'6"S, 148°33'35"E), with a 35-mm prawn net at a depth of ~30 m. Only whole specimens were used

in the study; direct contacts with commercial fishermen were made to ensure that they did not truncate specimens.

Species were identified using guidelines from Last and Stevens (2009). Morphometric measurements were based on measurements proposed by Last and White (2008) and used terminology from Francis (2006). In total, 50 measurements were taken for each sawshark and 38 for each angel shark; these were primarily fin and TL measurements, but included several anterior morphometric measurements (such as, for example, eye length, spiracle length, mouth width). Data were grouped by species, with the aim of estimating total lengths from other morphometric parameters.

Reproductive maturity for males was determined from the growth and calcification of claspers. Growth of elasmobranch claspers is related to the onset of maturity in sharks, and maturity is attained when clasper growth slows and claspers are fully calcified and rigid (Natanson and Cailliet 1986; Capapé *et al.* 2002, 2005). Immature males had small, soft claspers with little or no calcification. The size range of females and the number of mature females were too low to estimate reproductive trends in females for all species (*S. australis*, *S. albipunctata*, *P. cirratus*, *P. nudipinnis*). However, in the females that were caught, maturity was assessed through maximum oocyte diameter and the width of the uteri (Frisk *et al.* 2001).

Data were analysed to determine the most accurate measurement that would represent TL from truncated samples. A regression analysis was conducted between each morphometric measurement (e.g. the 50 measurements for each species of sawshark) and TL, with the expectation that the highest R^2 relationship would reveal the type of measurement that is the most useful for predicting TL. Length–weight relationships for each species were calculated using the following equation, which has been routinely used in other studies (Kohler *et al.* 1995):

$$W = a \times TL^b$$

where W is weight (g), a is a constant, TL the total length (mm) and b another constant.

Morphometric measurements were also compared between species within each group (*Squatina* spp. or *Pristiophorus* spp.) to assess whether a particular measurement on a truncated sample could be accurately used for species identification. Clasper inner length was plotted against TL to determine when clasper growth started, and when clasper length was indicative of mature individuals.

Results

Twenty-five *S. albipunctata* were obtained from the NSW SMP and from a commercial fishing trawler (*Maybelle*) operating out of Sydney harbour at ~80-m depth. Eighteen *S. australis* individuals were obtained from the New South Wales SMP, a commercial fishing trawler (*Maybelle*) operating out of Sydney harbour at an ~80-m depth, and from the Australian Maritime College research vessel *Bluefin* in eastern Tasmania at ~30-m depth. Thirty-eight *P. nudipinnis* individuals and 37 *P. cirratus* individuals were obtained from the Australian Maritime College research vessel *Bluefin* in eastern Tasmania at ~30-m depth.

Length–weight relationships

Squatina albigunctata sampling lacked small individuals, yet still allowed assessment of length–weight relationships ($y = 5 \times 10^{-8}x^{3.7265}$, $R^2 = 0.914$, $n = 25$; Fig. 1). The heaviest individual sampled was over 17 kg. *Squatina australis* individuals were generally smaller than were *S. albigunctata* individuals, growing to a maximum of ~1000 mm and just under 11 kg (Fig. 1). Neonates, as identified by the presence of visible yolk sacs and umbilical cords, were 253–260 mm in total length. Data followed an exponential curve ($2 \times 10^{-5}x^{2.9077}$, $R^2 = 0.9957$, $n = 18$; Fig. 1).

Pristiophorus cirratus grew to a maximum length of just over 1000 mm and a maximum weight of 1.9 kg (Fig. 2). The minimum recorded size was 460 mm, although neonates are expected to be smaller. Length–weight relationships followed an exponential curve ($y = 0.0002x^{2.319}$, $R^2 = 0.8363$, $n = 37$; Fig. 2). *Pristiophorus nudipinnis* grew slightly longer than *P. cirratus*, to a maximum length of 1092 mm, but were heavier at a maximum weight of 2.5 kg (Fig. 2). Minimum size was 460 mm, but again neonates are expected to be smaller. Length–weight relationships followed an exponential curve ($y = 4 \times 10^{-6}x^{2.8933}$, $R^2 = 0.9622$, $n = 38$; Fig. 2).

Morphometrics

Pectoral anterior margins, measured from origin to apex in a straight line, were the most accurate morphometric measurements for predicting total lengths in *S. australis*, *S. albigunctata*, *P. cirratus* and *P. nudipinnis* ($R^2 > 0.86$ in all cases, up to 0.994; Figs 2, 3). Table 1 presents the formulae that were used to calculate TL from pectoral fin margins. Eye length was the best morphometric parameter for separating the two angel shark species, although data were sufficient to determine this factor only for adults (mean lengths significantly different, $t = 4.1$, d.f. = 30, $P < 0.01$; Fig. 2). Sawshark rostrum length–TL ratio is already recorded as a species identification tool; all other morphometric measurements were too highly correlated with reliably separate species.

Reproduction

Because of the lack of juveniles, relationships with clasper length could not be assessed for *S. albigunctata*. All male *S. albigunctata* individuals sampled were mature and over 800 mm TL. *Squatina australis* clasper length started to increase on individuals from 500 mm TL, and all males over 800 mm TL were mature and had a clasper length of >120 mm (Fig. 4). An increase in the length of claspers in *P. cirratus* occurred from 500 mm and the length continued to increase in the larger individuals measured (Fig. 4). Individuals under 800 mm in length were immature, whereas all those over 900 mm were mature. The present study lacked mid-sized males of *P. nudipinnis*; however, clasper length increased after individuals reached 700 mm TL (Fig. 4). All individuals over 900 mm were mature, whereas all those under 700 mm were immature.

Discussion

Biological characteristics of south-eastern Australian angel sharks *S. australis* and *S. albigunctata* and sawsharks *P. cirratus* and *P. nudipinnis* were assessed. Females grew larger than

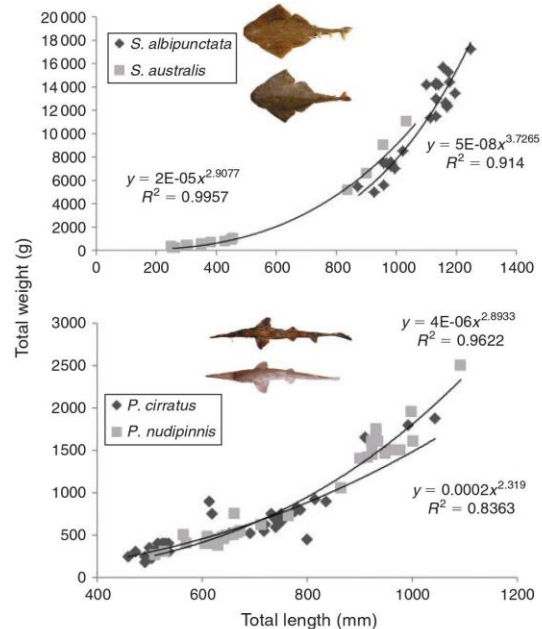


Fig. 1. Length and weight relationships comparing *Squatina albigunctata* ($n = 25$, both sexes), *Squatina australis* ($n = 18$, both sexes), *Pristiophorus cirratus* ($n = 37$, both sexes) and *Pristiophorus nudipinnis* ($n = 38$, both sexes). Exponential trend lines have been added.

males in all four species. Previous research trawling conducted by the *FRV Kapala* in the 1970s and 1990s also found female-biased TL ratios in the two angel shark species studied here, and the maximum sizes measured in the present study corresponded well with those results (NSW DPI 2015). Angel sharks as an Order display female-biased TL sexual dimorphism (Capapé *et al.* 1990, 2005; Bridge *et al.* 1998). Total lengths of *P. nudipinnis* and *P. cirratus* were similar, whereas *P. cirratus* individuals were relatively lighter than were similarly-sized *P. nudipinnis* individuals. The weaker length–size relationship in *P. cirratus* can be explained by the relatively longer rostrum, which can add length to specimens, without adding much weight (Nevatte 2015).

Pectoral anterior margins were highly correlated with total lengths in all four species. Pectoral anterior margin is the longest morphometric measurement in relation to TL that can be used in truncated angel sharks and sawsharks (aside from inter-dorsal fin distance), and this relationship may, therefore, have a lower measurement error rates than other relationships. Pectoral fin margins (the pectoral girdle and tip of fin) are easier to define and are stiffer than other structures used in morphometrics, which often have boundaries that are soft and pliable (i.e. caudal fin widths) and are thus more difficult to measure. Because of the large difference in morphology between sawsharks and angel sharks (fusiform v. flat), it is possible that anterior margins are good predictors of total lengths for a large number of shark species. Fishery managers should be able to determine TL in

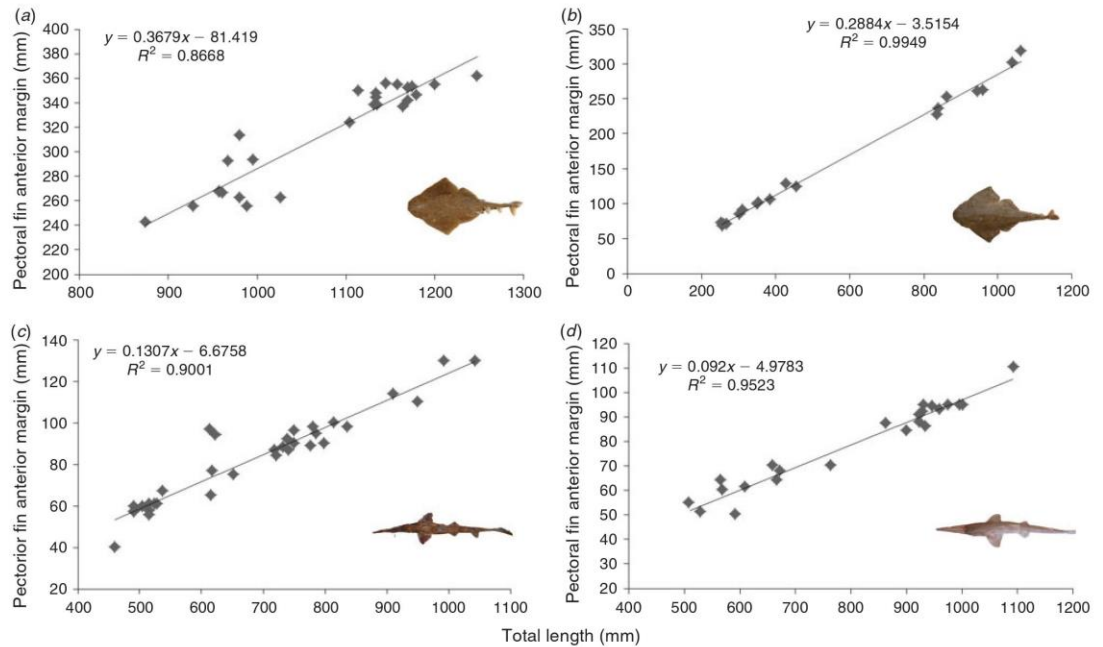


Fig. 2. Relationship between total length and pectoral fin anterior margin of (a) *Squatina albipunctata* ($n = 25$), (b) *S. australis* ($n = 18$), (c) *Pristiophorus cirratus* ($n = 37$) and (d) *P. nudipinnis* ($n = 38$). Linear trend lines have been added.

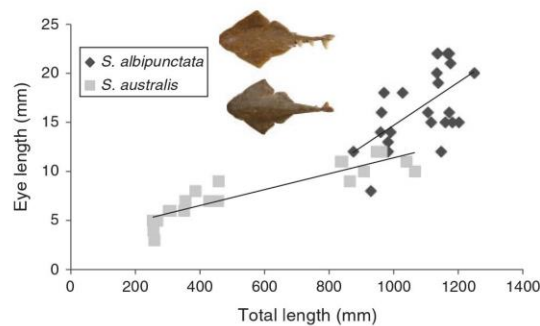


Fig. 3. Relationship between total length and eye length for *Squatina australis* and *S. albipunctata*.

Table 1. Formulae for calculating the total length (TL) from truncated specimens

Species name	Pectoral fin margin to total length conversion
<i>Squatina albipunctata</i>	$y = (x + 81.42) \div 0.368$
<i>S. australis</i>	$y = (x + 3.515) \div 0.288$
<i>Pristiophorus nudipinnis</i>	$y = (x - 4.98) \div 0.092$
<i>P. cirratus</i>	$y = (x + 6.68) \div 0.131$

truncated specimens with a high level of confidence by using pectoral anterior margins.

Eye length in adult *Squatina* spp. differed between species. *Squatina albipunctata* had larger eyes, and unidentified specimens with eye lengths greater than 15 mm are likely to be *S. albipunctata*. It is possible that eye length can, therefore, be used to separate species easily, although data were insufficient for juveniles. Eyes in larger elasmobranchs have a higher spatial resolving power than do smaller eyes (Litherland et al. 2009). *Squatina australis* and *S. albipunctata* have known depth segregation, with *S. albipunctata* occurring in deeper waters (V. Raoult, K. Rowling, V. Peddemors, J. E. Williamson, unpubl. data). Angel sharks are highly visual predators (Fouts and Nelson 1999) and, consequently, the larger eyes of *S. albipunctata* may facilitate predation at greater depths than for *S. australis* and add credence to predictions of depth segregation for these co-occurring species. Spatial segregation of size classes has been documented in other shark species (Borrell et al. 2011; Robbins and Booth 2012) and is suspected in *S. tergocellata* (Bridge et al. 1998). Commercial trawlers caught only large (>800 mm) *Squatina* spp. with a fish trawl net at 80 m, whereas immature individuals were caught only by research trawls with a prawn net (smaller mesh) at ~30-m depth. Smaller individuals may stay in shallower waters and larger ones may migrate to deeper waters as a result of the increased eye length in adults. Although this is just one explanation, similar morphologies can result in trophic separation (Raoult et al. 2015) and, therefore, these species may segregate as a result of diet preferences.

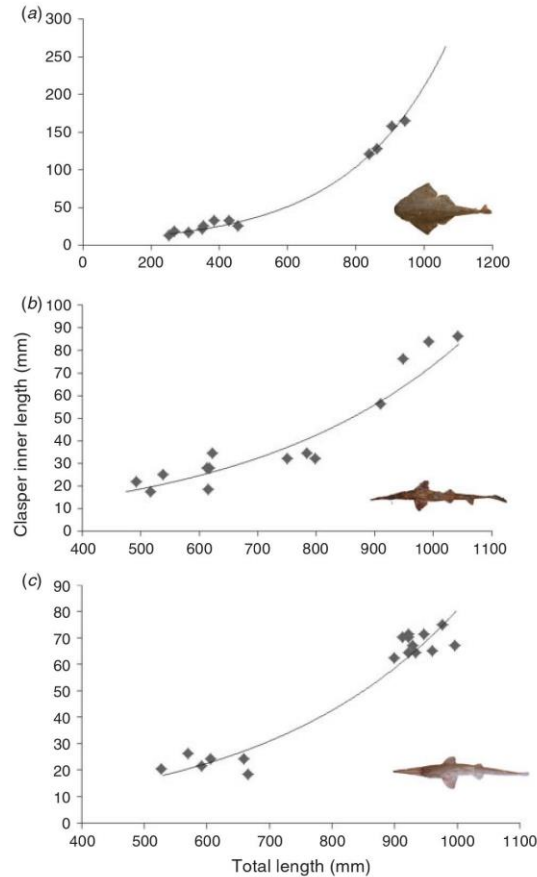


Fig. 4. Total length and clasper inner length relationship for (a) male *Squatina australis* ($n = 12$), (b) male *Pristiophorus cirratus* ($n = 14$) and (c) male *P. nudipinnis* ($n = 17$). Exponential trend lines have been added.

Embryos were present in one female *S. albiguttata*, with three young in each uterus. This female was caught in late autumn (April), and the embryos were 60 mm long, indicating they were not close to birth (minimum length estimated at completion of gestation is ~ 250 mm; the present study). As in some other *Squatina* studies (Capapé *et al.* 1990; Bridge *et al.* 1998), both ovaries were functional (producing oocytes) in *S. australis* and *S. albiguttata*. No egg cases were observed in the present study, although the observed embryos were likely past the egg-case stage for embryos under 60 mm long, as suggested by studies of *S. guggenheim* and *S. occulata* (Sunyem and Vooren 1997). Further research is needed to understand the gestation period and process in these sharks.

A crucial biological aspect that is missing from the species studied here is age. Studies have unsuccessfully attempted to determine the age of angel sharks by using vertebrae (Natanson and Cailliet 1990; Baremore *et al.* 2009); however, the vertebral structure of *Squatina* spp. is very different from that of other

elasmobranchs. *Squatina* spp. have very distinct banding in the vertebral centra that are related to girth rather than age, and no visible banding in the *corpus calcareum*, which is generally used to age elasmobranchs (Geraghty *et al.* 2014). The only reliable age estimates of *Squatina* spp. are from tag-recapture studies on *S. californica*, which suggested onset of maturity at ~ 5 years of age (Cailliet *et al.* 1992). A preliminary study that attempted to detect bands in the *corpus calcareum* of *S. australis* with a scanning X-ray fluorescence microscope found a result that correlated with the study of Cailliet *et al.* (1992) of five bands per year for a near-mature male (V. Raoult, V. M. Peddemors, D. Zahra, N. Howell, D. L. Howard, M. D. de Jonge and J. E. Williamson, unpubl. data). Accurate ageing of these sharks is necessary to predict the effects of commercial fisheries and to create accurate growth curves. Effort must be made to understand the development of shark vertebrae, and to develop new ageing techniques.

The principle limitation to the present study was the low number of study animals. Adequate reproductive analyses, particularly for females, were therefore difficult. Large adults and neonates were lacking for many of the study species. *Squatina albiguttata* length-weight relationships were the least reliable because of the absence of young individuals, and extrapolation of these curves may be inaccurate. Further research should aim to capture large females, so as to determine reproductive periodicity and fecundity. Numbers of subadult *P. nudipinnis* were low, possibly because of depth segregation (seasonal effects were unlikely because of the seasonal sampling patterns). High coefficients of determination (>0.85) for all relationships may be a result of the small sample size; future studies should add measurements to this dataset to verify whether it was an aberration.

The present study demonstrated that simple morphometric measurements can be used for a variety of applications, both biological and management related. The two species of angel sharks found in south-eastern Australia can be reliably separated according to their eye size, and TL can be inferred from anterior pectoral margins in these species with a high degree of accuracy. Fisheries identification guides should therefore use these facts to facilitate the separation of these species. Although the reproductive and size data presented here are sparse, they form the foundation for future studies assessing the biology of these little-known sharks.

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