An integrated approach to assess pupping areas and natal origins in a Conservation Dependent shark, *Galeorhinus galeus*

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For Grandad.

Declaration

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Matthew N. McMillan

25 September 2018

Cover image: A pregnant school shark *Galeorhinus galeus* with a PSAT tag prior to release by the author, South Australia.

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Abstract

Knowledge around reproductive movements and habitat use can be central to understanding the life histories of animal populations. Such knowledge can be especially important for managing recovery of populations depleted by human over-exploitation and habitat degradation. In marine environments, clarifying animal movements and habitat use can be difficult given the practical and logistical constraints of studying them. For my research I therefore integrate diverse techniques to cast light on reproductive movements and habitat use of the Conservation Dependent school shark *Galeorhinus galeus*, where conventional methodologies have left important knowledge gaps unanswered. A national rebuilding plan for the species highlighted a lack of knowledge around whether all female *G. galeus* migrate to historically identified pupping areas around Tasmania and Bass Strait in the south-eastern range of the species as current management assumes. Alternatively, reproductive movements and habitats may be more varied in extent and location, including pupping areas in South Australia to the northwest where aggregations of pregnant females occur. My overarching aim was to assess the spatial distribution of *G. galeus* pupping areas in southern Australia and the extent of shared natal origins among populations. I use: (1) element signatures in calcified shark vertebrae that derive from water chemistry and diet in birth areas as natural tags to test whether sharks from different populations recruit from common or different pupping areas, (2) energetic analyses to assess constraints on pup dispersal from pupping areas and whether pups caught in South Australia could feasibly have dispersed from known pupping areas around Tasmania and Bass Strait, and (3) satellite archival tags to track movements of pregnant *G. galeus* tagged in South Australia to assess pupping movements and the spatial distribution of likely pupping areas. My findings increase our knowledge of the extent and plasticity of reproductive movements and areas used by *G. galeus* and address several assumptions, on which current management is based, that conventional techniques such as mark-recapture studies and genetic investigations had left open to speculation.

A review of elasmobranch vertebral chemistry analysis and ground-truthing laboratory experiments establish the utility of shark vertebrae as sources for natural tags. Element signatures were consistent among related time-resolved portions of the same and adjacent vertebrae, while commonly used bleach preparation did not affect element signatures for a range of elements, validating use of elasmobranch vertebrae as biogenic archives for microchemistry analyses.

Post-natal element signatures from three cohorts of juvenile and sub-adult *G. galeus* were compared between populations in South Australia and Bass Strait. Signatures differed among populations, indicating use of different pupping areas and not supporting the previous assumption of uniform female migrations to common pupping areas.

Bioenergetic analyses established an energy budget and assessed constraints on dispersal of *G. galeus* pups from pupping areas. High energetic costs of growth, small energy reserves, and low concentrations of energy storage lipids relative to adults indicated a trade-off prioritising growth over dispersal in pups. Newborn pups in South Australia are shown to likely be born locally rather than migrants from distant, traditionally identified pupping areas.

Satellite archival tagging of pregnant females found that some remained resident in South Australia over the pupping season (November–January), some migrated to the region of known pupping areas around Tasmania and Bass Strait, and one migrated to New Zealand. Given that a single mixed stock is known to exist, this indicates partial female migration with likely pupping areas stretching from the Great Australian Bight to New Zealand that are far less spatially constrained than assumed.

This thesis therefore achieved its main aim of assessing whether the spatial distribution of *G. galeus* pupping areas and uniformity of female migratory behaviour in southern Australia conformed to current assumptions. Furthermore, it confirmed South Australia is a reproductively important area for school shark. Allocation of resources to future study of reproductive behaviours and habitats in South Australia would better inform management and enhance prospects for successful recovery of the species.

Acknowledgements

Hindsight has told me that designing a research project from the ground up, depending on securing extensive funding, and relying on the cooperation of wild animals probably wasn't the easiest way to do a PhD. However, the breadth of experience I have gained has made it more than worthwhile. The successful conclusion of this project is thanks in large part to my principal supervisor Bronwyn Gillanders, who didn't flinch when I put my proposal to her. She embraced it from the outset and always gave me clear and positive direction. I am heavily indebted for her knowledge, experience, professionalism, and support. I could not have asked for a better mentor.

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CHAPTER 1

General introduction

Leaving Backstairs Passage between Kangaroo Island and the Australian mainland heading into the Southern Ocean – home of the school shark.

General introduction

Since palaeolithic times, humans have devised tools and tactics to hunt and catch animals for consumption. Intervening eras are replete with examples of animals being over-exploited by humans, often to the brink of extirpation or beyond (Diamond 1989, Redford 1992, Steadman 1997). Ever expanding human populations drive growing demands for protein, which together with technological advances and shared knowledge have increased pressure on many animals. Anthropogenic habitat alteration can further compound challenges facing animals and can cause synergistic effects with over-exploitation (Martin & Klein 1989, Jackson 2008). The resilience of wild animal populations, i.e. their capacity to recover from population depletions, varies among species and may be influenced by a myriad of factors, e.g. reproductive biology and access to critical habitats (Fahrig 1997, Cardillo 2003, Reynolds 2003). Loss of critical habitats such as birthing or nesting areas from which depleted populations can be replenished (Fahrig 1997, Connelly et al. 2000, Gibbons et al. 2000), or the over-exploitation of females that bear future generations can heavily impact resilience in animal populations (Ford 1921, Bowen & Karl 2007, Mucientes et al. 2009). This may be particularly true for animals with high degrees of seasonality and selectivity in their habitat use, and whose movements are therefore predictable and easy to exploit.

In step with human population growth, the twentieth century saw exploitation of marine animals grow at unprecedented levels through expansion of fisheries, driven by enormous growth in demand for food and a surge in catches. Technological advances in vessel production and design drove the industrialisation and expansion of fishing fleets, adopting efficient new methods to capture, store and transport catches to distant markets (Grainger & Garcia 1996, Pauly et al. 2002). Imprudent management and the difficulty of predicting sustainable harvests contributed to almost a quarter of fish stocks being overexploited globally by the 1990s (Botsford et al. 1997). This decline in global fisheries drove scientific efforts to maintain and rebuild depleted fish populations (Essington et al. 2006). The stock concept came to the fore; self-replenishing groups of fish (stocks) connected by animal movement between sub-groups (populations) were identified as management units to estimate impacts on stock biomass and attempt to balance catches at sustainable levels against natural replenishment (Haddon 2007, Hilborn & Walters 2013, Secor 2013).

Stock depletion and rebuilding plans

Stock biomass becomes depleted when mortality exceeds growth and recruitment. Recognition of unsustainable stock depletion saw the adoption of the precautionary principle in fisheries management; the United Nations Food and Agriculture Organisation (FAO) developed guidelines for responsible fisheries that called for:

> prudent foresight to avoid unacceptable or undesirable situations, taking into account that changes in fisheries systems are only slowly reversible, difficult to control, not well understood, and subject to change in the environment and human values (FAO 1996).

Limit reference points were introduced as a tool for stock management, i.e. proportions of estimated stock biomass at pre-fishing levels or some past date, below which catches become unsustainable (F_x) or when biomass becomes so depleted as to present the risk of stock collapse (B_x). In the early 2000s, the FAO called for fish stocks globally to be maintained at levels that can withstand harvesting at maximum sustainable yields, and to restore stocks already depleted past this point urgently before 2015 (FAO 2003). A quarter of commercially exploited fish stocks were found to fall in the latter category; over-exploited and depleted beyond sustainable levels, with almost none of these in a state of recovery (FAO 2005). Stock rebuilding strategies were implemented with some success, however by 2007 at least 9% of marine fish stocks had collapsed with a general trend of failure in rebuilding efforts at the time despite reductions in catches (Worm et al. 2009).

Collapse of the school shark stock in Australian waters

One such stock that collapsed was the school shark *Galeorhinus galeus* in Australia (AFMA 2009). School shark inhabit temperate waters off Australia-New Zealand, South Africa, Brazil-Argentina, Chile, California, and in the northeast Atlantic (Walker 1999, Chabot & Allen 2009) and are variously known as soupfin shark, tope, liver oil shark, and vitamin shark throughout their range. Sharks have been targeted increasingly as global fishing pressure has increased, however they are often poorly equipped to cope with fishing pressure due to *K*-selected biological traits (Stevens et al. 2000, Barker & Schluessel 2005, Field et al. 2009).

This is true of school shark, a bentho-pelagic species that undertakes large-scale movements at times and an active predator of fish and cephalopods of both benthic (e.g. reef fishes and octopus) and pelagic origin (e.g. shoaling fishes and squid) (Olsen 1984, Walker 1999). Late onset of maturity (8–10 years), long reproductive cycles (2–3 years), discrete pupping seasons (November–January in Australia), and selective use of reproductive habitats (e.g. discrete pupping areas typically at depths <30 m) (Olsen 1984, Prince 1996, Walker 1999, Stevens 2005) impede rebound capacity from overfishing of school shark.

In Australia, school shark was the target species of one of Australia's oldest fisheries operating since the 1920s: the southern shark fishery (Stevens & West 1997). Schooling behaviour, from which their name derives, made school shark an attractive target; schools could be intercepted and systematically fished down yielding large catches. The fishery remains active, stretching from Bass Strait and Tasmania through South Australian waters into the Great Australian Bight (Fig. 1.1). However, throughout the fishery the focus has shifted to co-occurring gummy shark *Mustelus antarcticus* as the school shark stock became depleted (Prince 2005). Gummy shark is a demersal species that preys predominantly on crustaceans and cephalopods (Simpfendorfer et al. 2001). In contrast to school shark, gummy shark is better equipped to cope with fishing pressure due to faster growth and earlier maturity (4–5 years), shorter reproductive cycles (1–2 years), and non-selective pupping seasons or habitats with pups occurring throughout the year in depths from 0–150 m (Walker 1998, Prince 2005). However, school shark continues to be caught as bycatch in the southern shark fishery under incidental quota and marketed with other sharks under the generic umbrella term 'flake', ubiquitous fare in fish and chip shops of southern Australia.

Thus, while gummy shark is marketed as a sustainable fishery, school shark, the main bycatch species (and previous target species) of the fishery is far less resilient and has not coped well with intensive fishing pressure. Overfishing of school shark has occurred throughout the species' global distribution, e.g. in California (Walker 1998), Great Britain (Molfese et al. 2014), and Argentina (Cuevas et al. 2014). Originally targeted for their large livers rich in vitamin A, fishing pressure increased markedly during the Second World War when school shark were targeted as an alternative source of protein (Olsen 1959). Industrialisation and expansion of the shark fishing fleet led to catches peaking in the 1960s at around 2,500 t (Fig. 1.2). Catches declined briefly in the 1970s after bans on the sale of large school shark due to mercury content, but again increased in the 1980s with the lifting of the ban (SharkRAG 2009) (Fig. 1.2). To sustain stock biomass and maximise sustainable yields, fishing mortality should not exceed natural mortality (Braccini et al. 2009), however by the 1980s fishing mortality of school shark was estimated at four times natural mortality for age classes <10 years while catch per unit effort declined 87% between the 1970s and the 1990s (Walker et al. 2005). In the 1990s the school shark stock collapsed (Fig. 1.2).

Fig. 1.1. The core range of school shark *Galeorhinus galeus* in Australia and the operational area of the southern shark fishery that formerly targeted them and continues to take them as bycatch under incidental quota when targeting gummy shark *Mustelus antarcticus*. Females aggregate in the Great Australian Bight in austral spring (diamond, upper left) and are assumed to make obligate migrations (dashed line) to known pupping areas around Bass Strait and Tasmania (e.g. Port Phillip Bay, Westernport, Port Sorell, Georges Bay, and Pittwater) to pup in austral summer. Shark fishing closures designed to protect migrating and pupping school shark are marked in red.

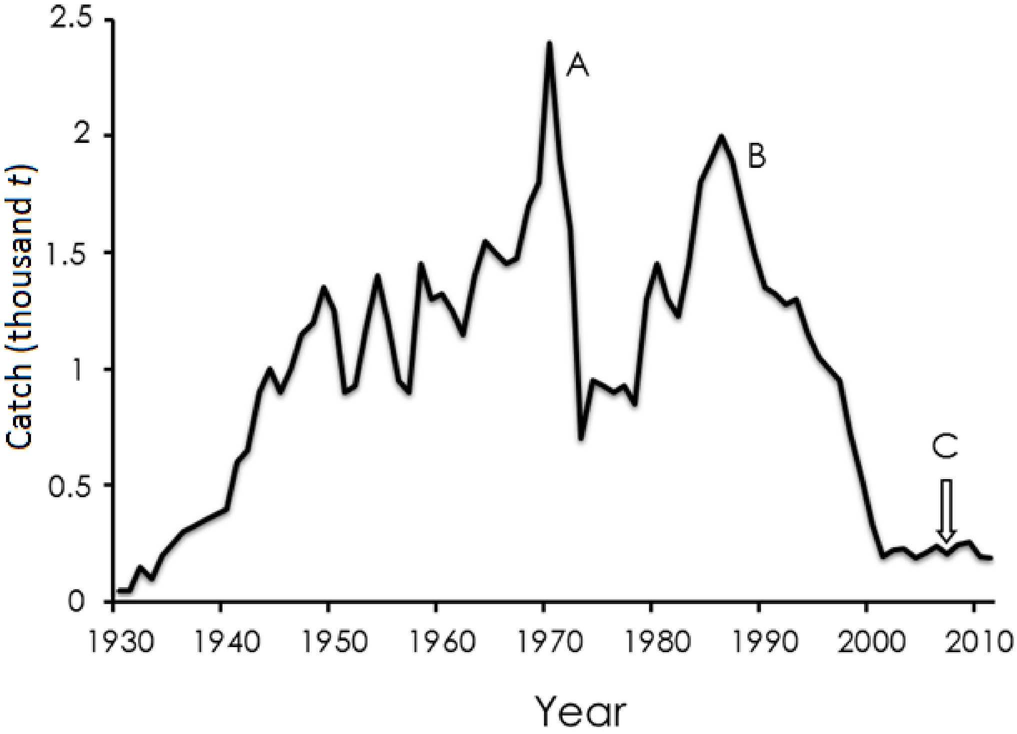

Fig. 1.2. Historic school shark catch landed in southern Australia since the fishery's inception in the 1930s. Primarily fished for liver oil initially, during world war two the species began to be targeted for food. In 1972, a ban on the sale of large school shark was introduced due to concerns about mercury content (A). After the ban was lifted, fishing effort rose in the 1980s leading to stock collapse throughout the 1990s (B). A national stock rebuilding strategy for school shark was introduced in 2008 (C). Data courtesy: *Australian Fisheries Management Authority*.

A stock rebuilding plan for school shark

Assessed at below 14% of pre-fishing levels (AFMA 2009), steps were taken to protect the remnant school shark stock, albeit slowly. Mesh sizes for gill nets used in the southern shark fishery were reduced from a maximum of 20 cm to 15–16.25 cm to reduce catches of mature females (Walker 1999). School shark were no longer permitted to be targeted commercially, but continued to be landed as bycatch (AFMA 2009). Quota limits on school shark bycatch were slowly introduced by 2001, with the aim of limiting catches to incidental levels, and ultimately aiming to restore the mature stock biomass to 1996 levels by 2011 (Bruce et al. 2002). The most recent estimates of school shark biomass are 8-17% of virgin stock levels (SharkRAG 2011).

The effectiveness of stock rebuilding efforts hinges on a number of factors (Wakeford et al. 2009, Hammer et al. 2010):

- Prompt and significant reductions in catches;
- Biological characteristics of the species (e.g. reproductive capacity);
- Environmental conditions (e.g. habitat loss);
- Demographic structure of the target stock (e.g. bias toward reproductive adults); and
- Effectiveness of management frameworks.

In a review of factors contributing to successful recovery plans, Wakeford et al. (2009) assessed stock recovery efforts for school shark in Australia. Although these efforts scored moderately well in environmental conditions and management frameworks (3/5), the slow reaction to the stock collapse and the previous decimation of mature age classes, particularly females, drove failures in the critical areas of demographic structure of the stock as well as prompt and significant reductions in catches (both scoring 1/5) (Wakeford et al. 2009). In 2008, a Commonwealth stock rebuilding strategy was introduced (Fig. 1.2) (AFMA 2009). Satisfying the criteria for listing as Endangered, the following year school shark received Conservation Dependent status in Australian waters under the *Environment Protection and Biodiversity Conservation Act 1999* (DEWHA 2009a). Conservation Dependent status was inserted into the *EPBC Act 1999* in 2006 as an alternative to listing, allowing for continued commercial and recreational fishing of listed fish species (DEWHA 2009b, DEE 2017).

A limit reference point for school shark was set at B_{20}, i.e. 20% of estimated virgin stock levels was considered the point below which the stock would be in continued danger of collapse (AFMA 2009). Under Australian fisheries management guidelines, catches should be low enough that stocks can rebuild to the limit reference point (B_{20}) within one generation and 10 years (32 years for school shark) and a target reference point of B_{40} should be achieved within an equal timeframe thereafter (DAFF 2007). However, modelling suggests that school shark biomass would require a catch of just 26 t to recover to B_{20} within this timeframe; seen as unrealistic given incidental captures in the gummy shark fishery (SharkRAG 2009). Instead, the Australian fisheries management guidelines were deemed unachievable and the timeframe for school shark recovery to B_{20} was pushed out to three generations, i.e. 66 years, to accommodate larger school shark catches (Huveneers et al. 2013). The current school shark incidental catch limit is set at 215 t (AFMA 2018).

In addition to overfishing, the stock rebuilding strategy noted habitat loss as a likely contributor to collapse and an impediment to recovery. Vast, previously highly productive nursery areas had been lost, e.g. in the Geelong Arm of Port Phillip Bay due to the loss of sea grass meadows, clearing of mangroves, and pollution (Williams & Schaap 1992, AFMA 2009). It therefore became a focus of the plan to assess sources of recruitment for school shark. An important lack of knowledge related to whether recruitment is entirely dependent on pupping grounds in Bass Strait and Tasmania identified in the 1950s or whether pupping occurs in other areas, notably South Australian waters that are unaccounted for in the stock assessment model and for which anecdotal evidence existed (Prince 1996, Stevens & West 1997, AFMA 2009).

School shark pupping areas – an enduring mystery

Many shark species use shallow, inshore areas such as bays and sheltered coastlines as pupping areas. These areas are thought to be selected for their high productivity, shelter, and protection from predation (Morrissey & Gruber 1993, Heupel & Simpfendorfer 2002). Recruitment to the breeding stock after surviving the first months of life is much more likely than neonate survival during this period (Heupel & Simpfendorfer 2002) and more critical to recovery than increases in fecundity (Stevens et al. 2000). Knowledge about pupping areas and recruitment from them is therefore useful to make accurate stock assessments in sharks (Punt et al. 2000), with implications for monitoring and estimating the effects of fishing pressure.

School shark pups were found in at least 17 locations in Bass Strait and Tasmania in the 1950s during surveys at the behest of the Victorian and Tasmanian state governments, where the school shark fishery and market were concentrated (Olsen 1954). Commercial fishers targeted both pupping females and juveniles at these grounds leading to steep falls in inshore catches and driving the protection of these grounds in the 1960s as shark refuge areas (Williams & Schaap 1992), among Australia’s first marine protected areas (Fig. 1.1). Some of these were highly productive pupping areas, but productivity has declined and only a handful remained productive by the 1990s (Stevens & West 1997). Olsen also queried fishers about pupping areas in South Australia in the 1950s expecting to find pups. However, the fishery was new there, having recently expanded westward after catches fell in Bass Strait, and he was unable to find fishers with sufficient experience of pupping activity (Prince

1996). Knowledge about school shark pupping areas has barely increased since Olsen's initial efforts and remains centred on a handful of grounds around Bass Strait and Tasmania (Stevens & West 1997). However, since it is estimated that these recorded pupping areas produce <10% of school shark pups, unidentified sources of recruitment must be sustaining >90% of the stock (Prince 1996, Stevens & West 1997).

In the period when school shark could be legally targeted prior to 2001, anecdotal evidence of school shark pupping grounds in South Australia emerged based on advice from commercial fishers who targeted reliable 'runs' of pregnant females during the summer pupping season at discrete locations where they displayed characteristic pupping behaviour (Prince 1996). Females would approach sheltered inshore areas and mill about close inshore before disappearing suddenly, after which nets were fouled with birth sacs recognised as those encapsulating the pups routinely found while cleaning pregnant females; this behaviour is consistent with that noted by commercial fishers at known pupping sites in Bass Strait and Tasmania (Prince 1996). Females were also caught during scientific surveys in immediate post-partum condition with irrigated and distended uteri consistent with having given birth very recently in western South Australia, far from south-eastern pupping areas (Braccini et al. 2009). Using gillnets of larger mesh size in South Australia (17.5–20 cm) than fishers in Bass Strait and Tasmania (15–16.25 cm) made it a rare occurrence to catch the small pups (~30–45 cm total length). When neonate school shark were caught in South Australia and reported to the appropriate authorities they were suggested to be probable migrants from Bass Strait pupping areas (Prince 1996, Risely pers. comm.). Given the considerable anecdotal evidence for school shark pupping in South Australia, there is a need to test whether pupping occurs outside traditionally identified pupping areas in Tasmania and Bass Strait. Such information would be useful to assist management of the species' recovery by clarifying population dynamics and allowing future stock assessments to incorporate these findings.

Thesis aims and scope

Research aims of the present study

This study aimed to integrate diverse approaches to address the knowledge gap around school shark reproductive habitats and movements outlined in the Commonwealth school shark stock rebuilding strategy (AFMA 2009). In particular, I aimed to determine if pupping was occurring in South Australia, or whether all school shark across their range shared common origins, i.e. derived from the same pupping areas, which would support the assumption that all pupping occurs around Tasmania and Bass Strait. A variety of techniques are used to assess the validity of current assumptions and seek evidence of the spatial distribution of pupping habitats:

- Ground-truthing experiments to test consistency of element signals in school shark vertebrae and effects of preparatory bleaching on microchemistry to assess utility of vertebral centra as biogenic archives and sources of natural tags (Fig. 1.3);
- Vertebral chemistry analysis to compare post-natal natural tags between populations in South Australia and Bass Strait and determine if these populations derive from common or different pupping areas (Fig. 1.3);
- Bioenergetic analyses to establish an energy budget and assess dispersal capacity of school shark pups to explore whether pups caught in South Australia could feasibly be migrants from Bass Strait pupping areas as has been suggested (Fig. 1.3); and
- Satellite-linked pop-up archival tags would track pregnant females tagged in South Australia seeking direct evidence of their movements to assess whether obligate migrations to pupping areas in Bass Strait and Tasmania occur as assumed (Fig. 1.3).

In addition, wide-scale liaison with the public, i.e. commercial and charter fishers past and present as well as recreational fishers, would be undertaken to help address my aims and ultimately arm conservation and fisheries managers with information to help best manage the recovery of school shark in Australian waters.

Thesis structure

Data chapters (chapters 2–5) are written as manuscripts that have all been published or submitted for publication in scientific journals. Hence, each chapter is structured as an independent study with its own introduction and discussion and uses the plural ‘we’ throughout, due to co-authorship. Nevertheless, data chapters are structured so as to flow as my argument builds from the proof-of-concept stage for using vertebral element markers (Chapter 2), through to using these markers as natural tags to assess contributions from pupping areas and discriminate populations with shared natal origins (Chapter 3), to determining bioenergetic constraints on pup dispersal to investigate if pups caught in South Australia are born locally (Chapter 4), and finally providing direct evidence of the spatial distribution of pupping areas and migration pathways in southern Australia (Chapter 5). Chapter 6 brings together the main findings and implications for management arising from this study as well as an outline of directions for future research in a concluding discussion.

Following is a brief synopsis of each chapter:

Chapter 2 – This chapter, published in the Journal of Fish Biology (McMillan et al. 2017), explores use of calcified elasmobranch hard parts as biogenic archives for elemental analyses. In addition to reviewing and summarising the existing literature as well as outlining key assumptions and approaches for elemental analyses, I conduct important ground-truthing laboratory work to verify the utility of school shark vertebrae as viable records of trace element uptake. Using element signatures as natural tags has the advantage that they are present in all individuals (unlike artificial tags) and can reveal movements and origins at ecological timescales (unlike genetic investigations which tend to focus on evolutionary timescales). Unlike otoliths as sources of natural tags in teleosts, elasmobranch vertebral chemistry analysis has only recently been taken up due to uncertainty about the suitability of these structures as biogenic archives. Reasons for this included uncertainty about:

- Stability of element concentrations in elasmobranch vertebrae over time;
- Uniformity of element distributions both within and among vertebrae; and
- Effects of preparation techniques (e.g. bleaching) on element signatures.

I establish that chemical signals are consistent throughout related time-resolved portions of the same vertebrae as well as among adjacent vertebrae while also providing important information on the effects of preparation (i.e. cleaning with bleach) on elemental analyses, thus laying the groundwork for confident use of this technique.

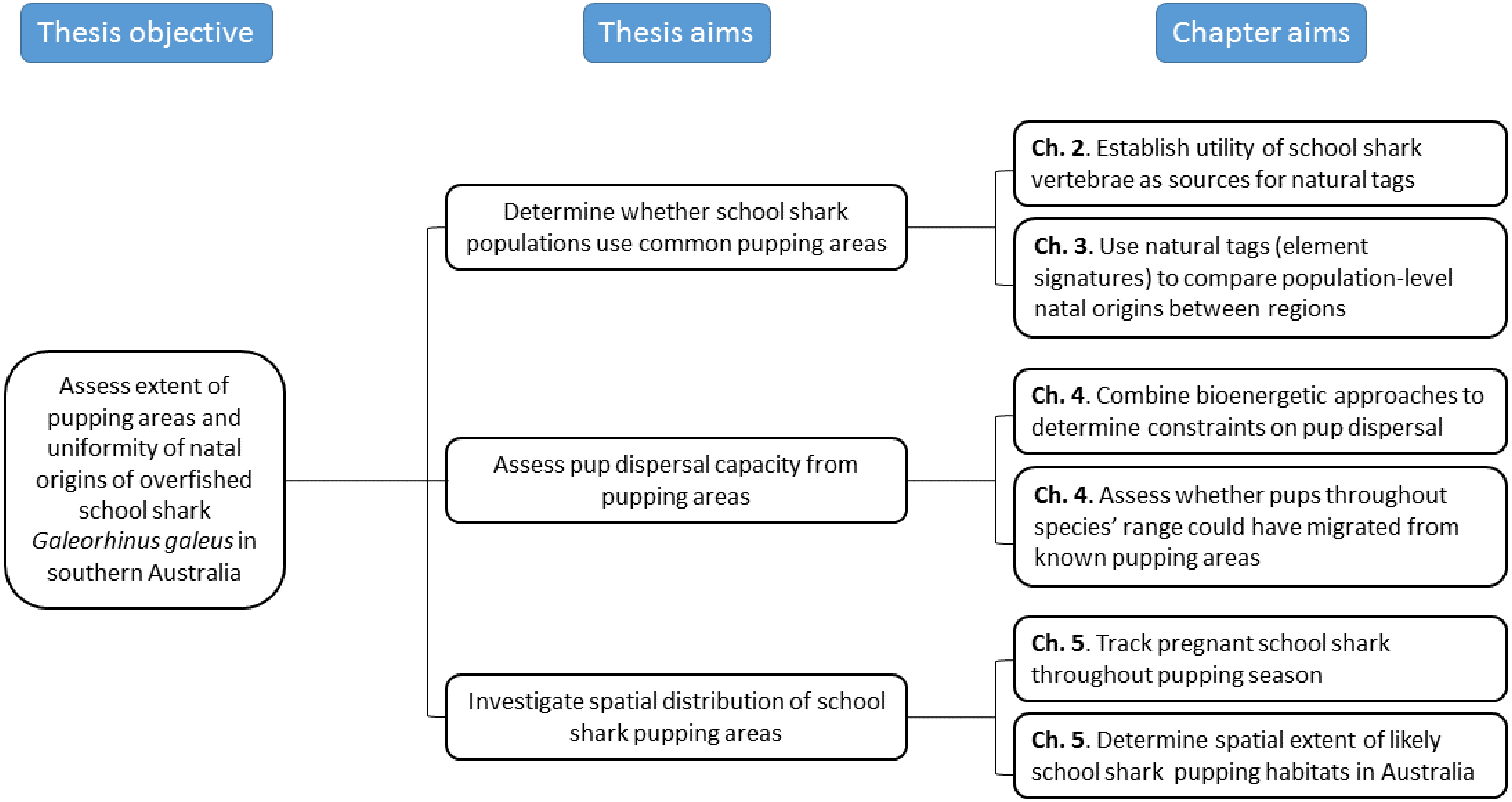

Fig. 1.3. Schematic representation of the overarching objective, thesis aims, and chapter aims for this thesis.

Chapter 3 – Following on from the proof of concept established in Chapter 2, in this chapter published in Marine Ecology Progress Series (McMillan et al. 2018a), I use natural tags in the vertebrae of juvenile and sub-adult school shark from an archival collection to compare natal origins of sharks from two populations: South Australia and Bass Strait. Vertebral chemistry provides an opportunity to assess whether these populations derive from different pupping areas, thereby ascertaining whether all females undertake obligate migrations to a handful of shared pupping areas.

Chapter 4 –Neonate school shark have been caught and reported by fishers in South Australia who related the presence of neonates as evidence for local pupping areas. However, it has been suggested that these newborn sharks must have rapidly dispersed from distant pupping areas ~500–2,500 km away around Bass Strait or Tasmania (Fig. 1.1). I investigate this claim by combining bioenergetic investigations including swim tunnel respirometry, bomb calorimetry, and lipid class analyses of pups from an important pupping area in Tasmania. This study is designed to ascertain whether bioenergetic constraints allow neonates from recorded pupping areas to disperse to South Australia at this early life stage, while also providing a unique study of constraints on shark pup dispersal more generally.

Chapter 5 – For the final data chapter, published in ICES Journal of Marine Science (McMillan et al. 2018b), I deploy satellite-linked pop-up archival tags to track the movement and behaviour of pregnant females from aggregating areas in South Australia and establish if obligate female pupping migrations to Bass Strait and Tasmania occur as assumed by management. Pregnant females are tagged inshore at the start of the pupping season in December or offshore prior to the pupping season (October). This study is designed to provide direct evidence as to whether partial- or obligate female migration occurs and determine migration pathways used by female school shark.

Chapter 6 – The concluding general discussion provides a synthesis of some of the key findings gained throughout this research. I also discuss conservation implications and future directions for research into school shark pupping areas in South Australia arising throughout the course of this study.

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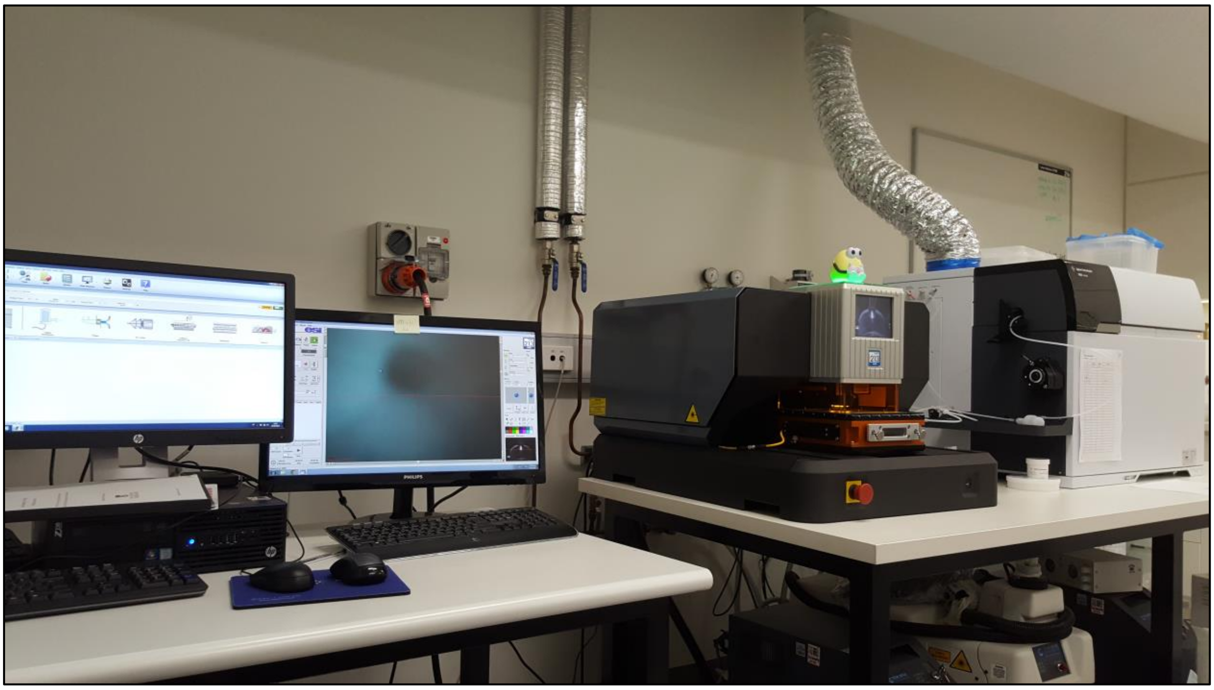
CHAPTER 2

Elements and elasmobranchs: Hypotheses, assumptions, and limitations of elemental analysis



The laser and mass spectrometer at Adelaide Microscopy used for laser ablation of shark vertebrae to conduct elemental analyses.

Statement of Authorship

Title of Paper	Elements and elasmobranchs: Hypotheses, assumptions, and limitations of elemental analysis
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Ed	Matthew McMillan		
Contribution to the Paper	Review of primary literature, sample collection, LA ICP-MS analysis of consistency of vertebral elemental signatures and preparation effects, authorship of hypotheses, assumptions and limitations, and shared authorship of remainder. Acted as corresponding author.		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Chris Izzo		
Contribution to the Paper	Review of primary literature, sample collection, LA ICP-MS analysis comparing otoliths and elasmobranch vertebrae, shared authorship of introduction and background, and feedback on manuscript.		
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Contribution to the Paper	Assistance with intellectual development, laser mapping, advice on LA ICP-MS analyses, and feedback on manuscript.		
Signature		Date	23/9/18

Name of Co-Author	Bronwyn Gillanders		
Contribution to the Paper	Assistance with intellectual development, advice and suggestions throughout, provided funding, and feedback on manuscript.		
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Elements and elasmobranchs: hypotheses, assumptions and limitations of elemental analysis

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Quantifying the elemental composition of elasmobranch calcified cartilage (hard parts) has the potential to answer a range of ecological and biological questions, at both the individual and population level. Few studies, however, have employed elemental analyses of elasmobranch hard parts. This paper provides an overview of the range of applications of elemental analysis in elasmobranchs, discussing the assumptions and potential limitations in cartilaginous fishes. It also reviews the available information on biotic and abiotic factors influencing patterns of elemental incorporation into hard parts of elasmobranchs and provides some comparative elemental assays and mapping in an attempt to fill knowledge gaps. Directions for future experimental research are highlighted to better understand fundamental elemental dynamics in elasmobranch hard parts.

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Key words: age validation; laser-ablation ICP-MS; movement and connectivity; population structure; vertebrae.

INTRODUCTION

Quantification of the elemental composition of teleost hard parts (*e.g.* otoliths, scales, fin rays and spines) has been invaluable in reconstructing environmental histories, inferring population structure and for tracking ontogenetic patterns of movement and habitat use (Campana *et al.*, 2000; Campana & Thorrold, 2001; Elsdon *et al.*, 2008). Conversely, the elemental composition of elasmobranch hard parts (*e.g.* vertebrae, jaw cartilage and dorsal fin spines) has only been occasionally investigated (Table I), which is surprising given these structures possess characteristics that make them suitable for chemical analyses (Hussey *et al.*, 2012). Moreover, increased use of isotopic and radiometric analyses of elasmobranch hard parts has yielded valuable ecological (*e.g.* trophic) and biological (*e.g.* age and growth) information for a range of species that, if employing conventional approaches, would not have been possible (Campana *et al.*, 2002; Estrada *et al.*, 2006; Hussey *et al.*, 2012).

This review focuses on elemental analyses of elasmobranch hard parts; literature can be found elsewhere regarding isotopic (Hussey *et al.*, 2012; Shiffman *et al.*, 2012) and radiometric (Fenton, 2001; Cotton *et al.*, 2014) analyses. Potential sources and

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TABLE I. Summary of studies that have applied elemental analyses to the hard parts of elasmobranchs

Species						
Common name	Scientific name	Region	Application	Hard part	Elements surveyed	Reference
Piked dogfish	*Squalus acanthias*	British Columbia, Canada	Age validation	Vertebrae	P, Ca	Jones & Geen (1977)
Grey reef shark	*Carcharhinus amblyrhynchos*	Northwest Hawaii, U.S.A.	Age validation	Vertebrae	P, Ca	Cailliet & Radtke (1987)
Common thresher shark	*Alopias vulpinus*	California, U.S.A.	Age validation	Vertebrae	P, Ca	Cailliet and Radtke (1987)
School shark	*Galeorhinus galeus*	Victoria, Australia	Age validation	Vertebrae	Ca, Sr, Ba, Pb	Fenton (2001)*
Piked dogfish	*Squalus acanthias*	Tasmania, Australia	Age validation	Fin spines	Ca, Sr, Ba, Pb	Fenton (2001)*
Golden dogfish	*Centroscymnus crepidater*	Tasmania, Australia	Age validation	Vertebrae	Ca, Sr, Ba, Pb	Fenton (2001)*
Southern dogfish	*Centrophorus uyato*	Victoria, Australia	Age validation	Vertebrae	Ca, Sr, Ba, Pb	Fenton (2001)*
Round stingrays	*Urobatis halleri*	California, U.S.A.	Age validation	Vertebrae	Ca, Sr, P, Mg, Na, Ba, Pb	Hale *et al.* (2006)
Small-tooth sawfish	*Pristis pectinata*	Southern Florida, U.S.A.	Age validation Environmental tracer	Vertebrae	Ca, P, Sr	Scharer *et al.* (2012)
Smooth hammerhead shark	*Sphyrna zygaena*	New South Wales, Australia	Age validation	Vertebrae	Sr	Raoult *et al.* (2016)
White shark	*Carcharadon carcharias*	New South Wales, Australia	Age validation	Vertebrae	Sr	Raoult *et al.* (2016)
Spinner shark	*Carcharhinus brevipinna*	New South Wales, Australia	Age validation	Vertebrae	Sr	Raoult *et al.* (2016)
Port Jackson shark	*Heterodontus portusjacksoni*	New South Wales, Australia	Age validation	Vertebrae	Sr	Raoult *et al.* (2016)
Dusky shark	*Carcharhinus obscurus*	New South Wales, Australia	Age validation	Vertebrae	Sr	Raoult *et al.* (2016)
Eastern Angel shark	*Squatina albipunctata*	New South Wales, Australia	Age validation	Vertebrae	Sr	Raoult *et al.* (2016)
Gummy shark	*Mustelus antarcticus*	Southwest Western Australia	Stock structure	Jaw cartilage	Al, B, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, S, Sr, Zn	Edmonds *et al.* (1996); Simpfendorfer *et al.* (1999)*

TABLE I. Continued

Species						
Common name	Scientific name	Region	Application	Hard part	Elements surveyed	Reference
School shark	*Galeorhinus galeus*	South eastern Australia	Stock structure Natal habitats	Vertebrae	Na, Sr, K, S, Cl, Ca	Stevens and West (1997)
Whiskery shark	*Furgaleus macki*	Southwest Western Australia	Stock structure	Jaw cartilage	Mg, Mn, Na, P, S, Sr, Zn, K, Ca	Simpfendorfer *et al.* (1999)*
Dusky whaler shark	*Carcharhinus obscurus*	Southwest Western Australia	Stock structure	Jaw cartilage	Mg, Mn, Na, P, S, Sr, Zn, K, Ca	Simpfendorfer *et al.* (1999)*
Scalloped hammerhead shark	*Sphyrna lewini*	Eastern Queensland, Australia	Stock structure	Vertebrae	Ba, Cu, Zn, Sr, Mg, Mn, P, Ca	Schroeder *et al.* (2010)*
Milk shark	*Rhizoprionodon acutus*	Eastern Queensland, Australia	Stock structure	Vertebrae	Ba, Cu, Zn, Sr, Mg, Mn, P, Ca	Schroeder *et al.* (2010)*
Australian blacktip shark	*Carcharhinus tilstoni*	Eastern Queensland, Australia	Stock structure	Vertebrae	Ba, Cu, Zn, Sr, Mg, Mn, P, Ca	Schroeder (2011)*
Bronze whaler	*Carcharhinus brachyurus*	Gulf St Vincent and Spencer Gulf, South Australia	Stock structure	Vertebrae	Ba, Mg, Sr, Cu, Mn, Zn	Izzo *et al.* (2016*b*)
Velvet belly lanternshark	*Etmopterus spinax*	Western France and Southern Norway	Stock structure	Vertebrae	Li, Mg, Co, Ni, Cu, Zn, Rb, Sr, Ba, Pb	McMillan *et al.* (in press)

TABLE I. Continued

Species						
Common name	Scientific name	Region	Application	Hard part	Elements surveyed	Reference
Scalloped hammerhead shark	*Sphyrna lewini*	Pacific coast Mexico and Costa Rica	Natal origins Population structure	Vertebrae	Li, Mg, Ti, V, Cr, Mn, Co, Rb, Sr, Zr, Cd, Ba, La, Pb, Ca	Smith (2013)*
Blacktip shark	*Carcharhinus limbatus*	Gulf of Mexico	Natal origins	Vertebrae	Li, Mg, Mn, Sr, Ba, Pb	Lewis *et al.* (2016)
Scalloped hammerhead shark	*Sphyrna lewini*	Pacific coast, Mexico and Costa Rica	Natal origins	Vertebrae	Li, Mg, V, Cr, Mn, Co, Rb, Sr, Cd, Ba, La, Pb	Smith *et al.* (2016)
White-edge freshwater whipray	*Himantura signifer*	Chao Phraya River, Thailand	Environmental tracer	Otoconia	Sr	Otake *et al.* (2005)
Scaly whipray	*Himantura imbricata*	Chao Phraya River, Thailand	Environmental tracer	Otoconia	Sr	Otake *et al.* (2005)
White-spotted whipray	*Himantura* (*gerarudii?*) *gerrardi*	Chao Phraya River, Thailand	Environmental tracer	Otoconia	Sr	Otake *et al.* (2005)
Pig-eye shark	*Carcharhinus amboinensis*	Northern Australia	Environmental tracer Natal habitats	Vertebrae	Li, Mg, Mn, Sr, Zn, Ba	Tillett *et al.* (2011)
Bull shark	*Carcharhinus leucas*	Northern Australia	Environmental tracer Natal habitats	Vertebrae	Li, Mg, Mn, Sr, Zn, Ba	Tillett *et al.* (2011)
Bull shark	*Carcharhinus leucas*	Southeast Queensland, Australia	Environmental tracer	Vertebrae	Ca, Mg, Cu, Sr, Ba, P, Mn	Werry *et al.* (2011)
Round stingray	*Urobatis halleri*	California, U.S.A. (*maintained in aquaria*)	Environmental tracer (in an experimental setting)	Vertebrae	Li, Mg, Mn, Ca, Zn, Sr, Ba	Smith *et al.* (2013)

*Denotes non-peer reviewed studies, *i.e.* reports or theses.

preparation of vertebral samples for elemental analysis are discussed and an overview of the applications of elemental analyses in elasmobranchs is provided, highlighting potential knowledge gaps in the field. This review also addresses several knowledge gaps, namely defining the distribution and range of concentrations of elements in the vertebral structure and provides direction for further applications of elemental analyses in elasmobranchs.

ELASMOBRANCH HARD PARTS

Elasmobranch hard parts include vertebral centra, as well as secondary hard parts such as neural arches of the vertebral column, jaws, rostral teeth, dorsal-fin spines and caudal thorns. These structures often possess growth-related banding patterns (Fig. 1) (Cailliet *et al.*, 2006) that have been used for determining ages of individuals (Cailliet & Goldman, 2004). Band formation is often annual in periodicity, comprising pairs of opaque and translucent bands deposited in summer and winter respectively following an initial birth band; growth prior to the birth band (*i.e.* from the focus to the birth band in vertebral centra) represents pre-natal tissue (Cailliet & Goldman, 2004; Cailliet *et al.*, 2006). It is, therefore, important that the annual periodicity of banding be validated for study species where elemental analyses are related to age. Where banding patterns are validated as annual, elasmobranch hard parts may provide time-resolved insights into patterns of growth, habitat use, migration and connectivity (Campana & Thorrold, 2001). In addition, elemental analysis of these structures has potential for past environmental reconstructions, as has been achieved using the isotopic composition of fossilized elasmobranch teeth (Fischer *et al.*, 2012; Kim *et al.*, 2014).

Some secondary structures (fin spines, barbs and rostral teeth) may be non-lethally sampled, which would be advantageous for endangered species (Gillanders, 2001; Field *et al.*, 2009). The external hard parts of elasmobranchs, however, may be subject to mechanical wear overtime (*i.e.* dorsal fin spines; Ketchen, 1975) or may be shed (*i.e.* caudal barbs; Thorson *et al.*, 1988), preventing the preservation of a continuous elemental record over an individual's life (Kerr & Campana, 2014). A greater understanding of the developmental processes and metabolic stability of these structures, as well as an assessment of their chemical properties is required.

Elasmobranch vertebral centra have been the most commonly utilized hard part for elemental analyses (Table I), probably because they have been the most widely used structure for age and growth studies (Cailliet & Goldman, 2004) and for this reason, their development and chemical properties are best understood. Vertebrae are composed of calcified cartilage surrounded by an extra-cellular matrix of proteins (proteoglycan and collagen), which is mineralized to varying extents by crystals of calcium phosphate hydroxyapatite (Applegate, 1967; Urist, 1976; Dean & Summers, 2006). Vertebral centra grow as dense tissue accreted distal to the vertebral focus in consecutive bands in a process of areolar calcification (Dean & Summers, 2006). Initially, centra grow continually throughout the life of individuals, but it is important to note that vertebral growth and the deposition of bands may become compressed at the vertebral edge in older individuals, confounding age validation and potentially elemental analyses. The age at which compression occurs is species specific; *e.g.* counts of band pairs to determine age may not be accurate beyond *c.*11 years in school shark *Galeorhinus galeus* (L. 1758) (Walker *et al.*, 2001) and *c.* 20 years in porbeagle shark *Lamna nasus*

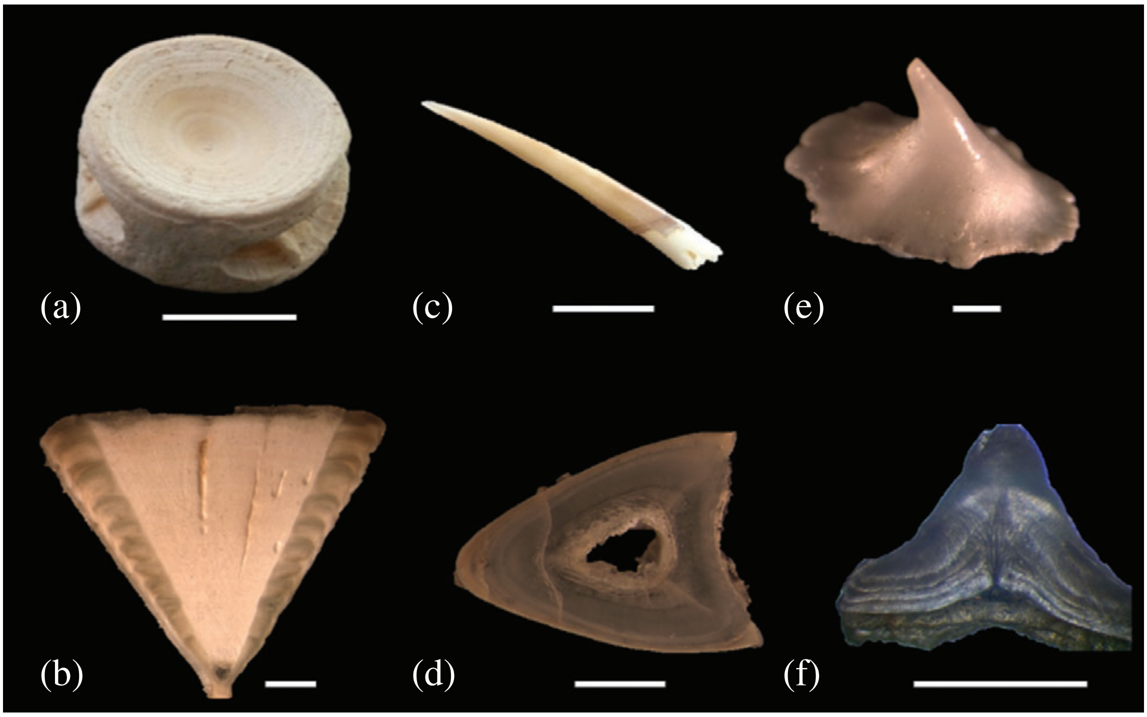

FIG. 1. Representative (a), (c), (e) whole and (b), (d), (f) sectioned hard parts from elasmobranchs. (a) , (b) vertebra of *Carcharhinus brachyurus*; (c), (d) dorsal fin spine of *Squalus megalops*; (e), (f) caudal thorn of *Spiniraja whitleyi* (courtesy of M. A. Treloar, Deakin University). Banding patterns are visible in the sectioned hard parts. Scale bars in panels (a), (c) 10 mm and 1 mm in the remaining panels.

(Bonnaterre 1788) (Francis *et al.*, 2007). The age at which bands become compressed at the vertebral edge should be taken into consideration, especially when elemental sampling is focused at the marginal edge or related to age.

The cartilaginous matrix of vertebral centra is considered metabolically inert (Clement, 1992; Ashhurst, 2004; Dean *et al.*, 2015). Suggestions to the contrary by authoritative authors including Campana (1999) and Cailliet *et al.* (2006) based on the speculation of Welden *et al.* (1987) have created some confusion around whether elasmobranch centra violate the assumption of a closed system immune to post-deposition resorption of elements that is fundamental to elemental analyses of calcified structures. Direct histological investigation, however, has found no evidence of skeletal calcium resorption in elasmobranchs (Clement, 1992). The long-term retention of temporally resolvable bomb radiocarbon (Campana *et al.*, 2002) also suggests that the chemical composition of elasmobranch vertebrae is probably retained or minimally reworked throughout an individual's lifetime; inferring the suitability of these structures for elemental analyses (Hussey *et al.*, 2012; Smith *et al.*, 2013; Kerr & Campana, 2014).

Elemental incorporation shows little variation among vertebrae from the same animal (Schroeder, 2011; Tillett *et al.*, 2011). Comparisons of elemental concentrations both within and among vertebrae from individual *G. galeus* indicate consistency in the elemental signal: (1) between the opposite sides of the *corpus calcareum*, in both the natal and edge portions of the same vertebra (Fig. 2) and (2) among multiple vertebrae from the same individual, with the exception of Zn (for methodology and detailed results see Appendix S1, Supporting information). The latter may be due to the mode of Zn incorporation into apatite; entrapment in interstitial spaces rather than direct substitution

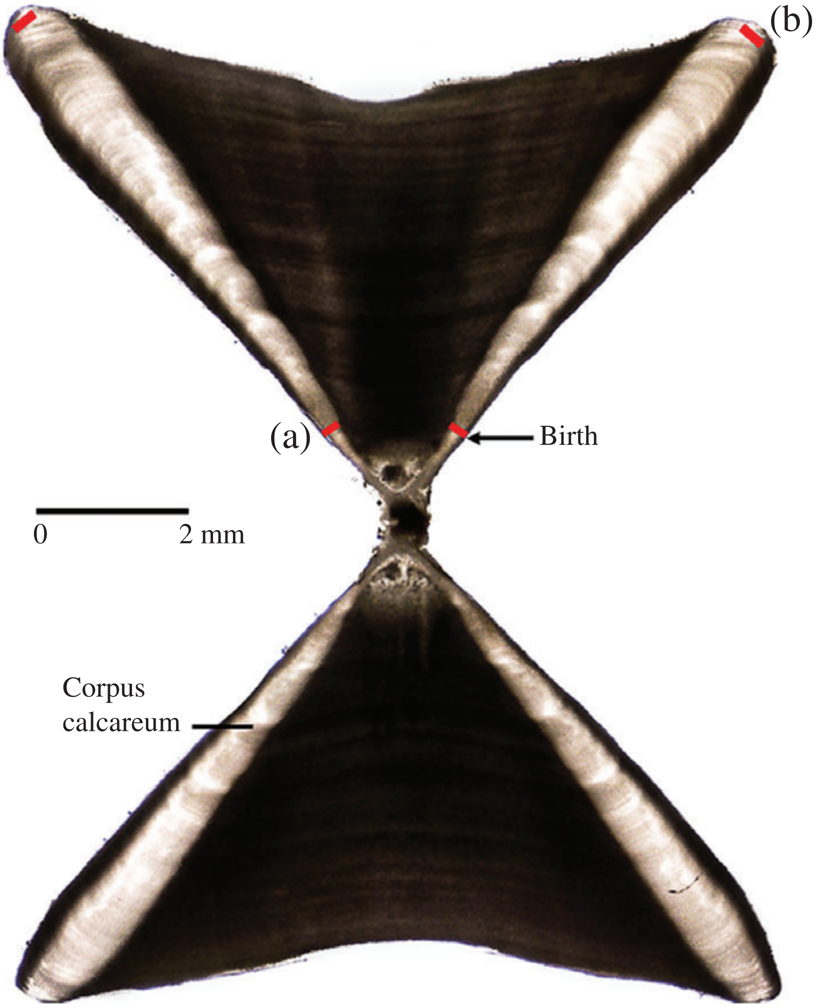

FIG. 2. Sectioned vertebra of school shark *Galeorhinus galeus* revealing visible age increments. Laser transect ablations (▬) were conducted across the corpus calcareum on the left and right side of the vertebra in both the (a) natal and marginal (b) edge areas to test consistency of elemental signals within vertebrae. Natal area was located distal to the birth band (←) relative to the vertebral focus.

for Ca (Tang *et al.*, 2009). These findings indicate that a range of elements (Li, Na, Mg, Mn, Sr, Ba and Pb) are deposited in a consistent manner within and among elasmobranch vertebrae from the same individual, inferring their suitability for elemental analyses.

SOURCES AND PREPARATION OF SAMPLES

Comprehensive collections of hard parts of elasmobranchs exist in fisheries and research institutions around the world owing to their use for age and growth studies (Cailliet & Goldman, 2004). Museums and other natural history collections may supplement more contemporary collections, through both space and time and provide samples for retrospective analysis of historic conditions (Rivers & Ardren, 1998; Izzo *et al.*, 2016*a*).

Methods of sample storage and preparation need to consider and evaluate potential elemental contamination or leaching and should broadly follow protocols developed in the study of otolith chemistry (Milton & Chenery, 1998; Proctor & Thresher, 1998). For example, when storing samples prior to preparation, it is recommended that samples be stored in a standardized manner, whether freezing or stored in ethanol solution. Storage of vertebrae in ethanol may reduce the contrast of banding patterns, increasing the potential for errors in age estimation (Wintner *et al.*, 2002); hence freezing may be preferable where chemical analyses are to be related to age. There is also evidence from isotopic studies of teleost tissues that storage in ethanol solution can lead to shifts in isotopic ratios (Sweeting *et al.*, 2004), but, as far as is known, no studies into effects of storage on elemental concentrations in elasmobranch hard parts have been conducted. Samples stored in formalin should be avoided owing to its decalcifying properties (Campana & Neilson, 1985; Nelson *et al.*, 1989). Samples should be prepared in class 100 laminar-flow cabinets or similar using acid-washed instruments and rinsed in ultrapure water to avoid contamination. Air drying in fume hoods is preferable to oven drying to avoid potential cracking of samples and elemental changes through heating (Kim & Koch, 2012; Smith *et al.*, 2013).

A common method of vertebral preparation is immersion in dilute bleach solutions to remove adjoining tissue. Preliminary evidence indicates that bleaching does not alter the elemental composition of the vertebral matrix (Tillett *et al.*, 2011). Direct testing of the effects of bleaching on elemental concentrations in the vertebrae of *G. galeus* indicate that moderate bleaching (40 min duration) had no effect on a range of elements, with the exception of Na, which increased. Extended bleaching (2 h duration) resulted in significant increases in Na and decreases in Mg and Mn (for methodology and detailed results see Appendix S1, Supporting information). Based on this work, it is recommended that Na be omitted from elemental analyses where bleaching preparation is employed and that the duration of immersion be minimized where possible; this may vary according to size and species of samples and preliminary testing of potential bleaching effects on the species of interest is recommended. Given that limited exposure to bleach does not affect most elements, the samples stored in existing collections may be suitable for elemental studies.

ELEMENTAL INCORPORATION INTO VERTEBRAE OF ELASMOBRANCHS

The chemical composition of elasmobranch vertebrae can be a useful tool to reconstruct movements and investigate habitat use where it reflects the chemistry of the external ambient environment. The extent to which ambient environmental conditions can be inferred from the elemental composition of elasmobranch hard parts, however, has not been fully investigated and there has been little evaluation of factors that influence hard part chemistry (Edmonds *et al.*, 1996; Smith *et al.*, 2013). As such, the application of elasmobranch hard-part chemistry is susceptible to running ahead of a sound fundamental understanding and full validation of the approach.

ELEMENTAL UPTAKE

In elasmobranchs, environmentally sourced elements entering the blood plasma are primarily regulated *via* the intestinal and gill interfaces and secondarily by the skin and nephron of the kidney (Pentreath, 1973; Dacke, 1979). Environmentally derived elements are then assumed to substitute for Ca or become trapped within the vertebral organic matrix (Dean & Summers, 2006; Tillett *et al.*, 2011). Pathways of elemental uptake into the fin rays and spines are thought to be similar to that of fish vertebrae (Gillanders, 2001; Kerr & Campana, 2014), but rates of element specific incorporation appear to differ among structures. Experimental evidence indicates that concentrations of Ba in vertebrae of round stingrays *Urobatis halleri* (Cooper 1863) reflect water Ba concentrations, but the relative uptake of Ba decreased with increasing availability (Smith *et al.*, 2013). It follows that Ba concentrations in elasmobranch vertebrae may be used as proxies to trace environmental Ba concentrations.

Dietary sourced elements probably contribute to the chemical composition of elasmobranch hard parts (Campana, 1999; Campana *et al.*, 2002; Estrada *et al.*, 2006), which may be interactively influenced by ambient water salinity and temperature (Izzo *et al.*, 2015). Examination of elemental uptake in the soft tissues of elasmobranchs indicates that ambient water provides a minor source of Zn, Mn, Co and Fe (Pentreath, 1973; Mathews & Fisher, 2009), but direct assessments of the relative contributions of water and dietary sourced elements to the chemistry of hard parts have not been addressed. Significant contributions of dietary sourced elements into hard parts may confound efforts to reconstruct the environmental life histories of individuals based on elemental signatures (Doubleday *et al.*, 2013). This may be particularly problematic for elasmobranchs as they may undergo ontogenetic or seasonal shifts in feeding habits (Braccini & Perez, 2005; Malpica-Cruz *et al.*, 2013) and may exhibit individual dietary specialization even within cohorts (Matich *et al.*, 2011; Matich & Heithaus, 2015).

ELEMENTAL DISTRIBUTION AND CONCENTRATIONS

Elements are incorporated within the cartilaginous matrices of elasmobranch hard parts as the latter are mineralized with deposits of calcium phosphate, $Ca_{10}(PO_4)_6(OH)_2$. Elasmobranch vertebral centra grow through accretion at the vertebral edge in the form of a permanently mineralized outer layer that does not alter the composition of the underlying mineralized matrix (Doyle, 1968; Clement, 1992). This distinguishes the apatite in elasmobranch centra from the transitional hydroxyapatite found in teleost bone and displays no evidence of post-deposition resorption as is found in bony apatite (Clement, 1992; Ashhurst, 2004). The resulting relatively poorly crystallized apatite differs from the highly crystallized aragonite of teleost otoliths; therefore it would be expected that the chemical properties of elasmobranch hard parts would differ to otoliths and other analogous calcified structures of aquatic taxa (*e.g.* coral skeletons and bivalve shells).

It is important to note that incorporation of elements into calcified structures of marine organisms may not directly correspond to ambient concentrations since uptake pathways may be regulated by physiological barriers (*e.g.* over the gills or intestines and into the plasma and finally into calcifying material) and uptake rates may be affected by factors like ontogeny, diet, temperature or salinity (Smith *et al.*, 2013). Furthermore, the relatively impure nature of elasmobranch apatite may allow for inclusion of elements in ways that differ to highly pure aragonite otoliths; this is an area requiring further research. Nevertheless, synthetic hydroxyapatites and the biogenic apatite of other marine taxa may provide proxies that help inform the understanding of inclusion processes in elasmobranch apatite. Studies involving these materials have found most metallic trace elements are incorporated *via* direct substitution for Ca, including Li

TABLE II. Species surveyed for comparisons of element concentrations in elasmobranch vertebrae and teleost otoliths

Species			
Common name	Scientific name	Family	Environment*
Elasmobranchs			
Southern shovelnose ray	*Aptychotrema vincentiana*	Rhinobatidae	Marine, demersal
Port Jackson shark	*Heterodontus portusjacksoni*	Heterodontidae	Marine, demersal
Southern fiddler	*Trygonorrhina dumerilii*	Rhinobatidae	Marine, demersal
Sparsely spotted stingaree	*Urolophus paucimaculatus*	Urolophidae	Marine, demersal
Shortnose spurdog	*Squalus megalops*	Squalidae	Marine, bathydemersal
Melbourne skate	*Spiniraja whitleyi*	Rajidae	Marine, demersal
Tiger shark	*Galeocerdo cuvier*	Carcharhinidae	Marine, benthopelagic
Bronze whaler	*Carcharhinus brachyurus*	Carcharhinidae	Marine, oceanodromous
Dusky whaler	*Carcharhinus obscurus*	Carcharhinidae	Marine, oceanodromous
Teleosts			
Australian sardine	*Sardinops sagax*	Clupeidae	Marine, pelagic-neritic
Skipjack trevally	*Pseudocaranx wrighti*	Carangidae	Marine, pelagic-neritic
Southern goatfish	*Upeneichthys vlamingii*	Mullidae	Marine, demersal
Australasian snapper	*Pagrus auratus*	Sparidae	Marine, reef-associated
Western striped trumpeter	*Pelates octolineatus*	Terapontidae	Marine, demersal
Degens leatherjacket	*Thamnaconus degeni*	Monacanthidae	Marine, demersal
Mulloway	*Argyrosomus japonicus*	Sciaenidae	Brackish, benthopelagic
Golden perch	*Macquaria ambigua*	Percichthyidae	Freshwater, demersal

*Environment as defined on FishBase (www.fishbase.org). All otolith and vertebral samples were collected in the Spencer Gulf, South Australia in 2006; with the exception of *C. brachyurus* and *C. obscurus* (caught in 2012). No catch data were available for the *G. cuvier* sample.

(Mayer *et al.*, 1986), Mg (Aoba *et al.*, 1992), Mn (Pon-On *et al.*, 2008), Fe (Pon-On *et al.*, 2008), Sr (Schoenberg, 1963; Wells *et al.*, 2000), Ba (Wells *et al.*, 2000), Cd (Bigi *et al.*, 1991; Wells *et al.*, 2000) and Pb (Bigi *et al.*, 1991). A less common route of incorporation is *via* entrapment in interstitial spaces of the expanding matrix during the accretion process, *e.g.* Zn is mainly incorporated this way (Tang *et al.*, 2009).

An exploratory assay of 12 elements in the vertebrae of a range of elasmobranchs and otoliths of teleost species (Table II and Fig. 3) provides a rapid comparison of relative elemental concentrations (methodology, Supporting information). Of the elements analysed, visual comparisons indicated Sr, Mn, Zn and Ba displayed comparable concentrations among the vertebrae and otolith samples. In general, elasmobranch vertebrae appear to have higher concentrations of all other elements investigated, with differences reaching up to several orders of magnitude, *e.g.* Mg (Fig. 3).

High contrast element maps have yielded important information regarding the spatial distribution of elemental incorporation in otoliths (Arai *et al.*, 2003; Limburg *et al.*, 2007), bivalve shells (Poulain *et al.*, 2015) and elasmobranch vertebrae (Raoult *et al.*, 2016). Such elemental maps have validated the homogenous distribution of Ca throughout teleost otoliths and bivalve shells, but more interestingly, the heterogeneous distribution of elements such as Ba and Sr (Limburg *et al.*, 2007; Schöne *et al.*, 2013). Elemental mapping of the Ca content of a Port Jackson shark *Heterodontus portusjacksoni* (Meyer 1793) vertebra showed that Ca was detected at intensities, *c.* 300 000–350 000 counts s^{-1} (cps), which was lower than that observed in the

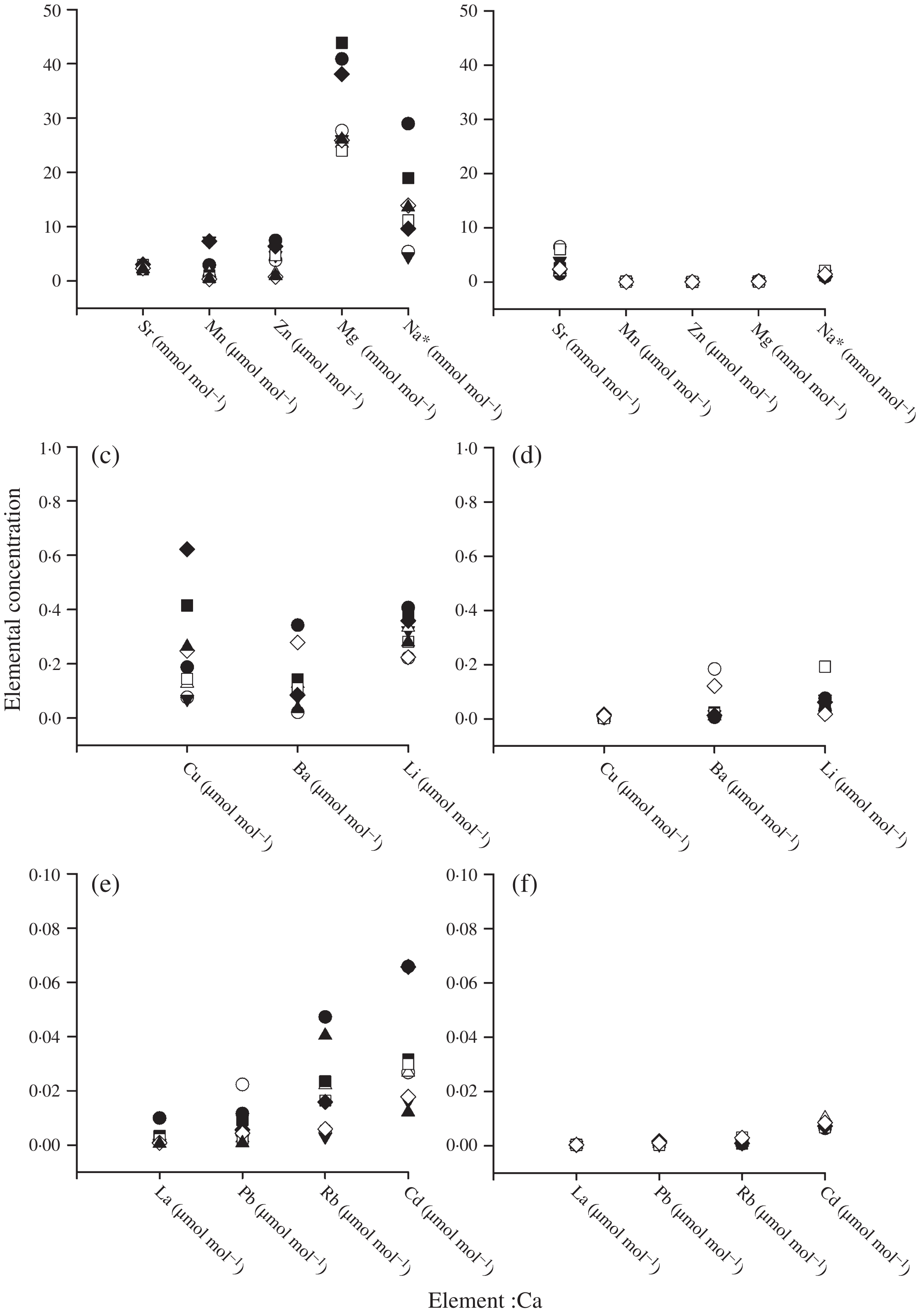

FIG. 3. Legend on next page.

otolith of an Australasian snapper *Pagrus auratus* (Forster 1801) (Fig. 4) (methodology, Supporting information). Overall, Ca declined in intensity from the vertebral edge to the focus, which differed from that seen in the *P. auratus* otolith (Fig. 4), or in other species of teleosts, *e.g.* whitefish *Coregonus lavaretus* (L. 1758), blueback herring *Alosa aestivalis* (Mitchill 1814) (Limburg *et al.*, 2007) and mottled eel *Anguilla marmorata* Quoy & Gaimard 1824 (Arai *et al.*, 2003).

Of the other elements mapped in the *H. portusjacksoni* vertebra, Mg and Ba intensities were greatest at the vertebral edge, relative to the rest of the structure. Minor variations in Mg intensity matched the vertebral banding pattern. In the *P. auratus* otolith, Mg intensity appeared to be greatest in the otolith core and Ba was heterogeneously distributed (Fig. 4). Lithium was largely homogenous within the vertebra. Strontium intensities were comparable to those seen in the otolith and for both structures peaks in Sr intensity appeared to correspond with growth bands (Fig. 4). In fact, there is evidence that growth bands may be more closely correlated with Sr concentrations than with Ca in some species, *e.g.* smooth hammerhead *Sphyrna zygaena* (L. 1758) and spinner shark *Carcharhinus brevipinna* (Valenciennes 1839) (Raoult *et al.*, 2016). Similarly, peaks in Mn intensity showed some agreement with growth bands in the vertebrae, however, signal intensities varied considerably throughout the vertebrae, potentially reflecting an environmental or physiological chemical signature (Fig. 4). Manganese was detected at higher concentrations in the focus and juvenile portion of the vertebra and in the otolith core (Fig. 4).

Concentrations of elements are generally standardized to Ca (expressed as element:Ca ratios in mmol mol^{-1}), thus providing relative concentration values for elements (as opposed to raw count data), facilitating ease of comparison among studies (Thorrold *et al.*, 1998; Campana, 1999). It is important, therefore, to quantify the Ca content (measured as the percentage mass of calcium in the structure: %Ca) of elasmobranch hard parts in order to accurately standardize elemental concentrations to the number of Ca ions in the structure. Vertebral %Ca did not differ greatly among elasmobranch species investigated, with mean %Ca values ranging from 39·6 to 48·0 (Table III; see Supporting information). No obvious differences were observed among taxonomic groups (Table III). The %Ca values for the elasmobranchs were consistent with mean %Ca values measured in teleost vertebrae [*Argyrosomus japonicus* (Temminck & Schlegel 1843): mean ± S.D. 45·86 ± 0·64% Ca) and teleost otoliths (38·8% Ca: Yoshinaga *et al.*, 2000], but were less than those reported for bivalve shells (*Plebidonax deltoides*: 56·78 ± 0·65% Ca).

FIG. 3. Comparison of elements from representative (a), (c), (e) elasmobranch vertebrae (●, *Aptychotrema vincentiana*; ○, *Heterodontus portusjacksoni*; ▼, *Trygonorrhina dumerilii*; △, *Urolophus paucimaculatus*; ■, *Squalus megalops*; □, *Spiniraja whitleyi*; ◆, *Galeocerdo cuvier*; ◇, *Carcharhinus brachyurus*; ▲, *Carcharhinus obscurus*) and (b), (d), (f), teleost otoliths (●, *Sardinops sagax*; ○, *Pseudocaranx wrighti*; ▼, *Upeneichthys vlamingii*; △, *Pagrus auratus*; ■, *Pelates octolineatus*; □, *Thamnaconus degeni*; ◆, *Argyrosomus japonicus*; ◇, *Macquaria ambigua*). All elemental data are expressed as ratios to ^{43}Ca. Note concentrations vary among elements in each of the panels, where: ^{24}Mg:Ca, ^{88}Sr:Ca and ^{23}Na:Ca shown in mmol mol^{-1}; and ^{55}Mn:Ca, ^{65}Zn:Ca, ^{64}Cu:Ca, ^{138}Ba:Ca, ^{7}Li:Ca, ^{112}Cd:Ca, ^{85}Rb:Ca, ^{207}Pb:Ca and ^{139}La:Ca shown in μmol mol^{-1}. *, all Na:Ca values were divided by 10 to fit the axes. All otolith and vertebral samples were collected in the Spencer Gulf, South Australia in 2006; with the exception of *C. brachyurus* and *C. obscurus* (caught in 2012). No catch data were available for *Galeocerdo cuvier*.

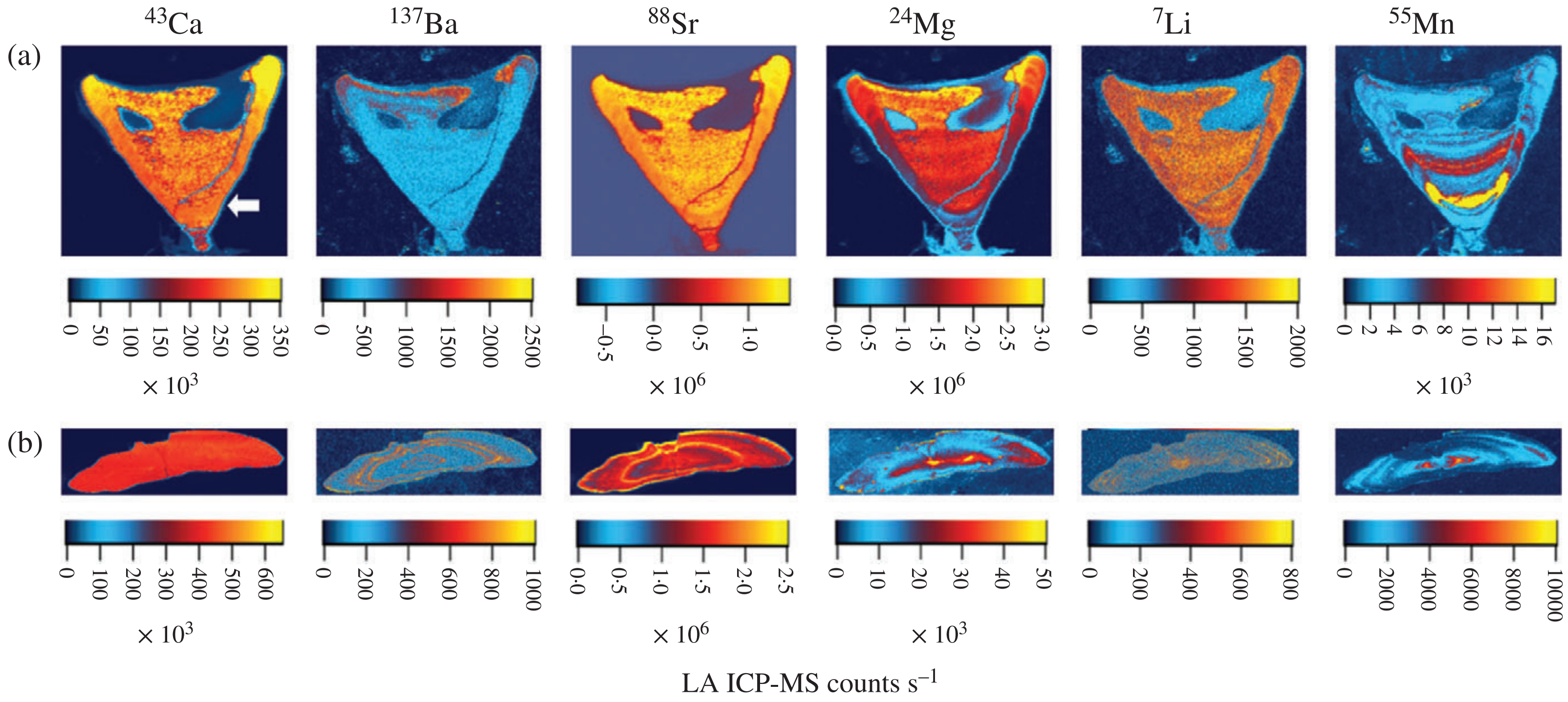

FIG. 4. Comparisons of the spatial distribution of six trace elements in (a) the vertebra of a Port Jackson shark *Heterodontus portusjacksoni* and (b) the otolith of an Australasian snapper *Pagrus auratus*. (a) ^{43}Ca: ⇦, Location of the birth band.

TABLE III. Comparisons of the mean ± S.E. percentage mass composition of calcium (%Ca) in the vertebrae of a variety of elasmobranch species

Species			
Common name	Scientific name	Family	%Ca
Tiger shark	*Galeocerdo cuvier*	Carcharhinidae	43·345 ± 0·177
Bronze whaler shark	*Carcharhinus brachyurus*	Carcharhinidae	43·117 ± 0·151
Dusky whaler shark	*Carcharhinus obscurus*	Carcharhinidae	43·507 ± 0·252
Pig-eye shark	*Carcharhinus amboinensis*	Carcharhinidae	35·160 ± 0·190*
Pig-eye shark	*Carcharhinus amboinensis*	Carcharhinidae	35·410 ± 0·185*
Bull shark	*Carcharhinus leucas*	Carcharhinidae	34·630 ± 0·210*
Bull shark	*Carcharhinus leucas*	Carcharhinidae	35·810 ± 0·183*
Shortnose spurdog	*Squalus megalops*	Squalidae	39·623 ± 0·117
Port Jackson shark	*Heterodontus portusjacksoni*	Heterodontidae	44·755 ± 0·140
Sparsely spotted stingaree	*Urolophus paucimaculatus*	Urolophidae	46·529 ± 0·161
Southern shovelnose ray	*Aptychotrema vincentiana*	Rhinobatidae	45·499 ± 0·135
Southern fiddler	*Trygonorrhina dumerilii*	Rhinobatidae	48·056 ± 0·083
Thornback skate	*Dentiraja lemprieri*	Rajidae	45·874 ± 0·120
Melbourne skate	*Spiniraja whitleyi*	Rajidae	40·083 ± 0·049

*Data from supplementary material of Tillett *et al.* (2011); where %Ca was quantified in two representative *C. leucas* and *C. amboinensis* using scanning electron microscopy with X-ray energy dispersive spectrometry.

Since most trace elements of interest are substituted directly for Ca in apatite (Schoenberg, 1963; Bigi *et al.*, 1991; Wells *et al.*, 2000), elemental concentrations are assumed to be dependent on Ca concentrations. A profile of %Ca from the outermost edge of the corpus calcareum to the focus of the vertebra of *H. portusjacksoni* showed a progressive decline in %Ca from the vertebral edge to the focus (Fig. 5). The change in %Ca profile over the vertebra is consistent with that observed in the 2D element map for Ca, both depicting Ca content varying spatially within the vertebral structure, with peak intensities at the edge region (Figs 3 and 4). Similar patterns have been reported for the grey reef shark *Carcharhinus amblyrhynchos* (Bleeker 1856), common thresher shark *Alopias vulpinus* (Bonnaterre 1788) (Cailliet & Radtke, 1987) and *U. halleri* (Hale *et al.*, 2006). An alternate pattern of Ca deposition, depicting a progressive decline from the focus to the vertebral edge was observed in small-tooth sawfish *Pristis pectinata* Latham 1794 (Scharer *et al.*, 2012). The observed spatial heterogeneity of Ca content in the vertebrae of elasmobranchs suggests an ontogenetic change in elemental uptake, which appears to be species specific. Ontogenetic trends in elemental incorporation have been reported in the calcified tissues of elasmobranchs (Eisler, 1967; Vas *et al.*, 1990; Fenton, 2001) and, if related to growth rather than habitat, potentially require statistical correction when examining patterns of age-related movement among individuals of varying ages or size (Morales-Nin *et al.*, 2012; Izzo *et al.*, 2016*b*). Ensuring that element values are normalized to Ca values obtained simultaneously will yield accurate element:Ca values and account for potential differences in %Ca between opaque and translucent bands.

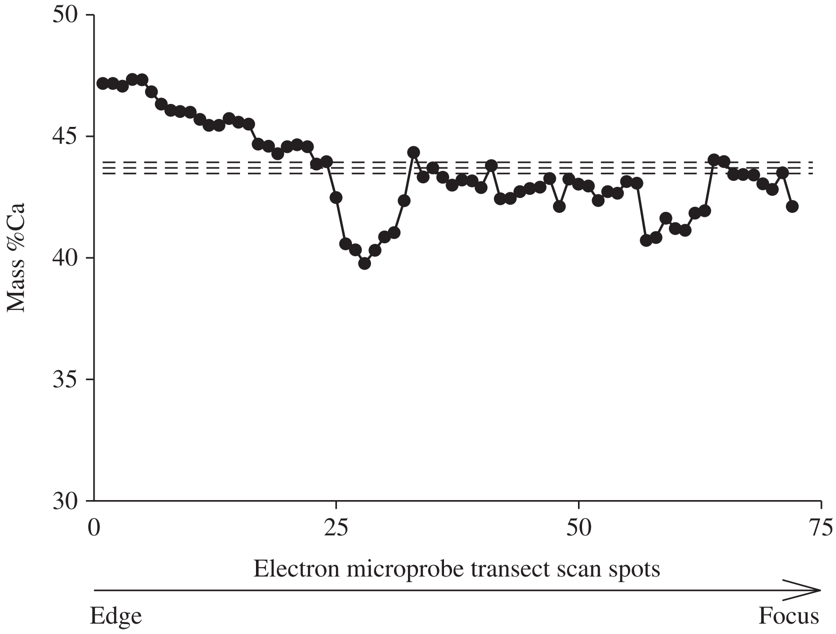

FIG. 5. Variability of mass per cent calcium (%Ca) content in the vertebrae of a *Heterodontus portusjacksoni*. —, The Ca profile from the vertebral edge to the foci; ●, individual measure of Ca; ≡ ≡, mean ± S.E. of the %Ca for all measurements across the vertebrae.

ENVIRONMENTAL INFLUENCES ON ELEMENTAL UPTAKE IN ELASMOBRANCHS

As far as is known, Smith *et al.* (2013) provide the only experimental data (*i.e.* direct evidence) illustrating the influence of temperature on vertebral chemistry. Temperature was negatively related to Mg:Ca and Ba:Ca and positively related to Mn:Ca and Zn:Ca. No relationship was detected for Li:Ca and Sr:Ca (Smith *et al.*, 2013). Based on element specific partition coefficients (which provide a measure of the elemental composition of a calcifying structure relative to that of a solution), Smith *et al.* (2013) demonstrated that temperature also had a variable effect on rates of elemental incorporation. These findings support the view that vertebral elemental chemistry reflects ambient temperature, but further studies are required to determine if similar patterns are found for other species. The lack of experimental studies probably reflects the difficulty of keeping elasmobranchs in captivity. In addition, field studies may be difficult as few elasmobranchs are territorial. Sharks are unlikely, therefore, to be spatially restricted and are potentially exposed to a range of environmental conditions that may influence elemental incorporation, confounding results.

Elasmobranchs primarily inhabit marine waters where there is little variation in salinity, with approximately 20% of the extant species of elasmobranchs utilizing habitats with reduced salinities (Martin, 2005). In euryhaline species, elemental analyses may be useful for tracing movements along salinity gradients. Bull sharks *Carcharhinus leucas* (Müller & Henle 1839) reared in environments of differing salinities showed differing multi-element signals (Werry *et al.*, 2011). Evidence for the effects of ambient salinity on the chemistry of elasmobranch hard parts is indirect (*i.e.* not formally examined), with several lines of evidence suggesting that Sr concentrations are negatively related to salinity (Otake *et al.*, 2005; Scharer *et al.*, 2012). Direct testing of the effects of salinity on hard-part chemistry, however, is required.

The limited available literature supports the application of elemental analyses to answer a range of ecological questions in elasmobranchs. Further manipulative experiments in controlled laboratory settings are required to gain a full understanding of the influence of ambient environmental conditions on elasmobranch hard-part chemistry. Research on teleost otoliths indicates that environmental influences on elemental uptake are often complex and show a high degree of species specificity (Gillanders & Kingsford, 2003; Elsdon & Gillanders, 2004). For example, while it is widely assumed that otolith Sr reflects ambient salinity, independent assessments of otolith Sr and salinity relationships yield a range of positive and negative correlations, as well as a lack of correlation between the two variables (Secor & Rooker, 2000; Gillanders, 2005*a*). In addition, temperature and salinity may interact to alter rates of elemental uptake, such that otolith Sr and salinity relationships are dependent on temperature (Elsdon & Gillanders, 2002). An improved understanding of environmental influences on the elemental composition of elasmobranch hard parts will aid in making more accurate generalizations about environmental effects among species.

APPLICATIONS OF HARD PART CHEMISTRY IN ELASMOBRANCHS

The natural occurrence and chronological capabilities of many elasmobranch hard parts (in particular vertebrae) make them attractive for age validation, investigating stock structure, investigating natal and juvenile habitats and as an environmental tracer. There are a number of instruments available to assay chemical composition of calcified structures, *e.g.* laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS), electron microprobe or proton induced X-ray emission (PIXE). Instrument selection, however, will probably be based on availability and the research questions to be addressed, since different elements are measured more precisely by different instruments (Campana *et al.*, 1997; Thresher, 1999).

The assumptions, limitations and interpretations involved in applications of elemental analysis of elasmobranch hard parts are highlighted here with methodological assumptions based on those identified for otolith chemistry applications in teleosts (Table IV; Elsdon *et al.*, 2008) and expanded with reference to unique considerations of elasmobranch biology and ecology.

APPLICATION 1: AGE VALIDATION

Band pairs in elasmobranch hard parts such as vertebral centra or dorsal spines were long assumed to be deposited annually and ascribed to differences in tissue density resulting from growth rates that vary during periods of alternating fast (summer) and slow (winter) growth (Cailliet *et al.*, 1981; Cailliet *et al.*, 1983; Cailliet, 1990) (Table I). Analysis of elemental profiles (*e.g.* Ca and P) in such hard parts has been used to verify the annual periodicity of band-pair deposition and validate age estimates derived from visual counts of band pairs by matching peaks and troughs in elemental concentrations with summer and winter bands, respectively (Jones & Geen, 1977; Cailliet & Radtke, 1987). Given the importance of age and growth data to fisheries and conservation management, verification of annual periodicity in band pairs in elasmobranch hard parts has been one of the most widespread applications of elemental analysis of elasmobranch hard parts to date (Table I).

Table IV. Assumptions relating to the four applications of hard-part chemical analysis in elasmobranchs (adapted from Elsdon *et al.*, 2008). Applications: 1, age validation; 2, determining stock structure; 3, assessing natal and juvenile habitats; 4, environmental tracer

	Application			
Assumption	1	2	3	4
1. There is no alteration of the elemental signal	x	x	x	x
2. Age of individuals is correctly determined	x	x	x	x
3. Sample collection is random and representative of the population	x	x	x	x
4. Elemental composition changes predictably with environmental variables	x			x
5. Interactive effects of environmental variables on elemental incorporation are understood	x			x
6. Ontogenetic effects on elemental incorporation are understood	x			x
7. Spatial and temporal variations in environmental variables are known	x			x
8. Correlations between structural development and chemistry are known	x			x
9. Elemental signals are representative of the group of interest and unique to it		x	x	x
10. Methods of sample collection are consistent		x	x	x
11. Groups segregate sufficiently in time and space to incorporate unique elemental signals		x	x	x
12. Methods of elemental analysis are consistent		x	x	x
13. Natal elemental signal source is known			x	

Hypotheses and assumptions

Age verification has been based on visually contrasting vertebral growth bands with intensity peaks along elemental profiles, which are assumed to represent seasonal fluctuations in ambient environmental conditions. Moderate to good concordance between elemental peaks and observed growth bands, inferring seasonality in banding-pattern formation, has been reported (Cailliet & Radtke, 1987; Hale *et al.*, 2006; Raoult *et al.*, 2016). The following assumptions apply to this application.

Assumption 1: there is no alteration of the elemental signal. In order to provide a reliable chemical record for interpretation, elemental composition should not be subject to reworking or alteration after accretion (Campana, 1999; Gillanders, 2001). Elements trapped in the growing structure of calcified material may be prone to contamination or leaching associated with method of storage or preparation (Campana *et al.*, 2000; Elsdon *et al.*, 2008); prolonged sample extraction and storage in either ethanol or hyper-saline solution has been shown to alter otolith elemental concentrations (Proctor & Thresher, 1998). Hence, timely extraction of vertebrae and standardized sample handling should be employed to minimize the potential for contamination or leaching (refer to section on sources and preparation of samples).

Assumption 2: age of individuals is correctly determined. Visual counts of band pairs should be accurate as a baseline against which to contrast element profiles. Counts can be assessed to detect imprecision or bias by comparing counts from multiple readers (Walker *et al.*, 2001).

Assumption 3: sample collection is random and representative of the population. Samples should reflect patterns of growth and ageing representative of the population as a whole, *e.g.* all available age classes should be sampled to uncover any potential age-related trends in elemental uptake or band deposition due to the compression of bands at the vertebral edge in older individuals.

Assumption 4: elemental composition changes predictably with environmental variables. The manner in which elemental incorporation changes shows a seasonality that can be used to infer periodicity of band pair deposition, *e.g.* different concentrations are routinely deposited in summer than in winter, allowing for contrasting concentrations in band pairs (Raoult *et al.*, 2016). Changes in elemental concentrations may be related to changes in environmental variables such as temperature (Smith *et al.*, 2013) or salinity (Scharer *et al.*, 2012).

Assumption 5: interactive effects of environmental parameters on elemental incorporation are understood. Any interactive effects of factors such as salinity and temperature on elemental uptake should be accounted for to ensure accurate interpretation of fluctuations in chemical profiles.

Assumption 6: ontogenetic effects on elemental incorporation are understood. Failure to account for age and growth-related changes in elemental incorporation may give rise to incorrect interpretations of temporal patterns in elemental uptake. In some species, compression of growth bands at the vertebral edge may confound ageing and elemental analysis beyond a certain age (likely to be species specific) and result in underestimation of ages of older individuals (Hale *et al.*, 2006).

Assumption 7: spatial and temporal variations in environmental parameters are known. Failure to account for spatial or inter-annual fluctuations in ambient chemistry may result in false-positives in estimation of specimen age; *e.g.* where peaks in elemental concentrations are deposited non-annually.

Assumption 8: correlations between structural development and chemistry are known. The accretion rate of elasmobranch cartilage is correlated with physiological or environmental factors (Smith *et al.*, 2013), which may cause variation in elemental concentrations complicating interpretations of elemental signals. Evidence suggests that vertebral precipitation rates do not generally alter elemental composition (Smith *et al.*, 2013); however, the generalities of these findings require further assessment (possibly on a species specific basis).

Limitations

Careful consideration as to which elements are employed for age verification purposes is required. For example, elements that are linked to physiological processes, such as Zn and K should be avoided. Non-essential elements that may be more closely related to environmental processes should be targeted (*e.g.* Sr or Ba), as these probably reflect seasonal patterns (Mugiya *et al.*, 1991; Campana, 1999; Sturrock *et al.*, 2014). An understanding of how different elements in elasmobranchs are physiologically regulated and incorporated into hard parts requires further investigation.

Example: growth of Squalus acanthias

Estimation of growth parameters based on the enumeration of elemental peaks has been investigated in spiny dogfish *Squalus acanthias* L. 1758 (Jones & Geen, 1977).

Resultant (von Bertalanffy) growth curves based on elemental analysis were consistent with those from an earlier, conventional ageing study. Uncertainties in identifying peaks at the edge of vertebrae (due to age-related compression of growth bands), however, probably results in underestimation of the ages of the oldest individuals and therefore, overestimating the species growth rates (Jones & Geen, 1977; Hale *et al.*, 2006).

APPLICATION 2: DETERMINING STOCK STRUCTURE AND CONNECTIVITY

Chemical analysis of teleost hard parts to delineate stock boundaries has become commonplace in fisheries science (Campana *et al.*, 2000; Kerr & Campana, 2014), but has been employed infrequently in elasmobranch species (Table I). Fisheries management involves the identification of management units, termed stocks, which can be defined as self-replenishing groups of fishes (Secor, 2013). Stocks may comprise groups of open populations connected by migration processes, allowing the movement of individuals between them, or single closed populations more susceptible to stressors that should be managed in different ways (Carvalho & Hauser, 1994). The stock concept thus provides a tool to help manage groups of fish that are affected homogenously by stressors such as fishing pressure and habitat degradation.

Hypotheses and assumptions

Where populations are connected by the movement of individuals, those individuals may incorporate similar elemental signals arising from exposure to shared water chemistries in the area over which they are distributed. These signals may be used to delineate boundaries between stocks or groups of connected populations. The elemental composition at the marginal vertebral edge (the region of most recent growth prior to capture) may be analysed to assess stock structure (McMillan *et al.*, in press). Alternatively, ecological connectivity among areas (*e.g.* estuary to open coast) may be investigated throughout the lifetimes of individuals (*i.e.* not restricted to time of capture) by analysing the elemental composition from the birth band to the marginal vertebral edge or discrete sections thereof as research questions require (Izzo *et al.*, 2016*b*). In addition to Assumption 1 (there is no alteration of the elemental signal), Assumptions 2 and 3 (as for Application 1) apply to this application with note to the following.

Assumption 2: age of individuals is correctly determined. To discriminate groups of fish based on hard-part chemistry where temporal variation in elemental signals exists (see Assumption 9), it is necessary to know the age of individuals to facilitate temporal cross-matching among corresponding sampling years and cohorts. Among elasmobranch species, however, variability in growth-band clarity and the decoupling of band-pair count–somatic growth relationships impede the accuracy and precision of age estimates for some species (Cailliet & Goldman, 2004). In addition, ageing precision may change with increasing specimen age as growth bands become compressed at the marginal edge of the vertebrae (Hale *et al.*, 2006).

Assumption 3: Sample collection is random and representative of the population. Sample collection should be random and representative of the population, such that the sample (and resultant elemental signal) is not influenced by size or sex segregation, a behaviour common among elasmobranch species (Wearmouth & Sims, 2008).

A series of variables (*e.g.* size range, mean size) should be investigated to ensure that significant differences do not occur among sample collection locations. In addition, the following assumptions apply to this application.

Assumption 9: elemental signals are representative of the group of interest and unique to it. Sample collection should be spatially and temporally comprehensive in order to develop elemental signals that are representative of the entire group rather than a component of the group; hence, it may be necessary to sample from multiple sites within a region of interest. Where geographically separate groups share common elemental signals it is impossible to differentiate them, suggesting that the spatial scale of sampling may be too fine or the environment homogeneous, such that chemical variation within groups exceeds that among groups (Elsdon *et al.*, 2008). In addition, temporal variation in elemental signals may occur within groups (*e.g.* inter-annual variation), confounding spatial interpretations. This can be assessed by sampling and comparing chemistries throughout time (*e.g.* over multiple years). Where temporal variation in group elemental signals is apparent, spatial comparisons among elemental signals should be limited to temporally matched cohorts.

Assumption 10: methods of sample collection are consistent. To gather data representative of natural populations and their densities, identical methods and effort should be employed in collecting samples from different sites. For example, fishery-dependent samples may result in a sampling bias favouring larger individuals, *i.e.* belonging to older cohorts that may not reflect the entire population (Xavier *et al.*, 2012). In order to reflect natural population densities and distributions, sample sizes should not be set arbitrarily but rather reflect the number of individuals collected at a given site using the same amount of effort used at all sampling sites (Elsdon *et al.*, 2008).

Assumption 11: groups segregate sufficiently in time and space to incorporate unique elemental signals. Groups should segregate sufficiently among different habitats for sufficient duration such that exposure to differing water chemistry gives rise to unique elemental signals that can be used to distinguish groups. Preliminary evidence suggests that spatially discrete vertebral elemental signatures may be formed in as little as 3 weeks (Werry *et al.*, 2011). Where groups cannot be distinguished they may be considered to comprise a single group. When specific sections of the vertebra are targeted for analysis, *e.g.* the natal vertebral portion, segregation should occur during the relevant (natal) period. Where whole structural profiles are analysed (*i.e.* profile analyses employing LA ICP-MS transect ablations from the birth band to the vertebral edge along the corpus calcareum), it suffices that segregation occurs at any period.

Assumption 12: methods of elemental analysis are consistent. The method of element quantification should be consistently applied to all samples to avoid introducing erroneous variation in the elemental signal (Campana *et al.*, 1997). Methodological standardization should be applied to both the region of the sample analysed, the technique employed to assay the chemical concentration (*e.g.* LA ICP-MS or PIXE) and the instrumental data acquisition methods in order to provide consistent elemental concentration data (Campana *et al.*, 1997; Secor *et al.*, 2002). It is preferable to randomize the order of samples prepared for elemental analyses and where possible analyse all samples in a single session to avoid inaccuracies that may arise from instrumental drift over time (Campana *et al.*, 2000; Swearer *et al.*, 2003) or ensure that any variation among sampling sessions can be corrected.

Limitations

Similar to other tagging approaches, an absence of significant differences in elemental signatures among areas does not necessarily imply that populations are the same (Campana *et al.*, 2000; Schroeder *et al.*, 2010). For example, individuals from adjacent populations may be freely migrating between similar regions, or ambient environmental conditions may be the same, producing similar elemental signatures. Hence, an understanding of the ecology of the species will ensure accurate interpretation of patterns of movement and population structuring based on elemental analyses.

The inherent migratory behaviour and varying patterns of habitat use of elasmobranch species requires consideration when employing elemental analysis of hard parts for delineating stock structure. Species that are philopatric (Hueter *et al.*, 2005) or that have life-history stages that are resident within habitats (*e.g.* nursery areas; Heupel *et al.*, 2007) would be amenable to having site-specific elemental signatures, facilitating assessments of stock structure and connectivity based on elemental analyses. It should be noted that elasmobranchs may exhibit ontogenetic shifts in habitat use (Knip *et al.*, 2011; Carlisle *et al.*, 2015) that drive changes in vertebral chemistry; this should be taken into consideration to avoid misattributing differences between juvenile and adult cohorts to differences in stock structure. In contrast, species that are widely distributed and highly migratory over relatively short time scales (*i.e.* days to weeks) (Heupel *et al.*, 2004; Ortega *et al.*, 2009), may be less well suited to elemental analyses, as individuals from potentially different stocks may co-inhabit some areas (Cortés, 2004; Speed *et al.*, 2010), making element-based assessments of population structure problematic. Approaches that integrate data from other techniques such as artificial tagging (Werry *et al.*, 2011) may facilitate interpretation of elemental data and *vice versa*, allowing for robust analyses (as has been applied in stable-isotope analyses; Papastamatiou *et al.*, 2010; Carlisle *et al.*, 2012).

Example: assessing stock structure in commercially exploited shark species

Elemental composition of jaw cartilage has been analysed to delineate three populations of commercially exploited gummy shark *Mustelus antarcticus* Günther 1870 in Western Australia (Edmonds *et al.*, 1996). A disc of jaw tissue was excised and dissolved for analysis by ICP-MS and ICP-AES (atomic emission spectrometry). This procedure is akin to the dissolution of whole otoliths (Campana *et al.*, 2000) or taking mean element concentrations from lifetime profile analyses of elasmobranch vertebrae (Schroeder *et al.*, 2010; Izzo *et al.*, 2016*b*). It provides insights into use of similar habitats among groups of fishes over lifetimes and is thus useful for investigating population connectivity and stock structure. In contrast, assays of chemistry at the vertebral edge reflect recent habitat use before capture and may be temporally limited, thus potentially missing periods of seasonal connectivity in wide ranging species that move among areas of different water chemistry.

APPLICATION 3: ASSESSING NATAL AND JUVENILE HABITATS

Several characteristics of the reproductive strategies of elasmobranchs (*i.e.* high degree of philopatry, use of nursery areas) infer the potential to develop unique elemental signatures in hard parts that may allow differentiation among pupping grounds (*i.e.* natal habitats) and juvenile nursery sites (Stevens & West, 1997). Furthermore, as many species show cohort and sex-based segregation (Heupel *et al.*, 2007), it may be possible to assess patterns of population connectivity and dispersal (Gillanders & Kingsford, 2000; Gillanders, 2002).

Hypotheses and assumptions

Periods of sufficient residency in nursery areas with unique water chemistry may give rise to unique elemental signatures in YOY and juvenile cohorts. In YOY cohorts, quantifying elemental composition of the natal portion of the hard part may provide a representative pupping-ground signature, assuming that new born individuals remain in a physically or chemically stable environment. For juveniles, the direct incorporation of elements from the ambient environment may be used to identify and differentiate among nursery habitats. Experimental evidence suggests that nursery signatures can be formed in vertebrae in as little as 3 weeks (Werry *et al.*, 2011), which is within observed residency times of newborn and juvenile elasmobranchs (Heupel *et al.*, 2007).

Assumptions 1–3 as for Application 1 (there is no alteration of the elemental signal; age of individuals is correctly determined; and sample collection is random and representative of the population) as well as assumptions 9–12 as for Application 2 (elemental signals are representative of the group of interest and unique to it; methods of sample collection are consistent; groups segregate sufficiently in time and space to incorporate unique elemental signals; and methods of chemical analysis are consistent) apply to this application, in addition to the following assumptions.

Assumption 13: natal elemental signal source is known. Elemental signatures from YOY pups known to have been sourced from particular natal areas are required to establish baseline signatures against which to establish natal origins of older individuals and link them back to natal areas (Tillett *et al.*, 2011). It is imperative in this case to match cohorts by age to account for any temporal variation in water chemistry in natal areas unless temporal variation in water chemistry has been ruled out by temporal sampling (*e.g.* inter-annual sampling in cases where natal residency is known to span a single season or inter-seasonal sampling where natal residency may span multiple seasons). Testing of water chemistry may be useful to discern elemental signatures unique to particular natal areas (for an example of water sampling methodology; Elsdon & Gillanders, 2006). It is imperative to discern and sample the natal portion of vertebrae (distal to the birth band) correctly and not to sample any pre-natal material that represents time spent within the mother (in viviparous species) and may not reflect the water chemistry of nursery areas.

Limitations

Many species of elasmobranchs exhibit protracted birthing and recruitment periods (Branstetter, 1987); hence, cohort specific elemental signatures may occur within a single year class, impeding classifications of animals to annually resolved natal or nursery regions (Gillanders, 2002; Smith, 2013). Separating annually resolved *S. lewini* natal signatures into an early and late season elemental signature marginally improved the classification success of YOY sharks to their putative pupping areas (Smith, 2013). These findings suggest that species-specific reproductive strategies of elasmobranchs and reproductive timing require consideration to ensure that sampling and analysis are conducted at appropriate temporal scales.

Example: delineating putative natal signals and assigning sharks to natal sites

Unique natal signatures have been successfully characterized for shark populations that differentiate natal habitats spatially based on vertebral chemistries (Tillett *et al.*, 2011; Smith, 2013; Lewis *et al.*, 2016). Vertebral chemistry, however, has shown mixed success in classifying individual sharks back to their putative pupping areas. Analysis of the natal region of vertebrae resulted in classification success to locations of capture varying among years and between sites, between 26 and 80% for YOY *S. lewini* (Smith, 2013) and 54–100% for juvenile blacktip shark *Carcharhinus limbatus* (Müller & Henle 1839) (Lewis *et al.*, 2016). Conversely, while Tillett *et al.* (2011) were unable to discriminate nursery areas for juvenile pig-eye shark *Carcharhinus amboinensis* (Müller & Henle 1839) they were able to do so for *C. leucas* (but they were unable to assign adult *C. leucas* to any sampled nursery areas because adults were not temporally matched to years of birth), indicating the effectiveness of this application may vary among species.

APPLICATION 4: ENVIRONMENTAL TRACER

When related to growth bands, element profiles can provide time-resolved records of environmental histories of individuals, providing insights into age-related patterns of movement and habitat use (Elsdon *et al.*, 2008; Hussey *et al.*, 2012). Moreover, validating relationships between ambient water chemistry and the elemental composition of elasmobranch hard parts infers the potential for these structures to be used as environmental proxies (akin to coral skeletons and bivalve shells; Schöne & Gillikin, 2013), which has been investigated using Sr concentrations in *P. pectinata* vertebrae (Peverell, 2010) and isotope analyses of shark teeth (Fischer *et al.*, 2012). When available, suitable vertebrae may provide novel palaeo-proxies for reconstructing past environmental conditions over extensive time spans.

Lifetime elemental records are assayed along transects encompassing the area of interest from the natal tissue distal to the birth band to the vertebral edge. In viviparous species, it is imperative to avoid ablating pre-natal tissue that may be associated with elemental concentrations derived from the mother rather than deposited during the lifetime of the individual being examined.

Hypotheses and assumptions

By incorporating elements from the surrounding environment in concentrations reflective of ambient water chemistry, elemental composition in elasmobranch hard parts can act as environmental tracers to help reconstruct periods of residency and movements between habitats (Gillanders & Kingsford, 1996; Elsdon *et al.*, 2008). All previously discussed assumptions apply to this application, except Assumption 13 (natal elemental signature is known), but note the following.

Assumption 4: elemental composition changes predictably with environmental variables. Reconstructions of habitat use are reliant on establishing relationships between hard-part chemistries and environmental variables, such as salinity and temperature. Laboratory experiments may assist in validating such relationships. Unfortunately, to date such knowledge in elasmobranchs extends only to laboratory testing on the effects of salinity on vertebral chemistry of juvenile *C. leucas* (Werry *et al.*, 2011) and of temperature and manipulated Ba water concentrations on juvenile *U. halleri* (Smith *et al.*, 2013). Natural environmental gradients could also be used for site-associated species, *e.g.* increasing Sr concentrations are indicative of transitions from fresh water to sea water (Gillanders & Munro, 2012). A summary of elements with potential utility as environmental tracers is given in Table V.

Assumption 5: interactive effects of environmental parameters on elemental incorporation are understood. Environmental variables may act independently or in combination on hard part elemental composition (Elsdon & Gillanders, 2002, 2004). Therefore, it is informative to identify the interactive drivers of elemental uptake. This may be achieved through laboratory validation experiments, *e.g.* testing interactive effects of various salinity and temperature treatments on elemental uptake (Elsdon & Gillanders, 2004; Reis-Santos *et al.*, 2013).

Assumption 6: ontogenetic effects on elemental incorporation are understood. Repeated measures of elemental composition along growth axes encompassing multiple life-history stages of individuals (*e.g.* profile analyses from the juvenile to adult portion of the structure) should be interpreted with caution, as variation in element profiles may reflect ontogenetic effects rather than environmental effects (Campana, 1999; Elsdon *et al.*, 2008; Walther *et al.*, 2010). If substantial differences in growth–incorporation rates exist among life-history stages, then it may be necessary to account for such differences. Ontogenetic trends in elemental incorporation may be statistically accounted for by subtracting the mean of a line of best fit from the time-series data (*i.e.* detrending the data; Izzo *et al.*, 2016*b*). Laboratory rearing of elasmobranchs will have some utility in assessing the effect of ontogeny on elemental uptake; however, these patterns will probably be species specific.

Assumption 7: spatial and temporal variations in environmental parameters are known. Localized environmental variables (*e.g.* salinity, temperature and water chemistry) that affect element variation probably vary over time, such that temporal changes in water chemistry may erroneously be attributed to movements of fishes between areas of differing water chemistry (Gillanders & Kingsford, 2000; Kraus & Secor, 2005). Hence, environmental variables should be quantified over time and ideally at a range of spatial scales (local and regional), since these are not necessarily uniform (Gillanders, 2005*b*).

Limitations

Interpretations of environmental histories of animals require an understanding of relations between the chemistry of the hard part and the surrounding water, for which little experimental work has been done for elasmobranchs (Smith *et al.*, 2013). Alternatively, sampling the water at the location of capture, or the most recently developed region of the structure may provide a regional signal to characterize the chemical properties of particular water masses. This technique has been applied using Sr:Ca profiles in vertebrae to infer age-related movements of euryhaline elasmobranchs through environments of differing salinities (Tillett *et al.*, 2011; Werry *et al.*, 2011; Scharer *et al.*, 2012). Element profiles in the vertebrae of the coastal *C. amboinensis* failed to distinguish movements between offshore and inshore habitats, as only subtle changes in elemental concentrations were detected (Tillett *et al.*, 2011). This suggests that inferring patterns of habitat use in obligate marine or freshwater species may be hindered by the stability of the chemical environment (*e.g.* sea water has maintained a global Sr concentration of 0·79 mg l^{-1} for the past 400 000 years: Ingram & Sloan, 1992).

TABLE V. Elemental tracers in fish hard parts that may assist in tracing shifts in environmental variables. Studies involving chondrichthyans are in bold; remaining studies involve teleosts

Environmental tracer	Environmental variable			
	Temperature	Salinity	Pollution	Upwelling
Barium	**Smith *et al.*** (2013)* Elsdon and Gillanders (2002) Miller (2009)*	Martin and Thorrold (2005)* Reis-Santos *et al.* (2013)* Barnes and Gillanders (2013)		Patterson *et al.* (1999) Bath *et al.* (2000) Woodson *et al.* (2013)
Strontium	Elsdon and Gillanders (2002) Clarke *et al.* (2010)* Collingsworth *et al.* (2010)	**Otake *et al.*** (2005) **Tillett *et al.*** (2011) **Scharer *et al.*** (2012)		
Magnesium†	**Smith *et al.*** (2013)* Miller (2011)* Barnes and Gillanders (2013)			
Manganese†	**Smith *et al.*** (2013)* Miller (2009)* Elsdon and Gillanders (2005)	Limburg *et al.* (2007)		
Lithium†	Tanner *et al.* (2013)	Hicks *et al.* (2010)*		
Rubidium†		Hicks *et al.* (2010)*		
Nickel			Geffen *et al.* (2003) Arslan and Secor (2005)	
Lead†			Geffen *et al.* (1998)* Arslan and Secor (2005) Ranaldi and Gagnon (2008)	
Copper†			Hanson and Zdanowicz (1999) Arslan and Secor (2005) Barbee *et al.* (2013)*	
Mercury			Geffen *et al.* (1998)*	
Cadmium			Arslan and Secor (2005) Ranaldi and Gagnon (2009)*	

*Denotes studies validated with laboratory experiments.

†Denotes elements that may be subject to physiological regulation (Ballatori *et al.*, 1988; Bury *et al.*, 2003; Mager, 2012; Smith *et al.*, 2013). Teleost citations are not exhaustive in some cases and are provided only as guidance where substantial chondrichthyan literature is not yet available.

Hence, the application of elements as an environmental tracer in elasmobranchs may be limited to those few euryhaline species (*e.g. C. leucas* and *P. pectinata*), as it may be easier to distinguish gross changes in elemental concentrations that delimit freshwater and marine habitats (Campana, 2005). Alternatively, use of other elements or multi-element signatures may help infer fine-scale movements between areas where water chemistry differs, *e.g.* areas with differing sedimentology, anthropogenic sources or seasonal influences like upwelling and terrestrial runoff. Such applications, however, remain largely unexplored. Given that some species of elasmobranchs have been shown to make transoceanic migrations, *e.g.* basking sharks *Cetorhinus maximus* (Gunnerus 1765) (Gore *et al.*, 2008), white sharks *Carcharodon carcharias* (L. 1758) (Bonfil *et al.*, 2005), elemental analysis may have some utility in broadly characterizing movements across ocean basins and between continental shelves.

Example: age-related movements in C. leucas

Age-related movements and periodic migrations of *C. leucas* to estuarine habitats to pup were investigated in northern Australia (Tillett *et al.*, 2011). Estuarine and coastal habitats were differentiated based on elemental signals. Profile analyses indicated that adult females had elemental signatures consistent with periodic returns to estuarine waters in 1–2 year cycles, consistent with known pupping related movements of the species into estuarine systems. Conversely, males had more uniform elemental profiles suggesting that they were less likely to return to estuaries throughout their lives.

FUTURE DIRECTIONS AND CHALLENGES

This review briefly summarizes research undertaken using elements in elasmobranch hard parts; however, there is potential for further application of elemental analyses in elasmobranch studies. Future applications of elasmobranch hard-part chemistry can be guided by the existing (and extensive) body of literature for otolith and bivalve shell elemental analysis. For example, a better understanding of the environmental sensitivity of hard parts may enable these structures to be used as indicators of persistent organic pollutants in the aquatic environment. This use of elasmobranch hard-part chemistry as a pollution indicator would require that the assumptions highlighted for Application 4 (environmental tracer) be met (Table IV).

Similarly, the elemental composition of elasmobranch hard parts may be used as palaeo-environmental tools, as has been performed using otoliths (Disspain *et al.*, 2016); *e.g.* reconstructing pre-historic salinity conditions of estuarine ecosystems (Disspain *et al.*, 2011; Izzo *et al.*, 2016*a*). Acquiring palaeo-samples of sufficient quality, however, may be problematic, as there is uncertainty around species identification and sample origin given the possibility of *post mortem* transport. In addition to meeting the assumptions for Application 4 (environmental tracer) (Table IV), further assumptions for palaeo-tracers would include knowledge about how digenetic–taphonomic processes may alter the elemental composition of samples over time (Disspain *et al.,* 2016).

Given the long gestation periods of many viviparous elasmobranchs and the potential for maternal contribution of elements to the developing embryo (Lyons *et al.*, 2013), vertebral chemistry may provide a maternal tag in pups that could help trace natal origins in these species (oviparous species that develop outside the mother will have less maternal elemental contribution or mediation). The potential for assessing transgenerational tags in elasmobranch hard parts would require that assumptions for Application 2 (determining stock structure and connectivity) are met (Table IV). In addition, the source of the maternal tag would need to be identified, either through water chemistry testing or comparisons with samples representative of the putative maternal habitat. Periods of elemental-tag retention in the mother and transfer to the embryo would also need to be known to be sure that the relevant portion of offspring hard parts is analysed. This would in turn require consideration of the modes of elasmobranch reproduction (placental viviparity, aplacental viviparity or oviparity) as each would entail differences in the passage of elements across physiological barriers between the mother and offspring (Dulvy & Reynolds, 1997). In oviparous species, retention of the embryo within the mother is of variable duration and much shorter than for viviparous species (Hamlett *et al.*, 2005), therefore any maternal tag would need to be identified and distinguished from elemental signatures acquired post-oviposition. Particularly in wide-ranging, highly mobile species, maternal contributions to the pre-natal chemistry of offspring may represent a range of habitats. Further research into how maternal chemistry translates into pre-natal chemistry in elasmobranch hard parts with respect to the various modes of elasmobranch reproduction would aid in refining the use of hard-part chemistry as a transgenerational tag in elasmobranchs.

In summarising the potential applications of elemental analyses in elasmobranchs, Edmonds *et al.* (1996) concluded that ' … the way is open for more detailed studies to be carried out'. Clearly there remains a need for further experimental work assessing the effects of abiotic and biotic factors on the uptake of elements in the hard parts of elasmobranchs, improving confidence in the interpretation of elemental data. Comparative studies among species with contrasting demographies and ecological niches are necessary to fully understand the utility of elemental chemistry in elasmobranchs more broadly. Owing to the logistical constraints of maintaining elasmobranchs in controlled experimental aquaria, however, future studies may be limited to relatively small sized, demersal species that are not obligate ram ventilators (*i.e.* do not require continual swimming to aerate their gills).

Future applications of elasmobranch hard part chemistry should be used in conjunction with existing biological (*i.e.* assessments of demographic parameters and molecular analyses) and ecological approaches (*i.e.* telemetry and isotopic studies) in order to provide a comprehensive understanding of patterns of habitat use, connectivity and movement in populations of elasmobranchs. Furthermore, the combined use of more conventional methodologies can aid in the interpretation of elemental data.

CONCLUSION

Elemental analysis of elasmobranch hard parts shows great potential to answer a range of ecological and biological questions at the individual and population level. With a greater understanding of the abiotic and biotic factors influencing rates and patterns of elemental uptake into elasmobranch hard parts, this underutilized approach will become more commonly employed given the information that can be obtained, particularly when complemented with alternate methodologies.

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Supporting Information

Supporting Information may be found in the online version of this paper:

Appendix S1. Consistency of elemental signals and bleaching effects on vertebrae.

Table S1. Experimental designs. Experiment 1 tested consistency of natal chemistry within vertebrae (left *v*. right sides) from the same individual (Exp. 1). Experiment 2 was as for Exp. 1 but comparing edge chemistry (Exp. 2). Experiment 3 tested consistency of natal chemistry among vertebrae from the same individual (Exp. 3). Experiment 4 tested the effect of bleaching on natal chemistry by running univariate ANOVAs on all vertebrae with treatment as the fixed factor and individual as a random factor (Exp. 4).

Table S2. Summary of results from paired *t*-tests comparing elemental concentrations taken from two locations within each of the natal and edge areas of vertebrae (area = portion of vertebrae ablated; *t* value = *t*-test statistic; d.f. = degrees of freedom; p = *P*-value).

Table S3. Summary of results from paired *t*-tests comparing natal elemental concentrations taken from two vertebrae from each of nine sharks (*t* value = *t*-test statistic; d.f. = degrees of freedom; p = *P*-value).

Table S4. Univariate ANOVA results comparing sampled element:Ca concentrations among three vertebrae preparation treatments: unbleached, bleached (for 40 min) and hyper-bleached (for 2 h) (d.f. = degrees of freedom; MS = means squared; *F* = ANOVA test statistic; P = *P*-value; 95% CI = upper and lower C.I. for each treatment). Significant *P*-values and 95% C.I. for treatments that differed are bolded.

Appendix S2. Exploratory comparisons of elasmobranch vertebrae and otoliths.

Table S5. Different combinations of elements analysed by laser ablation ICP-MS between collection periods. Element specific dwell times (in ms) are shown in parentheses.

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Appendix II.A: Supplementary material

Elements and elasmobranchs: Hypotheses, assumptions and limitations of elemental analysis

M.N. McMillan, C. Izzo, B. Wade and B.M. Gillanders

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Part I. Consistency of elemental signals and bleaching effects on vertebrae

Materials and methods

Sample preparation and treatments

Four post-cranial vertebrae were sampled from nine individual school shark (*Galeorhinus galeus*) (*N*=36). Vertebrae were manually cleaned of connective tissue, before being exposed to three cleaning treatments: (1) unbleached, samples were air dried in a fume hood for seven days and extraneous tissue allowed to dry and fall away; (2) bleached for 40 min, the maximum duration used at a commercial fish ageing facility; and (3) hyper-bleached for 2 h, three times the maximum duration of commercial bleaching. Commercially available White King premium bleach, with active ingredients 42 g L^{-1} NaClO and 9 g L^{-1} NaHO was used in cleaning treatments (2) and (3).

Samples were triple rinsed in ultra-pure water and air dried in a fume hood. Two vertebrae from each shark were unbleached and used as: (i) controls to examine effect of bleach on element concentrations; and (ii) to test for consistency in elemental concentrations within and among vertebrae from the same individual. Within vertebrae comparisons were made between the left and right sides of all vertebrae in the natal and edge regions (see Table S2.1, Fig. 2.2). Among vertebrae comparisons were made between the natal regions of the two unbleached vertebrae from each shark.

All treated vertebrae were set in an epoxy resin and sectioned sagittally into ~500 µm sections using a low speed diamond saw and mounted onto glass microscope slides in a randomised order using thermoplastic glue. Natal regions of vertebrae were identified distal to birth band relative to the vertebral focus under a dissecting microscope with transmitted light and marked by etching the adjacent resin.

Laser ablation

Elemental concentrations were assayed using laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS). Samples were ablated in 400 µm long transects with a width of 80µm at 10 Hz and 5 µm s^{-1} after pre-ablation at a width of 110 µm at 5 Hz and 100 µm s^{-1}. Elements analysed were: ^{7}Li, ^{23}Na, ^{24}Mg, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb and ^{43}Ca. Transects were ablated across the *corpus calcareum* on the left and right sides of the sample within both the natal and edge areas (see Fig. 2.2). Glass standard references (NIST 612) were ablated periodically to account for potential instrumental drift.

Statistical analysis

All element data (in counts per second) were normalised to Ca concentrations and expressed in mmol mol^{-1}. Consistency of individual elemental concentrations within vertebrae was tested using paired *t*-tests comparing concentrations in the left and right sides of all vertebrae (n=36) for both the natal and edge regions (see Table S2.1). Consistency of individual elemental concentrations among vertebrae was tested using the mean concentration of the natal regions of the two unbleached vertebrae from the same shark (n=18). Paired *t*-tests were used to compare the natal chemistries among the vertebrae.

Effects of bleaching on vertebral chemistry were tested using univariate ANOVAs for each element on all vertebrae (n=36) with ‘treatment’ (unbleached, bleached and hyper-bleached) as the fixed factor and ‘individual’ as a random factor. Tukey’s honestly significant difference (HSD) test was performed *post hoc* to determine which treatments differed.

Table S2.1. Experimental designs. Experiment 1 tested consistency of natal chemistry within vertebrae (left v right sides) from the same individual (Exp. 1). Experiment 2 was as for Exp. 1 but comparing edge chemistry (Exp. 2). Experiment 3 tested consistency of natal chemistry among vertebrae from the same individual (Exp. 3). Experiment 4 tested the effect of bleaching on natal chemistry by running univariate ANOVAs on all vertebrae with treatment as the fixed factor and individual as a random factor (Exp. 4).

	Region	***n***	**Treatments (*n*)**	**Test**
Exp. 1: Within vertebrae	Natal left v Natal right	36	Unbleached (18) Bleached (9) Hyper-bleached (9)	*t*-test
Exp. 2: Within vertebrae	Edge left v Edge right	36	Unbleached (18) Bleached (9) Hyper-bleached (9)	*t*-test
Exp. 3: Within individuals	Natal vert A v Natal vert B	18	Unbleached (18)	*t*-test
Exp. 4: Among individuals	Natal all	36	Unbleached (18) Bleached (9) Hyper-bleached (9)	ANOVA

Results

Testing for consistency within the vertebrae indicated that the only element whose concentrations differed in left and right portions of vertebrae was Na (Table S2.2), although this difference was only apparent in the natal area. Testing for consistency among vertebrae from the same animal indicated the only element to differ in concentration among vertebrae from the same animal was Zn (Table S2.3).

Table S2.2. Summary of results from paired *t*-tests comparing elemental concentrations taken from two locations within each of the natal and edge areas of vertebrae (area = portion of vertebrae ablated; *t* value = *t*-test statistic; df = degrees of freedom; p = *p* value).

Element	**Area**	***t* value**	**df**	**p**
Li	Natal	1.260	35	0.216
	Edge	-0.436	35	0.666
Na	Natal	3.101	35	**0.004**
	Edge	1.485	35	0.147
Mg	Natal	-1.800	35	0.080
	Edge	0.337	35	0.738
Mn	Natal	0.408	35	0.686
	Edge	-0.131	35	0.896
Zn	Natal	-0.884	35	0.383
	Edge	-1.295	35	0.204
Sr	Natal	-1.772	35	0.085
	Edge	-0.285	35	0.778
Ba	Natal	-0.580	35	0.565
	Edge	-0.025	35	0.980
Pb	Natal	-1.001	35	0.323
	Edge	0.090	35	0.929

Table S2.3. Summary of results from paired *t*-tests comparing natal elemental concentrations taken from two vertebrae from each of nine sharks (*t* value = *t*-test statistic; df = degrees of freedom; p = *p* value).

Element	***t* value**	**df**	**p**
Li	0.990	8	0.351
Na	1.611	8	0.146
Mg	-0.332	8	0.748
Mn	0.095	8	0.926
Zn	-2.978	8	**0.018**
Sr	-0.281	8	0.786
Ba	0.709	8	0.498
Pb	1.291	8	0.233

Among cleaning treatments, Na:Ca and Mg:Ca significantly increased with increasing bleach exposure, while Mn:Ca decreased with exposure to bleach (Table S2.4). The concentrations of the remaining elements were not affected by the cleaning treatments. *Post hoc* Tukey's HSD tests found significant differences for Na:Ca concentrations among all treatments ($p < 0.05$); however Mg:Ca and Mn:Ca only differed between unbleached and hyper-bleached treatments.

Since Na:Ca showed significant treatment results from bleaching, a further paired *t*-test was conducted on the natal regions of only unbleached vertebrae to account for any bleaching effects. Results for this test showed no difference in Na:Ca concentrations in different areas of the natal portion of vertebrae ($t_{17} = 1.778$, $p = 0.093$).

Table S2.4. Univariate ANOVA results comparing sampled element:Ca concentrations among three vertebrae preparation treatments: unbleached, bleached (for 40 mins), and hyper-bleached (for 2 h) (df = degrees of freedom; MS = means squared; F = ANOVA test statistic; P = *p* value; 95% CI = upper and lower confidence intervals for each treatment). Significant *p* values and 95% CIs for treatments that differed are bolded.

						95% CI		
Element	**Model**	**df**	**MS**	**F**	**P**	Treatment	Lower	Upper
Li	Treatment	2	<0.001	0.959	0.404	Unbleached	0.018	0.020
	Residual	16	<0.001			Bleached	0.017	0.020
						Hyper-bleached	0.017	0.020
Na	Treatment	2	0.059	9.711	**0.002**	Unbleached	**1.654**	**1.680**
	Residual	16	0.006			Bleached	**1.688**	**1.719**
						Hyper-bleached	**1.716**	**1.756**
Mg	Treatment	2	0.008	4.290	**0.032**	Unbleached	1.238	1.279
	Residual	16	0.002			Bleached	**1.215**	**1.262**
						Hyper-bleached	**1.214**	**1.257**
Mn	Treatment	2	0.191	12.336	**0.001**	Unbleached	-1.813	-1.700
	Residual	16	0.015			Bleached	**-1.848**	**-1.694**
						Hyper-bleached	**-1.947**	**-1.753**
Zn	Treatment	2	0.042	2.025	0.165	Unbleached	-1.343	-1.279
	Residual	16	0.021			Bleached	-1.370	-1.306
						Hyper-bleached	-1.406	-1.333
Sr	Treatment	2	<0.001	0.018	0.982	Unbleached	0.303	0.318
	Residual	16	<0.001			Bleached	0.300	0.323
						Hyper-bleached	0.300	0.316
Ba	Treatment	2	0.005	0.423	0.662	Unbleached	-2.323	-2.222
	Residual	16	0.012			Bleached	-2.357	-2.217
						Hyper-bleached	-2.326	-2.008
Pb	Treatment	2	0.092	0.985	0.395	Unbleached	-4.554	-4.419
	Residual	16	0.094			Bleached	-4.641	-4.501
						Hyper-bleached	-4.618	-4.453

Part II. Exploratory comparisons of elasmobranch vertebrae and otoliths

Materials and methods

Sample preparation

For LA ICP-MS analysis, preparation of vertebrae was as for Part I (above) with the minimum bleaching duration required to remove adjoining tissue, while otolith preparation was identical minus bleaching. For electron microprobe analysis, structures were embedded in indium spiked epoxy, halved using the lapidary saw, and polished with progressively finer grades of lapping film, before a final surface polish using 15 µm diamond paste. Samples were then carbon coated prior to analysis.

Electron microprobe analysis

Measures of the percent weight of calcium (%Ca) were quantified using a Cameca SX-51 electron microprobe. Beam conditions consisted of 15 kV and 20 nA, with an Astimex calcium carbonate reference material for standardisation. For all samples, multiple (n = 4) spot ablations were performed across the vertebrae. For a single vertebral sample from a representative Port Jackson shark *Heterodontus portusjacksoni* specimen, a profile consisting of 72 points, from the outermost edge of the corpus calcareum to the focus of the vertebrae, was made to examine longitudinal variation in the calcium profile of the vertebra.

Multi-element mapping using laser ablation ICPMS

LA-ICPMS mapping was conducted using a Resonetics M-50-LR 193-nm Excimer laser coupled to an Agilent 7700cx quadrupole ICPMS. Mapping was performed by ablating sets of parallel rasters in a grid across the sample. A beam size of 14 µm and a scan speed of 25 µm s^{-1} provided the desired sensitivity of elements of interest and adequate spatial resolution. The spacing between the lines was kept at a constant 14 µm to match the size of the laser beam used. A laser repetition of 10 Hz was selected at a constant energy output of 100 mJ, resulting in an energy density of approximately 7 J cm^{-2} at the target. A set of six elements were analysed (Table S2.5). To correct for instrument drift, identical rasters were done on the standard glass NIST 612 and the USGS reference material MACS-3 at the start and end of a mapping run. Standards were not run during the mapping scans as this may create a mismatch of the stage position after standard analysis resulting in a blurring or distortion of the element maps (Cook *et al.*, 2013). Elemental concentrations were normalised to Ca before 2D element maps were compiled using the Iolite software package (Woodhead *et al.*, 2007) in conjunction with the data analysis program Igor (WaveMetrics). Element maps are shown in counts per second (cps) as the values indicated are semi-quantitative due to the large errors associated with them relative to spot analysis (Cook *et al.*, 2013).

Table S2.5. Different combinations of elements analysed by laser ablation ICPMS between collection periods. Element specific dwell times (in ms) are shown in parentheses.

Element comparison among species of elasmobranchs and teleosts (refer to Figure 2)	
Element	^{7}Li, ^{23}Na, ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{111}Cd, ^{138}Ba, ^{139}La, & ^{208}Pb (dwell times for all elements set to 100 ms), and ^{115}In (50 ms)
Elemental mapping (refer to Figure 3)	
Element	^{7}Li, ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, and ^{137}Ba (dwell times for all elements set to 5 ms)

Spot analysis using laser ablation ICPMS

Spot analyses of sectioned vertebrae and otoliths was undertaken using a New Wave Q-switched Nd Yag 213nm UV laser connected to an Agilent 7500cs ICP-MS. For data acquisition, a laser spot size of 30 µm at the outermost edge region of the structure, with a repetition rate of 5 Hz was selected at a constant energy output of 80 mJ, resulting in an energy density of approximately 4 J cm^{-2} at the target. The ICPMS was operated in time resolved mode. A set of 13 elements were analysed (Table S2.5).

To correct for instrument drift, spot ablation were performed periodically on the standard glass NIST 612 and the USGS reference material MACS-3, as well as at the start and end of each session. Based on species specific mean %Ca values, determined via electron microprobe analysis (refer to Table III for values used), element concentrations (in ppm) could be calculated from raw count per second data using the software package Glitter (Griffin *et al.*, 2008). All element concentrations were then normalised to ^{43}Ca.

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CHAPTER 3

Natural tags reveal populations of Conservation Dependent school shark use different pupping areas

Late-term school shark pups dissected from a female caught inshore at Kangaroo Island, South Australia in January 2017 at the end of the pupping season.

Statement of Authorship

Title of Paper	Natural tags reveal populations of Conservation Dependent school shark use different pupping areas
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Ed	Matthew McMillan		
Contribution to the Paper	Study concept and design, sample selection and preparation, LA ICP-MS analysis, statistical analyses, comparison of vertebral elemental signatures between populations, lead authorship of manuscript. Acted as corresponding author.		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Natural tags reveal populations of Conservation Dependent school shark use different pupping areas

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ABSTRACT: Knowledge of reproductive movements and sources of recruitment in highly mobile species is important to understand population-level resilience and to manage recovery in populations depleted by human interference. Management of the school shark *Galeorhinus galeus* (Linnaeus, 1758), a Conservation Dependent species in Australia subject to a national recovery strategy after stock collapse from overfishing, has long assumed obligate female migration to pupping areas in the southeast of their range. We used post-natal elemental signatures of individuals from 3 cohorts born in 1996 to 1998 as a proxy to test whether females use common pupping areas. Environmental or biological factors that differ among pupping areas can give rise to unique trace element signatures in shark vertebrae that act as natural tags and can be used to assess relative contributions from recruitment sources to adult populations. We compared post-natal signatures from sharks caught in 2 regions, South Australia in the northwest of the species' range and Bass Strait in the southeast, using laser ablation inductively coupled mass spectrometry. Signatures were similar between regions for 1 cohort, suggesting high use of shared or similar pupping areas, but differed for the 2 remaining cohorts. Region of capture could also be accurately predicted (>75%) based on post-natal signatures, refuting the long-held view that all females use common pupping areas. We conclude that female movements and reproductive strategies are likely more plastic than previously assumed, highlighting the need to clarify them and their potential effects on resilience and conservation.

KEY WORDS: Behavioural plasticity · Laser ablation inductively coupled mass spectrometry · LA-ICP-MS · Partial migration · Reproductive strategies · Recovery · Resilience

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INTRODUCTION

Migratory species present considerable challenges for conservation management (Thirgood et al. 2004, Martin et al. 2007, Dulvy et al. 2008) that are amplified when variability in migrations occurs and direct observations are difficult or impossible to make. Knowledge about spatial ecology, e.g. connectivity among populations and sources of recruitment, is central to conservation planning (Webster et al. 2002, Simpfendorfer et al. 2011), particularly for managing the recovery of species depleted by overharvesting or habitat degradation (Martin et al. 2007, Petitgas et al. 2010). Furthermore, conservation efforts can be confounded where there is incomplete knowledge about reproductive movements; adult migrations to recruitment areas may be modeled on incomplete information. Widespread use of nursery areas by sharks (Feldheim et al. 2002, Heupel et al. 2007) can magnify the importance of knowledge about repro-

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ductive migrations for conservation efforts. Many sharks and other elasmobranchs are unique in the marine realm for combining internal fertilisation with limited parental care, investing substantial resources into small numbers of offspring for which they select pupping habitats providing shelter, food, and protection from predators to maximise recruitment (Branstetter 1990, Simpfendorfer & Milward 1993).

School shark *Galeorhinus galeus* were heavily exploited in one of Australia's oldest commercial fisheries from the 1920s to the 2000s, leading to stock collapse, by which time stock size had declined to <14% of original pup production levels (McLoughlin 2007). The species was listed as Conservation Dependent under the Environment Protection and Biodiversity Conservation Act 1999. A stock rebuilding strategy was introduced after the stock failed to recover, despite no longer being targeted commercially (AFMA 2009, Huveneers et al. 2013, McAllister et al. 2017). School shark are long-lived, ~60 yr (Walker 1999), and reach lengths up to 174 cm (Olsen 1954) in Australia. They have low reproductive capacity arising from late onset of maturity (>120 cm for males and >135 cm for females, equating to ages of ~8 and 11 yr, respectively), low fecundity (~28 pups), and a bi-annual reproductive cycle (Olsen 1954, Walker 1999), leading to a limited recovery potential. Despite these conservation challenges, a lack of knowledge about their reproductive movements and pupping areas persists.

Seventeen pupping areas were identified in the southeast of the species' range in the 1950s with help from the established commercial fishery in the region, while the fishery to the northwest in South Australia (SA) was in its infancy and had yet to locate such areas (Olsen 1954). However, productivity varied widely among these sites and more recently is concentrated in a handful of estuaries and sheltered bays (Stevens & West 1997) (Fig. 1). Combined with the absence of mature females from the southeast of their range during winter when they aggregate in SA, this led to a belief that persists today that all pregnant females migrate to southeastern pupping areas to pup during summer (Olsen 1954, Punt et al. 2000, Walker et al. 2008). However, recruitment from recorded pupping areas is vastly insufficient to support the population (Prince 1996, Stevens & West 1997, Walker 1999); >90% of pupping activity is occurring elsewhere (Stevens & West 1997). Despite limited scientific investigation of other potential pupping areas since the 1950s, recent evidence of pupping in SA has emerged and includes presence of neonates <45 cm up to >1500 km from recorded nurseries (Knuckey et al. 2014, Rogers et al. 2017), presence of late-term pregnant females (Prince 1996, West & Stevens 2001, M. N. McMillan unpubl. data), and presence of females in immediate post-partum condition (Braccini et al. 2009). If females also pup in SA, the currently assumed obligate female migration must be inaccurate, instead entailing partial female migration.

Differences among pupping areas give rise to unique elemental signatures retained throughout the lives of sharks in the post-natal portion of their calcified vertebrae that may be driven by differences in water chemistry, diet, or environmental factors moderating elemental uptake (e.g. temperature or salinity) (Smith et al. 2013, McMillan et al. 2017a). These signatures act as natural tags present in all individuals that allow demographic connectivity among populations to be assessed at ecological timescales (Tillett et al. 2011, Lewis et al. 2016, Smith et al. 2016). The present study aimed to investigate (1) the validity of the obligate female migration hypothesis in *G. galeus* by analysing post-natal natural tags in

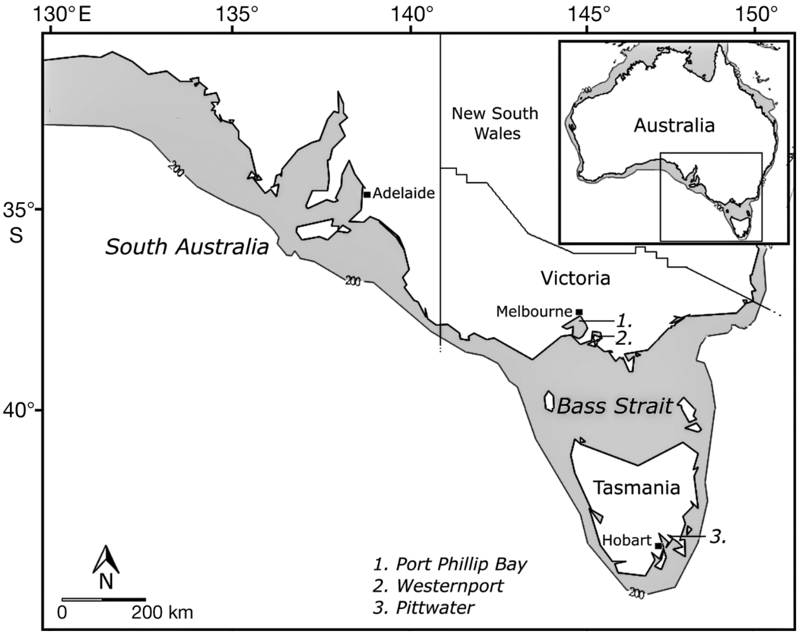

Fig. 1. Study area in southeastern Australia. The area depicted covers the core range of *Galeorhinus galeus* in Australian waters, although individuals are sometimes encountered further north and west. Main recorded nursery areas are numbered (1–3). Samples were compared between sharks caught in South Australia and Bass Strait. Inset shows study area (boxed) relative to Australia

vertebrae as a proxy of female reproductive movements and (2) whether sharks from SA and Bass Strait (BS) demonstrated evidence for use of common or different pupping areas. We predicted that post-natal elemental signatures would be similar for sharks caught in both regions if females from across their range undertake obligate migrations to common pupping areas in the southeast of their range. If post-natal signatures differed between regions, this would suggest that pupping areas differ between SA and BS and that pupping is likely not as spatially confined as currently assumed.

MATERIALS AND METHODS

Experimental design and sample collection

Cervical vertebral centra from 154 individuals were sourced from archival collections available at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Hobart. Vertebrae were collected from 2000 to 2003 and stored frozen until processed for age determination. Processing involved removal of adjoining tissue by immersion in bleach solution (active ingredients: 42 g l^{-1} sodium hypochlorite and 9 g l^{-1} sodium hydroxide) for up to 40 min. Bleaching of *Galeorhinus galeus* vertebrae using this method does not affect trace element concentrations for a range of elements (Ba, Li, Mg, Mn, Pb, Sr, Zn; exception Na) (McMillan et al. 2017a). Na was therefore not analysed in the present study.

Several cervical vertebrae were sampled from each shark, one of which was selected for age determination and embedded in polyester resin and then cut sagittally through the vertebral focus into 250 µm thick sections. Age was estimated by counting growth increments under a dissecting microscope using transmitted light and elevated contrast and used to determine birth year since year of capture was known. Age determination using this method has been validated for *G. galeus* <140 cm total length (TL) (Walker et al. 2001), and increment counts do not vary among adjacent vertebrae (Officer et al. 1996). Age estimation was conducted by 2 readers with precision of age estimates between readers well within acceptable limits (CV = 2.7%) (Chang 1982, Campana 2001). Samples from 3 cohorts (birth years: 1996, 1997, and 1998) were selected for further analysis (Table 1). Specimen size ranged from ~82 to 138 cm TL and age from 2 to 7 yr. In Australia, *G. galeus* have a well-defined pupping season beginning in November, peaking in December or January, and concluding by February (Olsen 1954, Stevens & West 1997), with neonates dispersing from pupping areas from March to June (McAllister et al. 2015). An arbitrary birth date of 1 January is thus assigned to *G. galeus* in the southern hemisphere (Moulton et al. 1992, Francis & Mulligan 1998); a shark of the 1996 cohort would therefore be considered 1 yr old at 1 January 1997, having been born in austral summer 1995–1996, and any migrations to pupping grounds by its mother would have occurred in 1995.

Table 1. Summary of sampling information and biological data including region of capture, cohort (birth year), sample size (n), sex ratio (male:female, M:F), age range, and total length (TL) range

Region	Cohort (birth year)	n	Sex (M:F)	Age (yr)	TL (cm)
South Australia					
	1996	27	1:1.25	4–6	90–131
	1997	27	1:0.93	3–5	88–121
	1998	25	1:2.13	2–4	82–117
Bass Strait					
	1996	24	1:0.71	4–7	92–138
	1997	26	1:1.36	3–6	85–138
	1998	25	1:1.08	2–5	86–126

Sample preparation for laser ablation

After individuals were assigned to cohorts, one of the remaining vertebral centra from each shark was embedded in an epoxy resin and cut sagittally into 500 µm thick sections with a low-speed diamond saw. Sections were polished with lapping film of decreasing grade (30, 9, and 3 µm) before rinsing in ultrapure water and air drying in a fume cupboard. Sections were then mounted onto glass microscope slides using thermoplastic glue. Birth bands were identified using transmitted light and elevated contrast under a dissecting microscope and marked by etching the adjacent resin to allow for accurate location of post-natal tissue during laser ablation.

Laser ablation inductively coupled mass spectrometry

Element concentrations were assayed using laser ablation inductively coupled mass spectrometry (LA-ICP-MS) employing a New Wave 213 laser coupled to an Agilent 7500cx mass spectrometer. Ca was used as an internal standard at a percent mass composition of 43% (equivalent to other carcharhiniform sharks from temperate Australian waters, e.g. *Carcharhinus brachyurus*: McMillan et al. 2017a). Transects of 200 µm length were ablated across the corpus calcareum perpendicular to its axis and immediately distal to the natal band, representing approximately the first month of growth after birth (Fig. 2). Transects were checked post ablation to ensure that only post-natal material was sampled. Transects were scanned at a speed of 5 µm s^{-1}, a width of 80 µm, and a frequency of 10 Hz. Glass reference standards (NIST 612) were ablated before and after each session and periodically after every 10 samples to account for any instrument drift. Elements analysed included ^{7}Li, ^{24}Mg, ^{55}Mn, ^{88}Sr, ^{138}Ba, and ^{203}Pb as well as ^{43}Ca against which element:Ca ratios could be calculated for statistical analysis by normalising raw element count data to Ca (mmol mol^{-1}). Element concentrations for all samples were detected at levels >3 SDs greater than mean limits of detection calculated for each session. CVs were calculated for each session and were <5% for all elements (range: 0.7–4.8%).

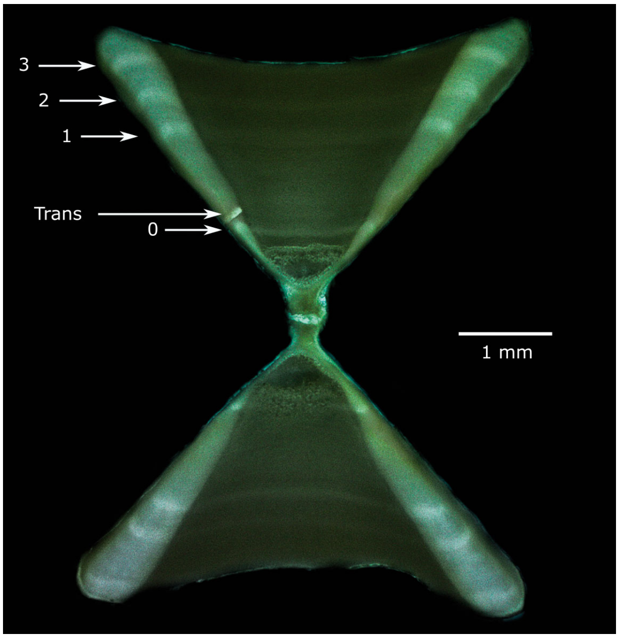

Fig. 2. Sectioned vertebral centrum of a 3 yr old school shark showing the ablated 200 µm laser transect (Trans) used to sample natal elemental signatures immediately distal to the birth band (0). Annual growth bands are numbered 1 to 3. Scale bar = 1 mm

Statistical analysis

Element:Ca ratios were log(x + 1) transformed to normalise elemental distributions and ensure all elements were on a similar scale to relativise effects of abundant elements and then analysed using the PRIMER Permanova software package. To determine if differences occurred between regions and birth year, the multi-element signature (all 6 element:Ca ratios) was analysed using permutational MANOVAs (Anderson 2001) with both factors treated as fixed factors; a Euclidean distance matrix was used. Where significant differences were found, post hoc *t*-tests were used to determine which region or birth years differed. Similar analyses were used for individual elements using univariate ANOVAs. Canonical analysis of principle coordinates (CAP) (Anderson & Willis 2003) was used to assess spatial variation among regions using a leave-one-out approach to predict the region of origin of samples based on their post-natal signatures.

RESULTS

Significant variation in post-natal elemental signatures in vertebrae of sharks occurred with interactions between year and region (Table 2). Sharks born in 1996 caught in both regions (SA and BS) had similar multi-element signatures ($t = 0.41$, $p = 0.74$), while corresponding signatures of sharks born in 1997 and 1998 differed between regions (1997: $t = 2.70$, $p < 0.01$ and 1998: $t = 2.29$, $p = 0.03$). There were also differences in multi-element signatures between regions (with sharks from all cohorts pooled: Table 2) and between years (1996–1997: $t = 2.08$, $p = 0.04$ and 1997–1998: $t = 2.11$, $p = 0.04$), validating the approach of comparing post-natal signatures within cohorts. The interaction between year and region observed for the multi-element signature (Table 2), indicating variation among years for spatial patterns, was reflected by a corresponding interaction for Mg:Ca that was similar for sharks born in 1996 ($t = 0.39$, $p = 0.71$) but that differed for sharks born in 1997 ($t = 2.73$, $p < 0.01$) and 1998 ($t = 2.30$, $p = 0.03$) (Table 2). Concentrations of Mg, Mn, and Li all varied between regions (Table 2), with Mn:Ca and Li:Ca generally higher in sharks caught in SA and Mg:Ca higher in sharks caught in BS born in 1997 and 1998 (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m599p147_supp.pdf). There was no significant variation in signatures between sexes ($F_{1,152} = 0.02$, $p = 0.96$).

CAP successfully predicted the region of capture for 75.3% of sharks based on their post-natal signatures (72.2% of sharks caught in SA and 78.7% of

Table 2. Permutational MANOVA results for the multi-element signature (Multi) and univariate ANOVAs for individual elements (Element:Ca) with region (i.e. South Australia vs. Bass Strait), cohort (birth years 1996, 1997, and 1998), and interactions thereof as factors. Significant differences ($p < 0.05$) are in **bold**

Element	Region			Cohort			Cohort × Region			Residual	
	df	MS	p	df	MS	p	df	MS	p	df	MS
Multi	1	0.501	**0.003**	2	0.198	**0.042**	2	0.189	**0.046**	148	<0.01
Li:Ca	1	<0.001	**<0.001**	2	<0.001	0.32	2	<0.001	0.412	148	<0.01
Mg:Ca	1	0.496	**0.002**	2	0.191	**0.038**	2	0.187	**0.046**	148	0.06
Mn:Ca	1	0.002	**0.016**	2	<0.001	0.306	2	<0.001	0.987	148	<0.01
Sr:Ca	1	0.003	0.241	2	0.006	**0.038**	2	0.002	0.289	148	<0.01
Ba:Ca	1	<0.001	0.116	2	<0.001	**0.022**	2	<0.001	0.947	148	<0.01
Pb:Ca	1	<0.001	0.450	2	<0.001	0.695	2	<0.001	0.570	148	<0.01

sharks caught in BS). Multi-element signatures showed considerable mixing of sharks from both regions in quadrant I of the CAP plot, whereas quadrants II and III were dominated by sharks caught in SA and quadrant IV was dominated by sharks caught in BS (Fig. 3). When individual cohorts were isolated from the CAP plot, similar patterns were discernible in all plots; however, signatures for sharks born in 1996 (Fig. 4A) were more clustered than for those born in 1997 (Fig. 4B) and 1998 (Fig. 4C).

DISCUSSION

Our results showed that sharks from both regions came primarily from different pupping areas in 2 years (1997 and 1998) and similar pupping areas in another year (1996). It is unclear whether the similarity of signatures from 1996 arose from use of common or similar pupping areas for this cohort. In relatively homogeneous marine environments, where most *Galeorhinus galeus* pupping likely occurs (Stevens & West 1997), there may be little variation in elemental signatures among regions. Where no differences exist, this should not automatically be taken for evidence of a single group, since the drivers of variability in signatures are not known (Campana et al. 2000). In marine environments, differences may emerge at broad regional scales such as in the present study, which may be of particular ecological relevance for studying wide-ranging species (Smith 2013, McMillan et al. 2017b). To date, little experimental work has validated drivers of elemental signatures in elasmobranchs (but see Smith et al. 2013). Such drivers may include regional differences in water chemistry, diet, temperature, salinity, physiology, or ontogeny (Smith et al. 2013). Ontogeny, however, is unlikely to have influenced differences in signatures here since all signatures were derived from the same cohort. Knowledge about specific drivers of elemental signatures is not required to distinguish groups of fish where the aim is simply to determine if such differences exist (Thorrold et al. 1998).

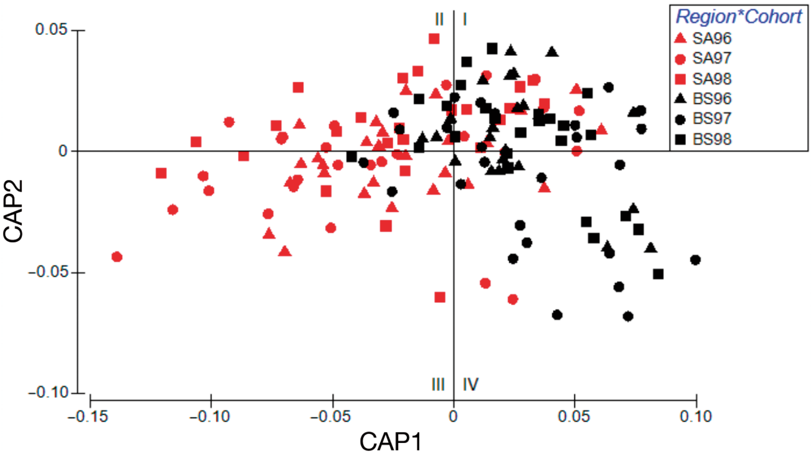

Fig. 3. Canonical analysis of principle coordinates (CAP) plot with cohorts (1996: triangles; 1997: circles; 1998: squares) pooled by region. Red and black symbols denote sharks caught in South Australia (SA) and Bass Strait (BS), respectively. Quadrants are numbered I to IV

Differences in post-natal signatures between regions indicate different pupping areas make major contributions to the SA and BS populations, at least in some years. One possibility is that pupping remains restricted to the southeast of the species' range but that females bearing pups destined for SA or BS select different pupping sites in the same general region. Alternatively, undiscovered pupping areas in other locations may make major contributions to the SA population, e.g. in

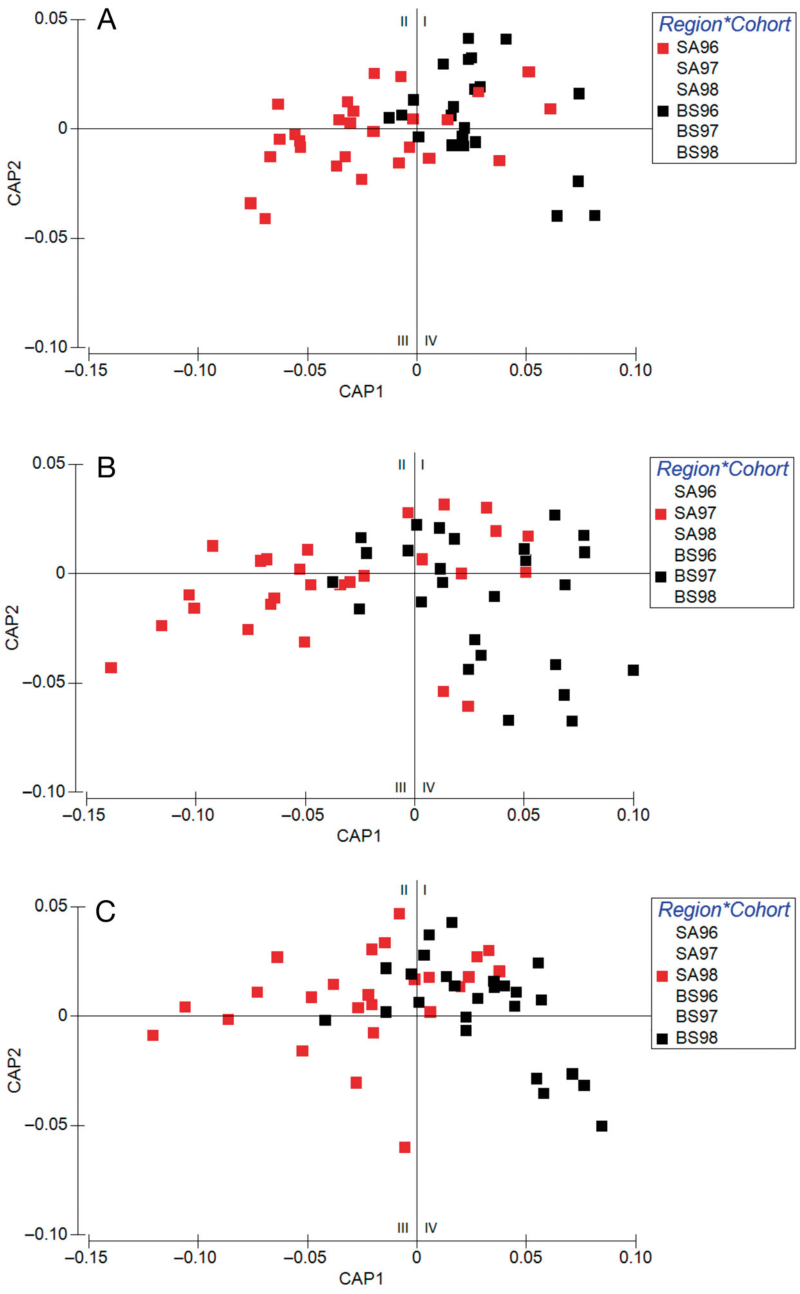

Fig. 4. Individual cohorts (A) 1996, (B) 1997, and (C) 1998, isolated from the canonical analysis of principle coordinates (CAP) plot of pooled cohorts (Fig. 3). Red and black symbols denote sharks caught in South Australia (SA) and Bass Strait (BS), respectively. Quadrants are numbered I to IV

Western Australia or New South Wales at the fringes of the species' range, although there is no evidence for pupping in these areas. Recruits from New Zealand (NZ) may also contribute to differences in post-natal signatures between SA and BS. Recent genetic evidence has established the Australian and NZ populations form a connected panmictic stock (Hernández et al. 2015, Bester-van der Merwe et al. 2017) and individuals from NZ move to Australia; 8 to 10% of recaptures of sharks tagged in NZ occurred in Australia (Hurst et al. 1999, Francis 2010). However, movements to Australia appear to increase with age (Francis 2010) and NZ emigrants appear to be roughly equally distributed throughout the Australian population (Hurst et al. 1999), suggesting they are probably not responsible for differences in post-natal signatures observed between SA and BS.

A more likely driver of differences is that pupping occurs in SA, as has long been considered plausible (Stevens & West 1997) or likely (Prince 1996). Discovery of pupping areas in the southeast in the 1950s led to a concentration of scientific effort there, e.g. a large-scale investigation of potential pupping areas in the 1990s consisted of >1300 scientific longline and gillnet sets surveying areas in BS and Tasmania, compared to only 7 in SA (all opportunistically and without scientific observation: Stevens & West 1997). Most pups born in southeastern pupping areas mix completely throughout the BS population (Olsen 1954, Stevens & West 1997) where juveniles form large aggregations, e.g. in eastern BS (Olsen 1954, Walker 1999). Juveniles in this region typically move short distances, e.g. tagged individuals <65 cm in BS and Tasmania travelled a mean distance of 22 ± 10 km at recapture (Brown et al. 2000), and the majority of sharks aged 0+ to 3 yr remain within 100 km of pupping areas (Stevens & West 1997). Although some dispersive individuals make long movements, e.g. as far as central SA (Olsen 1954, McAllister et al. 2015), movements >100 km generally do not occur until 3 to 4 yr of age (Stevens & West 1997). There is some recent evidence that neonates from Tasmania may disperse further from pupping areas than previously thought, however mostly northwards toward BS and NSW (J. M. Semmens unpubl. data). Yet, juveniles of these age classes are not uncommonly caught in SA, typically forming schools of similarly sized individuals (Fig. 5A), e.g. around Pearson Island in western SA

Fig. 5. (A) Juvenile school sharks caught near Pearson Island, western South Australia, in October 2017. Individuals this size rarely move more than 100 km from pupping areas. (B) Neonate school shark caught in Marion Bay, South Australia, in early February 2017, when neonates are yet to disperse from pupping areas

and off the Coorong, suggesting pupping likely occurs within 100 km of these areas.

Presence of neonates (Fig. 5B; see also Rogers et al. 2017), females in immediate post-partum condition (Braccini et al. 2009), and late-term pregnant females (Prince 1996, West & Stevens 2001), including late-term pregnant females remaining in SA throughout the peak pupping season in December and January (M. N. McMillan unpubl. data), also supports undiscovered pupping areas in SA. In addition to post-natal signatures differing between regions, region of capture was predicted for >75% of sharks based on post-natal signatures (where prediction by chance alone would equal 50%: White & Ruttenberg 2007), suggesting different sources of recruitment for most sharks in each region with some mixing of dispersive individuals. Such a model is consistent with the experience in NZ, where most individuals (76%) make localised movements <500 km with long distance movements by a minority of dispersive individuals (Hurst et al. 1999). In Australia, comprehensive tagging in the 1990s (the first to include all of SA) also found that most individuals of the size classes examined in this study (<140 cm TL) travelled <500 km at recapture (Brown et al. 2000), again suggesting mostly relatively localised movements for these age classes with a minority of dispersive individuals. Although knowledge about drivers of element incorporation in elasmobranch vertebrae is currently limited, incorporation of Mn increased and Mg decreased in response to increasing temperature in another elasmobranch, the round stingray *Urobatis halleri* (Smith et al. 2013). Elevated Mn:Ca and lower Mg:Ca in vertebrae of SA sharks would therefore be consistent with pupping in SA, where lower latitudes give rise to generally warmer coastal water temperatures than those in BS during the summer pupping season (Fig. S2). Pupping in SA would entail partial female migration, whereby some pregnant females remain resident in SA over the pupping season while others migrate to southeastern Australia or NZ pupping areas.

One of the key drivers of pupping site selection is predator avoidance (Branstetter 1990, Morrissey & Gruber 1993, Heupel et al. 2007). Shallow estuaries provide scarcity of predators and turbidity refuges, making them attractive nurseries for many fishes (Blaber & Blaber 1980). While some female *G. galeus* select such habitats, leading to high densities of pups in confined areas, others may employ more dispersed pupping in coastal marine habitats as an alternative strategy, relying on benthic cover and dispersal of young to limit predation. Use of alternative pupping strategies may confer population-level resilience; if one strategy fails, the other may still yield recruits. Habitat availability is known to drive reproductive strategies in sharks; the same species may pup in estuarine or marine habitats depending on availability (Knip et al. 2010). Marine pupping areas are used by *G. galeus* throughout their global range including in California (USA) (Ripley 1946), NZ (Hurst et al. 2000), South Africa (Freer 1992, M. McCord pers. comm.), and Argentina (G. Chiaramonte pers. comm.). The use of marine pupping areas thus appears to be an underinvestigated source of recruitment in Australia, particularly in SA, where all neonate records derive from marine areas (Fig. 5B; see also Knuckey et al. 2014, Rogers et al. 2017). Vertebral Sr concentrations have been used as a salinity tracer and found to decline with decreasing salinity in elasmobranchs, e.g. bull sharks *Carcharhinus leucas* (Tillett et al. 2011) and smalltooth sawfish *Pristis pectinate* (Scharer et al. 2012). However, in the present study, Sr:Ca did not differ between regions, supporting the idea that most pupping may occur in relatively homogeneous marine habitats. Due to the large extent of marine habitat available, marine pupping areas may yield lower densities of pups than estuaries, making them less conspicuous and less likely to be detected than within-estuarine pupping areas, but occur over larger areas and could therefore yield more biomass. This may explain why recruitment from recorded estuarine pupping areas is estimated at <10% of that required to maintain the population (Stevens & West 1997). Female reproductive behaviour therefore appears divided between alternate pupping strategies; the impacts of these strategies on the resilience of the species and on the ability to accurately monitor its status should be better understood to best manage the species' recovery.

Divergent modes of movement and habitat use across a species' range may be a bet-hedging strategy (Kerr et al. 2010, Chapman et al. 2011). Temporal fluctuations in resource availability may offer benefits to both migrants and residents at different times, potentially conferring long-term resilience (Gillanders et al. 2015). Varying degrees of mixing from year to year, driven by opportunistic exploitation of regional fluctuations in prey abundance, may also allow for replenishment of overexploited populations from source populations (Secor et al. 2001). However, variable patterns of movement may also lead to differing vulnerability among regions to stressors, e.g. overharvesting or habitat loss, and variable capacity among sub-populations to recover from population depletions (Secor et al. 2001, Parsons et al. 2011). This may explain the apparent loss of a population of *G. galeus* formerly present off New South Wales or account for the varying abundances noted between SA and BS over time (Punt et al. 2000). At any rate, our findings provide evidence that contributions to the populations in SA and BS derive largely from different pupping areas, at least in some years, with probable admixture of some dispersive individuals. If, as recent evidence suggests, pupping is also occurring in SA, this is not consistent with the current model under which recovery of *G. galeus* is managed in Australia, which assumes obligate female migration with all pupping occurring in the southeast of their range (Punt et al. 2000, Walker et al. 2008). Our evidence suggests female movements are likely more plastic than previously assumed and that, in concert with a preponderance of anecdotal and emerging scientific evidence, sources of recruitment remain unaccounted for, particularly in SA. Greater understanding of the species' reproductive strategies and habitats should therefore be sought to best direct and improve conservation measures, specifically via rigorous and overdue investigation of pupping activity in SA. Our study illustrates the complexity and variability of reproductive strategies that can occur in highly mobile species, presenting challenges for managing the conservation and recovery of such species depleted by human interference.

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Appendix III.A: Supplementary material

Natural tags reveal use of different pupping areas by populations of the Conservation Dependent school shark

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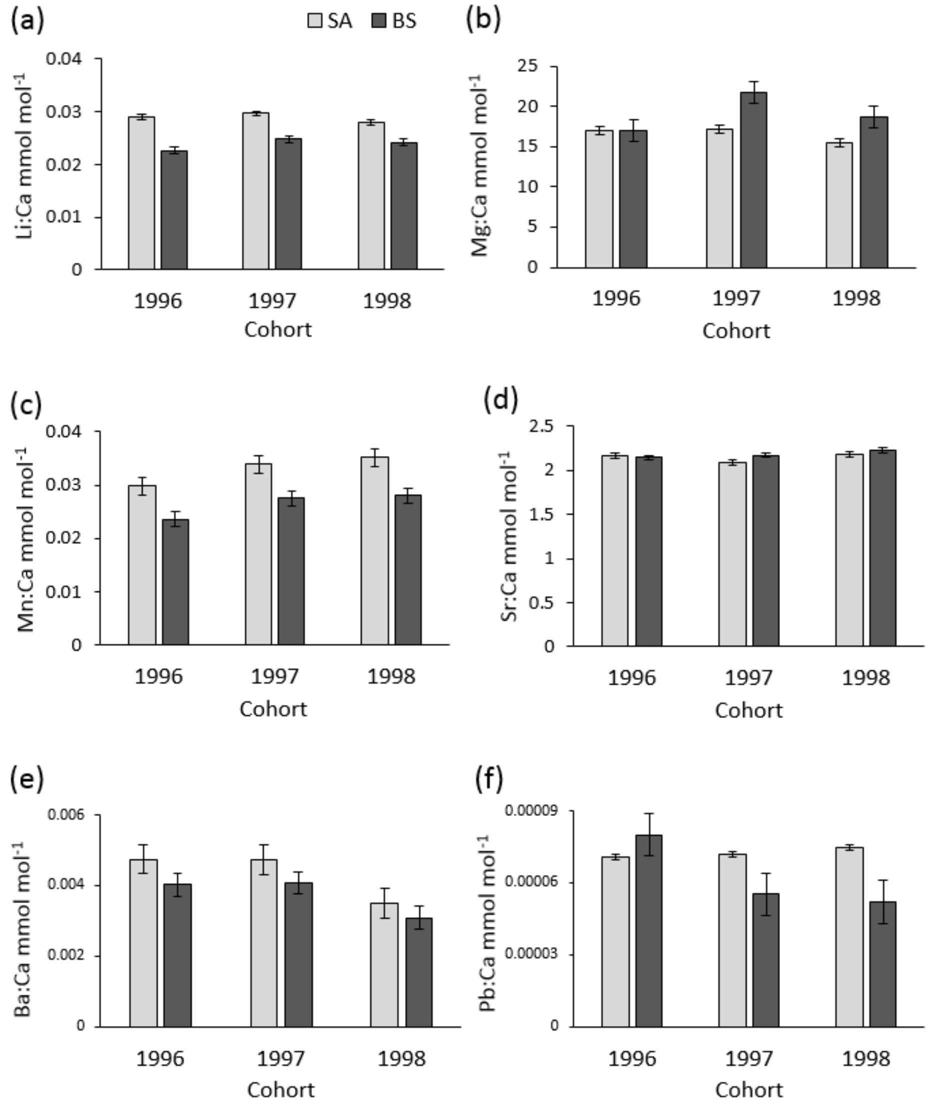

Fig. S3.1. Mean element:Ca concentrations in vertebrae of *G. galeus* for six elements: Li (a), Mg (b), Mn (c), Sr (d), Ba (e) and Pb (f) with standard error. Columns are clustered by cohort (born in 1996, 1997 and 1998). Sharks caught in SA are light shaded and BS is dark shaded.

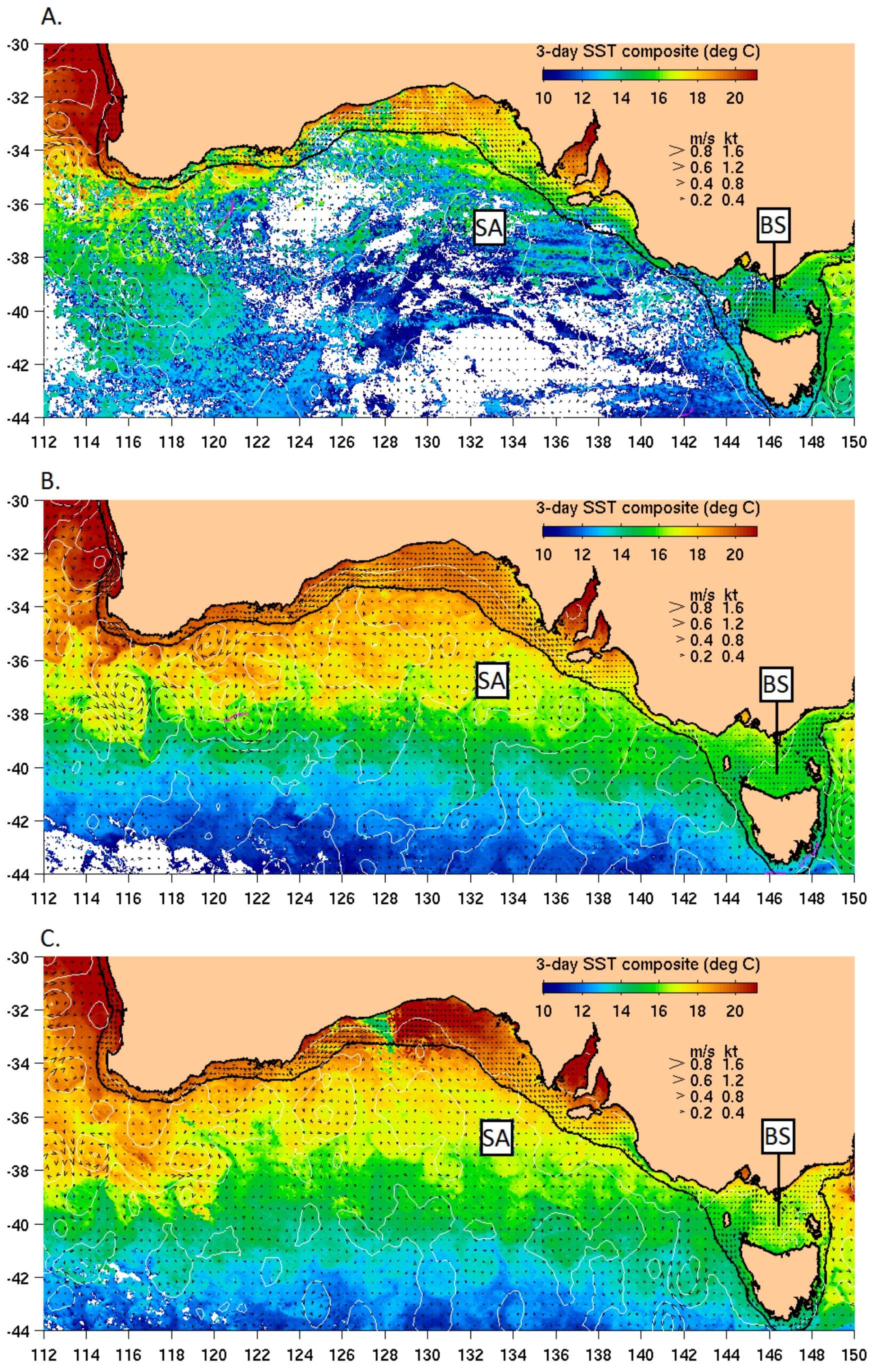

Fig. S3.2. Sea surface temperature (3-day composite SST) in southern Australia as close as possible to the 1st January arbitrary birth date for all cohorts: A = 1996 (29th December 1995), B = 1997 (31st December 1996) and C = 1998 (30th December 1997), showing the generally warmer coastal water temperatures at the lower latitudes of South Australia (SA) compared to Bass Strait (BS) (IMOS, 2016).

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CHAPTER 4

When to leave home? Constraints on shark pup dispersal from pupping areas

Extracting lipids from liver tissue of school shark pups in the laboratory of the Institute for Marine and Antarctic Studies, Taroona, Tasmania.

Statement of Authorship

Title of Paper	When to leave home? Constraints on shark pup dispersal from pupping areas
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	This is a co-authored paper prepared for publication in a scientific journal and is therefore written in plural.

Principal Author

Ed	Matthew McMillan		
Contribution to the Paper	Study concept and design, preparation of samples, bomb calorimetry and lipid extraction, energy budget calculations and statistical analyses, lead authorship of manuscript. Acting as corresponding author.		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Jayson Semmens		
Contribution to the Paper	Assistance with study concept and design, capture of specimens, swim tunnel respirometry, provided funding, and provided suggestions, comments and feedback on manuscript drafts.		
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Contribution to the Paper	Assistance with intellectual development and provided suggestions, comments and feedback on manuscript drafts.		
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Name of Co-Author	David Sims		
Contribution to the Paper	Swim tunnel respirometry, assistance with intellectual development and provided suggestions, comments and feedback on manuscript drafts.		
Signature		Date	20/09/2018

Name of Co-Author	Kilian Stehfest		
Contribution to the Paper	Capture of specimens, swim tunnel respirometry, respirometry data analysis, and assistance with intellectual development and provided suggestions, comments and feedback on manuscript drafts.		
Signature		Date	20-09-2018

Name of Co-Author	Bronwyn Gillanders		
Contribution to the Paper	Assistance with intellectual development, advice and suggestions throughout, provided funding, and provided suggestions, comments and feedback on manuscript.		
Signature		Date	25/09/2018

When to leave home? Constraints on shark pup dispersal from pupping areas

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Abstract

Natal areas are used by many marine taxa to maximise survival of young, but dispersive movements away from natal areas to other areas may be costly. The school shark *Galeorhinus galeus* is a broadly distributed species that uses pupping areas in south-eastern Australia, for which knowledge of pup dispersal to distant populations is lacking. For the first time, we combined swim-tunnel respirometry, bomb calorimetry, and lipid class analysis to investigate how energy requirements affect timing and speed of dispersal from an important pupping area: Pittwater estuary, Tasmania. Metabolic rate of pups at an optimal swimming speed of 1.4 body lengths s^{-1} was 149 mg O_2 kg^{-1} h^{-1} at 20°C, giving a cost of transport of 0.9 J g^{-1} km^{-1}. Seasonal variations in water temperature lowered daily ration requirements by 39% from 2.3% wet bodyweight (wbw) on the coastal dispersal route in early autumn to 1.4% wbw in winter, yielding clear benefits in delaying dispersal after the summer pupping season. Energy storage in pups was limited by small livers and low stores of energy storage lipids (e.g. triacylglycerols) relative to adults, with energy stores sufficient to sustain routine requirements for 1.4–4.5 days (mean: 2.7 ± 0.9 days). Field observed dispersal rates were slower than theoretical dispersal capacity based on continuous swimming at optimal speed. We conclude that dispersal capacity is likely constrained by allocation of resources to growth (the largest energetic cost for pups), bouts of resting and foraging to replenish small energy stores, and possible behavioural mediation, e.g. nocturnal activity and daytime refuging. Neonate *G. galeus* caught in the northwest of their Australian range long distances from known pupping areas are therefore likely to be born locally. Integrated bioenergetic approaches similar to the one applied here may help quantify dispersal capacity for other taxa from natal areas, e.g. many teleost fishes, elasmobranchs, and invertebrates.

Key words: cost of transport, dispersal, energetic constraints, energy storage, habitat shifts, metabolic rate, nursery areas, ontogeny, optimal swimming speed

Introduction

Natal areas play important roles in the life histories of many marine taxa by providing shelter, food, and protection from predation to maximise recruitment of young into adult populations (Beck et al. 2001, Heithaus 2007, Nagelkerken et al. 2015). These areas are often characterised by size segregation, such that there is little or no overlap between young and older age classes that may present intraspecific competition or predation risks (Dahlgren et al. 2006, Speed et al. 2010, Guttridge et al. 2012). In such cases, recruitment of young into the broader population is dependent on dispersal from natal areas into habitats used by older age classes (Simpfendorfer & Milward 1993, Eggleston 1995, Gillanders et al. 2003). Such ontogenetic habitat shifts may entail substantial movements, requiring energy intensive dispersal to forge connectivity between natal- and other habitats.

In these situations, dispersal from natal areas relies on overcoming energetic constraints that govern the dispersal capacity of young. In sharks, the liver is the primary organ of energy storage (Sargent et al. 1973, Zammit & Newsholme 1979, Rossouw 1987). As such, individuals with large livers rich in energy storage lipids are considered in good condition and best prepared to undertake long range movements (Rossouw 1987, Hoffmayer et al. 2006). Variation in the effects of season and location on metabolic demands, e.g. due to varying water temperature and other factors that assist or hinder dispersal such as ocean currents, may play important roles in the cost and timing of dispersive movements. Ecological characteristics and lifestyles of shark species can also influence energy flow between shark populations and their communities, e.g. pelagic and migratory species are likely to require more energy to fuel more active lifestyles and wide-ranging movements than less dispersive species (Cortés & Gruber 1990, Killen et al. 2010, Whitney et al. 2016).

The school shark *Galeorhinus galeus* (aka: tope, soupfin) is a bentho-pelagic species distributed circumglobally including in Australian waters where they are known to undertake large-scale movements (Olsen 1954, Walker 1999). *Galeorhinus galeus* is commercially fished throughout its distribution (Walker 1999), with overfishing having occurred in California (Walker 1998), Great Britain (Molfese et al. 2014), and Argentina (Cuevas et al. 2014). The species was heavily overfished in Australia up to the 1990s, leading to stock collapse from which it has not recovered despite a moratorium on targeted commercial fishing of *G. galeus* since 2001, the introduction of a national rebuilding plan in 2008, and conferral of Conservation Dependent status in 2009 (AFMA 2009, Huveneers et al. 2013, McAllister et al. 2018). In Australia, *G. galeus* use pupping areas from which young-of-the-year pups disperse (Olsen 1954, Stevens & West 1997) (Fig. 1) and therefore represents a model species to investigate constraints on shark pup dispersal from pupping areas.

To investigate constraints on shark pup dispersal capacity, we conducted bioenergetic analyses on *G. galeus* pups from their most important recorded pupping area in south-eastern Australia, the Pittwater estuary in Tasmania (Stevens & West 1997) (Fig. 1). We used swim tunnel respirometry to examine optimal swimming speed (U_{opt}) and costs of transport (COT) for dispersing pups and conducted bomb calorimetry and lipid class analysis to assess energy storage and dispersive fitness. Finally, we calculated an energy budget for *G. galeus* pups to gain insight into their energetic requirements and related foraging demands. To our knowledge, this is the first study using such a combined approach to investigate dispersal capacity from natal areas into other habitats.

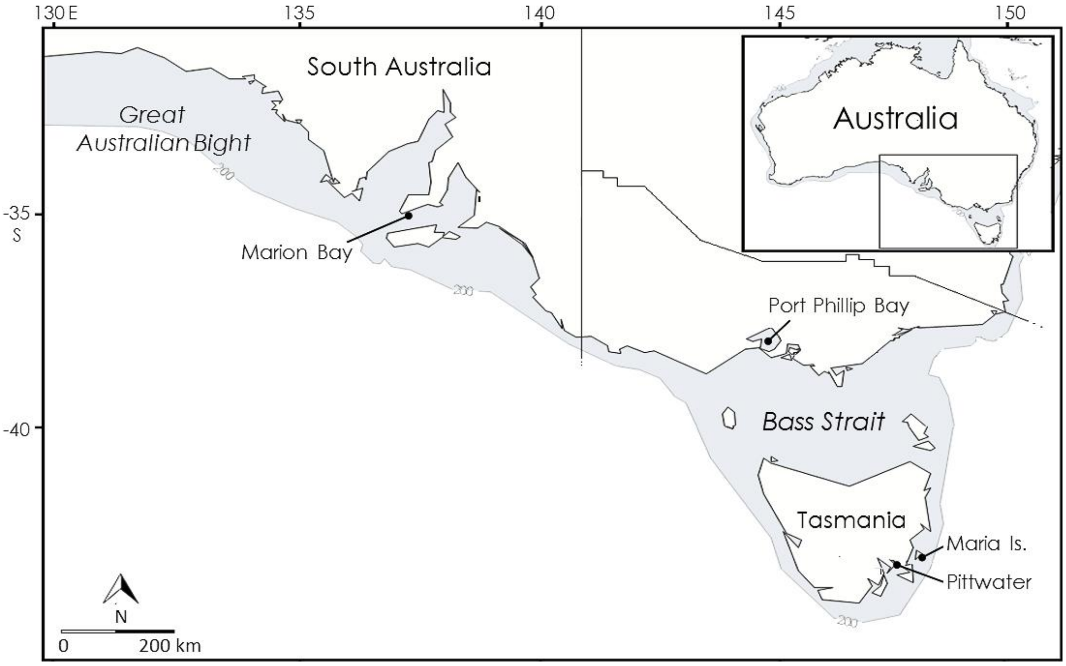

Fig. 1. Map of the study area capturing the core range of *Galeorhinus galeus* in Australia. Marked are the Pittwater estuary pupping area where this study was conducted, the Maria Island monitoring station, Port Phillip Bay – the most westerly recorded *G. galeus* pupping area, and Marion Bay – the closest location to known pupping areas in Bass Strait and Tasmania where neonate *G. galeus* have been recorded in South Australia. Shaded area is the continental shelf. Inset shows the study area (boxed) relative to Australia.

Materials and methods

Sample collection and preparation

School shark pups (*n* = 23) were caught with baited longlines in Pittwater estuary, Tasmania, over a three week period in early austral autumn (15th March to 7th April 2017). Pup collection was after the pupping season of *G. galeus* in austral summer (occurring mainly in December or January: Stevens & West 1997) and coincided with the first departures of pups from Pittwater that continue throughout autumn and winter (McAllister et al. 2015). The estuary has an area of 20.7 km^2 and is characterised by shallow flats (depth: ~4 m) draining at low tide into a main channel (depth: ~8 m) (McAllister et al. 2018). For bomb calorimetry and lipid analyses combined, 13 neonates were either euthanased on the capture vessel (sharks 1 and 2, Table 1), or after live transport to the Institute for Marine and Antarctic Studies facility at Taroona, Hobart (sharks 3–13, Table 1). All procedures were carried out under research permits issued by the Animal Ethics Committees of the University of Tasmania (A0016274) and the University of Adelaide (S-2016-134) in accordance with the Australian Code for the use and care of animals for scientific purposes (NHMRC 2013). Shark total weight (M_T: g), total length (TL: cm), sex, liver whole wet weight (M_L: g), and hepato-somatic index (M_L/M_T: HSI) were determined (see Table 1). Liver sub-samples were desiccated in a freeze dryer for five days, then homogenised and stored frozen in sealed vials.

Cost of transport

We conducted respirometry trials on 10 pups in a 175 L, sealed recirculating Brett-type swim tunnel respirometer with a swim chamber 875 mm long, 250 mm wide, and 250 mm deep (Loligo Systems, Denmark). Before trials sharks were housed in a 10,000 L holding tank exposed to environmental temperatures (16–18.6°C) and fed jack mackerel *Trachurus declivis* once daily, except for the period prior to respirometry trials, when food was withheld for 24 h. During trials, dissolved oxygen was measured using a Witrox oxygen meter, with an optical fibre oxygen sensor (Loligo Systems, Denmark) and recorded throughout to determine oxygen consumption rate (i.e. metabolic rate). Respirometer water was flushed and refreshed whenever oxygen saturation levels fell below 80% (Clark et al. 2013). Sharks were introduced into the respirometry chamber and acclimated at low speeds (0.3–0.4 body length s^{-1}) for between 30–47 minutes before starting oxygen consumption measurements. Trials were run under constant red-light conditions to minimise disturbance and water temperature maintained at 20°C. Starting at 0.5 body length s^{-1}, swimming speed was increased in increments of 0.1 body length s^{-1} periodically at intervals of 15 min (Payne et al. 2011).

To account for the increased water speed caused by the profile of the animal in the respirometry chamber, a solid blocking correction was applied as per Bell & Terhune (1970): $U_F = U_T(1 + \varepsilon_s)$, where U_F is the speed of the corrected flow and U_T is flow speed in the swim chamber absent a pup. Fractional error caused by solid blocking (ε_s) was calculated as: $\varepsilon_s = 0.8\lambda(A_O/A_T)^{0.5}$, where λ is a constant for pup shape (= 0.5*body length/body thickness), A_O is maximum cross-sectional area of the pup, and A_T is the cross-sectional area of the swim chamber (Bell & Terhune 1970, Payne et al. 2011). For each 15-minute speed trial, a linear regression was fit to the decrease in oxygen in the respirometer. Only trials where linear regressions yielded R^2 values greater than 0.8 were retained for analysis. Metabolic rates were then calculated as the product of the linear regression slope of pup oxygen consumption corrected for background oxygen consumption and the respirometer volume divided by the wet weight of the fish scaled using an exponent of 0.86 (Sims 2000). Resulting metabolic rates (mg O_2 kg^{-1} hr^{-1}) were divided by swim speed in km hr^{-1} to derive cost of transport (COT). A second order polynomial was then fit to the relationship between COT and swim speed (m s^{-1}) for all experimental animals and the minimum of the function determined to obtain the swim speed at which COT was lowest (U_{opt}).

Juvenile teleost fishes associated with inshore flats and benthic vegetation, e.g. whiting (Sillaginidae), flounder (Pleuronectidae), and flathead (Platycephalidae), are important prey for *G. galeus* pups departing pupping areas (Stevens & West 1997). On this basis, it is assumed dispersing pups move along the coastal shelf in relatively shallow water within the photo-benthic zone. While most pups depart Pittwater in autumn and winter, around a third return to adjacent bays the following spring suggesting relatively small-scale movements by these individuals (McAllister et al. 2015). However, dispersive individuals undertake longer movements, mostly northward along the coastal shelf of eastern Tasmania, as evidenced by detections at the Maria Island acoustic monitoring station 155 km from Pittwater (McAllister et al. 2015) (Fig. 1). Cost of transport during dispersal was thus adjusted for water temperature, season, and current flow at the Maria Island monitoring station. Here, the East Australia Current flows in a mean poleward direction during autumn-winter (at 20 m depth, mean current direction: 161°, and mean current flow: 0.21 m s^{-1}: IMOS 2018). It is possible that some pups disperse via a longer route south from Pittwater, around Tasmania's southern tip and north along the west coast against the Zeehan current, however there are no data to support this; here we focus on the observed east-coast dispersal route.

Bomb calorimetry

Energy content of livers was determined using a semi-micro oxygen bomb calorimeter (Parr model 6725, Parr Instrument Company, Illinois, U.S.A.) coupled with a calorimetric thermometer (Parr model 6772). Sub-samples of dried and homogenised liver (~40 mg) were pressed into pellets with a 200 mg spike of known energy content (standardised benzoic acid, Parr Instrument Company, Illinois, USA) to act as a fuse, and burned in the bomb calorimeter yielding measures of gross heat (MJ kg^{-1}). Heat produced by burning the liver sample was calculated by subtracting known heat production from the fuse material. The bomb calorimeter was calibrated prior to each session by burning a benzoic acid pellet of known energy content. Dried liver mass (D_L) was derived from the equation $D_L = D_S M_S^{-1} M_L$, where D_S was dried sub-sample mass (g), M_S was wet sub-sample mass (g) and M_L was wet liver mass (g) (Hoffmayer et al. 2006). Total liver energy (E_L) was then calculated from $E_L = D_L E_S$, where E_S was dried sub-sample energy. To optimise parsimony of the linear model employed (E_L ~ TL + HSI + lipid content), weight was omitted as a predictor variable of total energy storage since TL and weight were highly correlated ($r = 0.85$).

Lipid extraction and class analysis

Lipids were extracted from sub-samples of dried and homogenised liver tissue (~0.1 g) using a modified Bligh and Dyer (1959) technique. A two-phase extraction process was followed. Phase 1: the sub-sample was added to a solvent mixture of 9 ml purified H_2O and 20 ml methanol in a valve-sealed glass funnel then agitated gently and left to stand for 1 h before 10 ml dichloromethane (DCM: CH_2Cl_2) was added, agitated gently and left overnight. Phase 2: the funnel contents were shaken then 10 ml DCM and 9 ml saline purified H_2O was added and left for 2 h. Contents were drained and concentrated using a rotary evaporator before adding 2 ml DCM and pipetting the contents into pre-weighed sealed vials. Moisture was expelled using N_2 flow and total lipid extract weighed prior to adding 0.5 ml DCM and storing in a freezer. Lipid classes (HC/WE/SE: hydrocarbons/wax esters/sterol esters, TAG: triacylglycerols, ST: free sterols, DMAG: di/monoacylglycerols, and PL: phospholipids) were analysed using an Iatroscan Mk V TLC-flame ionization detector (Iatron Laboratories, Tokyo) after spotting of total lipid on silica rods and solvent development. The detector was calibrated using a standard mixture containing lipid classes. Lipid classes were quantified using the Iatroscan integrating software v7.0 (Iatron Laboratories, Tokyo). Lipid class data were analysed by applying a Bray Curtis similarity matrix and running a complete linkage cluster analysis and principal component analysis in Primer v6.0 (Primer-E, Plymouth, UK) to determine groups with similar lipid compositions.

Energy budget

An energy budget for *G. galeus* pups was calculated using a formula adapted from Ricker (1975): $C = M + Ms + G + W$, where C (energy consumed) is equal to the sum of energy used in metabolism (M), energy used in specific dynamic action (M_s, i.e. energetic costs of digestion), energy devoted to growth (G), and energy lost as waste (W). Metabolic energy consumption (M) was derived from COT at U_{opt} (COT at U_{opt} * U_{opt}), as a proxy for routine metabolic rate (Ikeda 2016) and scaled to mean pup size (g) using a mass scaling exponent for ectothermic sharks of 0.86 (Sims 2000). Because fish routinely swim at optimal speeds where energetic costs are minimal (U_{opt}) (Videler 1993, Clark & Seymour 2006), COT at U_{opt} provides an ecologically relevant measure of energy demands in the natural environment (Steffensen 2005). Since dispersal from Pittwater begins in autumn and is completed in winter (McAllister et al. 2015), we used a temperature coefficient (Q_{10}) of 2.51 from related leopard shark *Triakis semifasciata* in a similar thermal range (Miklos et al. 2003) to adjust for spatial and seasonal changes in ambient temperature. Adjustments were made for mean water temperatures in: early autumn (1st March – 15th April: 17.2°C) and late autumn (16th April – 31st May: 12.6°C) in Pittwater (Semmens unpublished data), and in early autumn (17.4°C), late autumn (15.3°C), and winter (13°C) at the Maria Island monitoring station (depth: 20 m, IMOS 2018) (Fig. 1), assumed to represent conditions on the coastal shelf of eastern Tasmania. This station is on a dispersal route used by *G. galeus* pups from Pittwater (McAllister et al. 2015). Specific dynamic action costs were estimated at 6% of metabolic energy consumption, consistent with young-of-the-year of another carcharhiniform shark: bonnethead shark *Sphyrna tiburo* (Bethea et al. 2007). Given a mean monthly growth rate for *G. galeus* pups of 2.3 cm (Stevens & West 1997) and that male and female growth curves do not differ (Moulton et al. 1992), monthly growth was derived from the weight-length relationship for *G. galeus*: $y = 4.86(10^{-6}x^{3.18})$, where y = weight (lb) and x = length (cm) (Olsen 1954) and converted to g. Since *G. galeus* pups are immature, all energy devoted to growth was calculated as somatic rather than reproductive growth and was multiplied by a caloric tissue value of 1.5 kcal g^{-1} from ecologically similar *Squalus acanthias* (Eder & Lewis 2005, Barnett et al. 2017) to estimate daily energy devoted to growth. Energy lost to waste was estimated at 27% (Lowe 2002, Dowd et al. 2006).

Results

Swimming performance and energy budget

Metabolic rate at U_{opt} was 149 mg O_2 kg^{-1} h^{-1} at 20°C from six neonates that produced usable respirometry data. Adjusting for seasonal differences in ambient water temperature on the coastal dispersal route yielded a decrease in metabolic rate at U_{opt} from 117 mg O_2 kg^{-1} h^{-1} in early autumn to 78 mg O_2 kg^{-1} h^{-1} in winter. At 20°C, U_{opt} was 0.6 m s^{-1} (Fig. 2) equating to a mean of 1.4 bl s^{-1}. Cost of transport at U_{opt} on the coastal dispersal route decreased from 0.7 J g^{-1} km^{-1} in early autumn to 0.5 J g^{-1} km^{-1} in winter. Adjustment for swimming headlong into the poleward flowing East Australia Current (mean flow rate: 0.21 m s^{-1}) along the observed dispersal route on the coastal shelf of eastern Tasmania gave a COT of 0.9 J g^{-1} km^{-1} in early autumn decreasing to 0.6 J g^{-1} km^{-1} in winter. Growth was the largest energetic cost; daily growth ranged from 2.5–3.1 g demanding 15.7–19.2 kJ day^{-1} (Table 2). Metabolic energy consumption ranged from 11.5–14.8 kJ day^{-1} and energy lost to waste ranged from 7.4–9.2 kJ day^{-1} yielding a total routine energy consumption including specific dynamic action of 35.3–44 kJ day^{-1} (Table 2). Whiting (Sillaginidae, the most important prey item for *G. galeus* pups by occurrence and weight: Stevens & West 1997), yields a mean caloric value of 5.9 kJ g^{-1} (McCluskey et al. 2016). Pups would therefore need to consume 6–7.5 g whiting day^{-1} to satisfy routine energy requirements, equivalent to a daily ration of 1.4–2.3% wet bodyweight (Table 2).

Bomb calorimetry and lipid class analyses

Livers of *G. galeus* pups were small with a mean HSI of only 3.6% wet bodyweight (range: 2.4–4.8%). Bomb calorimetry yielded mean energy values for liver sub-samples of 28.42 ± 2.42 MJ kg^{-1} (range: 25.5–32.36 MJ kg^{-1}) and for whole livers of 120.9 ± 54.8 kJ (range: 59.8–249.8 kJ) (Table 1). The most important driver of total liver energy in the linear model was HSI with an effect size of 34.7 kJ per percentage increase in HSI ($t = 2.7$, $p = 0.02$), while total length had a marginally significant effect (t = 2.4, $p = 0.04$) and lipid content had no significant effect (t = 1.4, $p = 0.21$). Lipid content averaged 38.7 ± 8.6% (range: 26–56%). Lipid class profile was broadly similar with triacylglycerols (TAG) and phospholipids (PL) the most abundant lipids, however proportions of these classes varied among individuals (Fig. 3). Mean content of lipid classes were: HC/WE/SE = 3.47 ± 2.1%, TAG = 62.71 ± 13.9%, ST = 4.56 ± 5.4%, DMAG = 3.23 ± 2.8%, and PL = 26.52 ± 9.7%. Cluster analysis yielded four groups with >80% similarity and two groups with >60% similarity in lipid class content; one group characterised by high TAG content and a more mixed group with high PL content (Fig. 4). Membership of these two groups was not driven by sex ($t_{11} = 0.33$, $p = 0.75$) or total length ($t_{11} = -1.75$, $p = 0.11$).

Table 1. Sampling information and results of bomb calorimetry and lipid extraction from livers of *G. galeus* pups from Pittwater estuary including shark identification (ID), total length (TL), weight, sex (male/female), liver wet weight, hepato-somatic index (HSI), lipid content (percentage of liver tissue composed of lipid), energy stored per kilogram of dried liver tissue, total stored energy (total energy stored in livers of each pup), and number of days energy stores are calculated to last without further feeding when sampled in Pittwater in early autumn.

ID	TL (cm)	Weight (g)	Sex (M/F)	Liver wet wt (g)	HSI (%)	Lipid content (%)	Energy (MJ/kg)	Total stored energy (kJ)	Energy stores (days)
S1	42	323	M	9.1	2.8	34.73	26.82	67.0	2.0
S2	42	325	M	7.7	2.4	29.72	26.01	61.5	1.8
S3	46	384	F	18.3	4.8	44.69	32.37	247.0	4.5
S4	41	333	M	10.4	3.1	26.05	26.32	85.5	3.6
S5	47	386	M	13.2	3.4	56.23	32.33	175.0	2.8
S6	41	306	F	11.2	3.7	33.90	26.83	90.6	3.0
S7	44	363	F	12.3	3.4	35.55	30.60	133.2	3.3
S8	40	253	F	11.0	4.4	44.71	31.14	127.5	3.6
S9	39	228	F	7.7	3.4	37.54	25.50	68.6	2.0
S10	46	401	M	18.9	4.7	52.40	28.47	186.5	3.8
S11	45	344	M	14.7	4.3	34.12	27.04	117.3	2.2
S12	46	369	F	11.7	3.2	39.63	28.81	126.9	2.2
S13	44	282	M	10.1	3.6	34.10	27.20	83.8	1.4

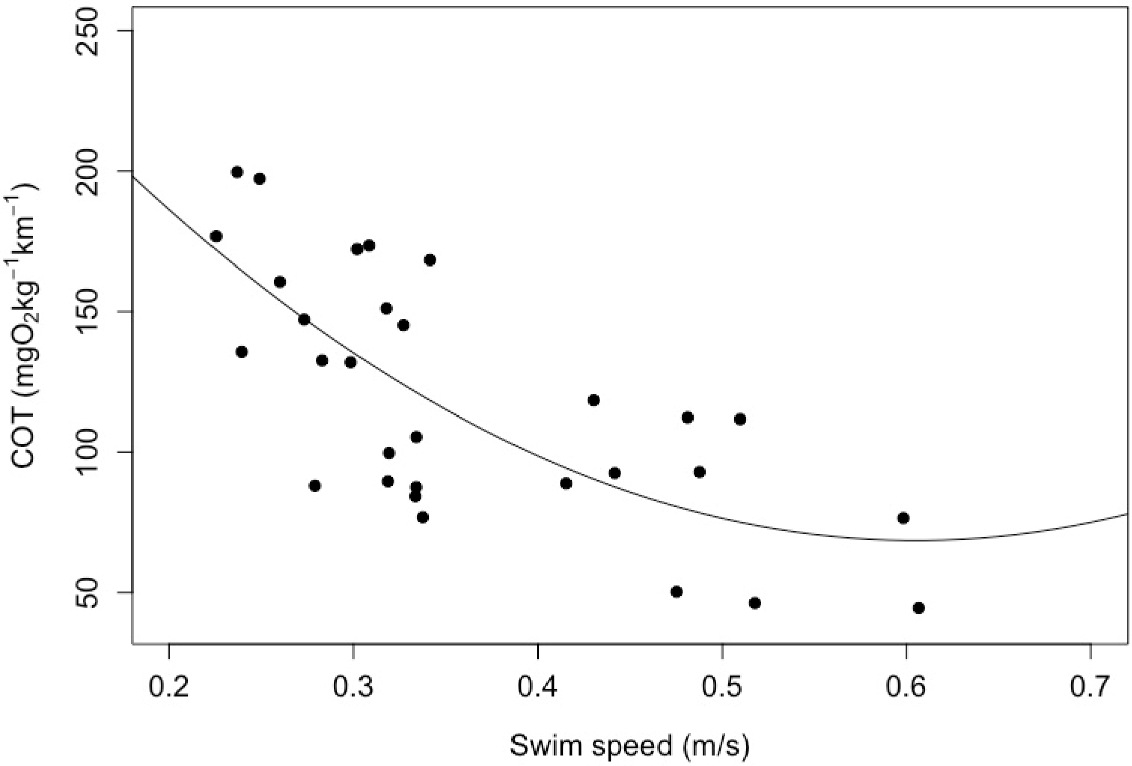

Fig. 2. Cost of transport (COT: mgO_2 kg wet weight^{-1} km^{-1}) as a function of swimming speed (m s^{-1}) for *G. galeus* pups from swim tunnel respirometry trials. A polynomial trendline is fit to derive optimal swimming speed at which COT was lowest (U_{opt}).

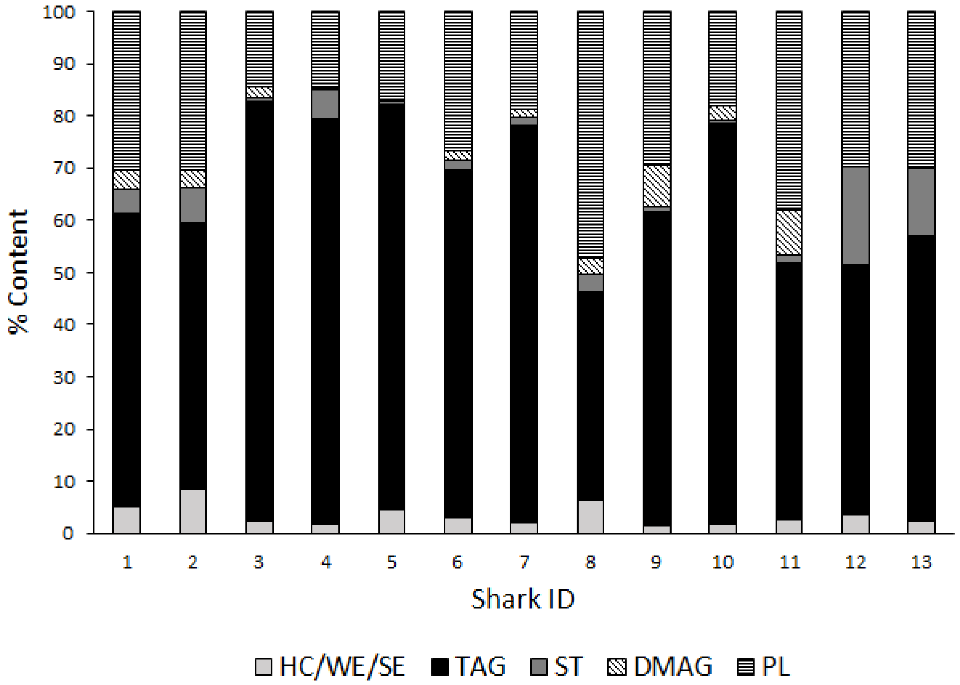

Fig. 3. Lipid classes as % total liver lipid content for *G. galeus* pups (*n* = 13) from the Pittwater estuary collected in austral autumn 2017. Identification numbers for each shark are given on the *x*-axis. HC = hydrocarbons, WE = wax esters, SE = sterol esters, TAG = triacylglycerols, ST = free sterols, DMAG = di/monoacylglycerols, and PL = phospholipids.

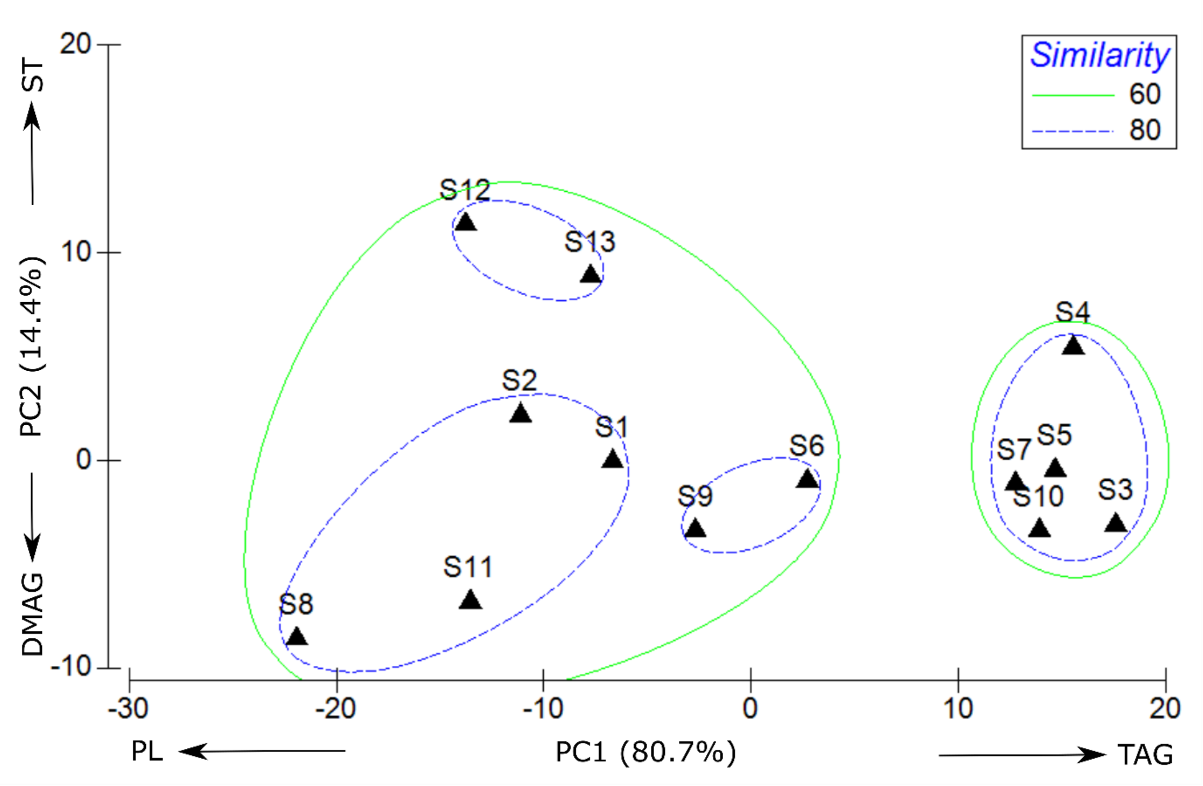

Fig. 4. Principal component analysis of lipid classes for *G. galeus* pups (*n* = 13) from Pittwater estuary in austral autumn 2017. Eigenvalues in brackets give the amount of variance explained by axes (PC1 and PC2). Lipids labelled on axes are principal eigenvectors constituting each principal component; PL = phospholipids, TAG = triacylglycerols, ST = free sterols, and DMAG = di/monoacylglycerols. Continuous (green) and dashed (blue) lines encompass groups with >60% and >80% similarity respectively based on complete linkage cluster analysis.

Table 2. Estimated energetic parameters for *G. galeus* pups of mean size dispersing from Pittwater estuary with adjustments for changes in site, season, and growth. Sites: Pittwater estuary and Maria Island. Seasons: early austral autumn (1st March – 15th April), late austral autumn (16th April – 31st May), austral winter = 1st June – 31st August. Temp = mean water temperature, MR at U_{opt} = metabolic rate at optimal swimming speed at mean weight, TL = mean total length, Wt = mean weight, Metabolism = metabolic energy consumption, SDA = energy used in specific dynamic action, Growth = energy devoted to growth, Waste = energy lost to waste, Total = total daily routine energy consumption, and daily ration required to fuel routine energy consumption based on caloric value of preferred prey (whiting Sillaginidae: 5.9 kJ g^{-1}) expressed as % wet bodyweight.

Site	Season	Temp °C	MR at U_{opt} mg O2 h^{-1}	TL cm	Wt g	Metabolism kJ day^{-1}	SDA kJ day^{-1}	Growth kJ day^{-1}	Waste kJ day^{-1}	Total kJ day^{-1}	Daily ration % wbw
Pittwater	Early autumn	17.2	44	43.3	330	14.4	0.9	19.2	9.1	43.6	2.2
	Late autumn	12.6	35	45.6	416	11.5	0.7	15.7	7.4	35.3	1.4
Maria Is.	Early autumn	17.4	45	43.3	330	14.7	0.9	19.2	9.2	44.0	2.3
	Late autumn	15.3	45	45.6	416	14.8	0.9	15.7	8.2	39.6	1.6
	Winter	13.0	42	47.9	486	13.7	0.8	17.6	8.4	40.5	1.4

Discussion

Our results revealed clear energetic benefits for *G. galeus* pups to delay dispersal from the pupping area until ambient water temperatures decrease in late autumn and winter to capitalise on lower routine energy costs. With cooler temperatures, daily ration required by dispersing pups decreased by over a third on the coastal dispersal route from early autumn to winter, such that pups dispersing in winter require less prey despite growing by almost 50% body mass since early autumn. Growth was the largest energetic cost for pups. Energy stores were small compared to adults in terms of liver size relative to body mass, liver lipid content, and lipid classes, suggesting immature development of energy storage capacity and prioritised allocation of energy to growth rather than metabolic expenditure. There were two distinct groups of pups in terms of dispersive fitness; those with high stores of energy storage lipids who may be best equipped for dispersal and those with high stores of structural lipids who may be prioritising growth. There were also differences at the individual level with energy stores at the time of sampling sufficient to sustain routine energy requirements for ~1.4–4.5 days (mean ± SD: 2.7 ± 0.9 days) without further feeding.

The liver is the main site of energy storage in elasmobranchs, where lipids are synthesised and stored prior to export to the serum and transport to muscle tissues to fuel metabolic activity (Sargent et al. 1972, Zammit & Newsholme 1979). As such, the liver of many sharks is a particularly energy rich organ; e.g. livers of white sharks *Carcharodon carcharias* have higher energy density than whale blubber (Pethybridge et al. 2014). Depletion of liver lipid content (and hence liver weight/HSI) after undertaking energy intensive migrations highlights the energy storage role of the liver, whose reserves are depleted to fuel long distance movements (Bone & Roberts 1969, Rossouw 1987). Consistent with this, mature *G. galeus* have significantly smaller livers after long migrations than prior to migrating (Olsen 1954). Liver lipids are also used to offset starvation with individuals in poor condition having lower HSI than those in good condition (Bone & Roberts 1969, Hoffmayer et al. 2006). The low HSI of pups (mean ± SD: 3.6 ± 0.7%; range: 2.4–4.8%) contrasted greatly with adults (HSI range: 10–20%: Ripley 1946b), indicating the limited energy reserves and dispersal capacity of pups relative to adults. Low HSI is also indicative of high relative body density and low hydrostatic lift, suggesting a predominantly benthic lifestyle in pups (Bone & Roberts 1969, Rossouw 1987). In addition to increased energy stores, greater HSI in adults would provide increased static buoyancy (lift), reducing dynamic lift costs of more active swimming. Adults would therefore be assisted in movements into the water column during diel vertical movements, a ubiquitous behaviour in adult *G. galeus* (West & Stevens 2001; McMillan et al. 2018a) but unlikely in pups, whose diet comprises mainly benthic taxa (Stevens & West 1997, McAllister et al. 2015). Pups also had lower liver lipid content (~39%) than adults (~60% and ~75% in males and females respectively: Ripley 1946a). Low HSI and lipid content in pups may therefore be a function of low energy storage, possibly a trade-off to prioritise growth, and appear to be key constraints limiting dispersal.

High proportions of structural lipids *v*. energy storage lipids in pups relative to adults further supports a trade-off prioritising growth and is reinforced by energy devoted to growth being the largest energetic cost for pups. Growth may increase competitive and foraging advantages while minimising predation risks at this vulnerable life stage (Morrissey & Gruber 1993, Heupel et al. 2007). Individual variation in lipid profiles was noted with two main groups whose membership was not driven by size or sex; those rich in energy storage TAG, the most abundant lipid (range: 40–80.6%, mean: 62.7%), and those rich in structural PL, the second most abundant lipid (range: 14.4–47.2%, mean: 26.5%). Intraspecific variation in lipid content and classes in sharks may result from differences in age, diet, condition, or season (Bakes & Nichols 1995, Jayasinghe et al. 2003). In the present study, given similar ages and season of collection, variation likely resulted from differences in diet and/or condition. Although the most abundant lipid in pups, concentrations of energy storing TAG were far lower than in adult *G. galeus*, where TAG comprises >95% of liver lipids (Nichols et al. 1998). Conversely, PL are structural lipids, important components of cell membranes and thus growth (Janse et al. 2004, Pethybridge et al. 2010). There was a strong negative correlation between concentrations of TAG and PL ($r = -0.95$). It is unclear whether this relationship represents a trade-off between energy storage and growth based on individual physiology or stems from dietary specialisation as can occur in sharks (Matich et al. 2011). Crustaceans and cephalopods have low lipid content compared to teleost fishes and cephalopod flesh in particular yields mainly structural lipids (Semmens 1998). Although size had a marginally significant effect on energy storage, the most important driver was HSI, suggesting individual variation plays a key role in dispersal capacity. Teleost fishes become increasingly important in the diet of juvenile *G. galeus* (Stevens & West 1997), suggesting a transition from generalist foraging in inexperienced neonates to a focus on teleost prey with higher energy value as foraging experience increases. Variation in foraging experience yielding different dietary proportions of teleost *v.* less energy-rich prey may thus drive differences in lipid classes among pups observed here.

Seasonal and spatial changes in ambient water temperature can have strong effects on energy consumption in ectothermic sharks (Carlson & Parsons 1999, Miklos et al. 2003, Bethea et al. 2007). We found clear energetic benefits for pups in delaying dispersal from pupping areas until periods of cooler ambient temperatures in late autumn and winter consistent with thermal effects of decreasing water temperature lowering metabolic rate and COT (Clark & Seymour 2006). Daily ration requirements decreased by over a third from early autumn to winter in response to declining metabolic demands; a similar range as in other ectothermic sharks, e.g. young lemon shark *Negaprion brevirostris* (1.5–2.1% bw day^{-1}: Cortés & Gruber 1990). Ration levels may also increase with decreasing latitude as increasing ambient temperatures elevate metabolic demands (Bethea et al. 2007). Increasing energetic costs for *G. galeus* pups as they move north along the Tasmanian coast into warmer waters thus provide further reason to delay dispersal until temperatures at lower latitudes have fallen.

The optimal swimming speed of 1.4 bl s^{-1} was comparable to sustainable swimming speeds in other ectothermic sharks of similar size (0.9–1.7 bl s^{-1}) including scalloped hammerhead *Sphyrna lewini* (Lowe 1996), lemon shark *N. brevirostris*, and leopard shark *T. semifasciata* (Graham et al. 1990). This suggests a relatively fast theoretical dispersal capacity of up to 52 km day^{-1} given continuous swimming and absent current, decreasing to ~41 km day^{-1} when swimming into the East Australia Current at mean flow. Yet, these speeds exceed even adult dispersal speeds, e.g. female *G. galeus* making purposeful pupping migrations moved at a maximum of 24 km day^{-1} (McMillan et al. 2018a). Such high speeds are therefore unlikely to be maintained by pups. Like other carcharhiniform sharks, *G. galeus* are not obligate swimmers; they are capable of both ram ventilating while swimming and buccal pumping while at rest (Carrier et al. 2012). Pups were also observed resting in holding tanks, suggesting continuous swimming by pups is unlikely in the wild. Acoustically tracked pups dispersing from Pittwater required 44–358 days to cover the 155 km to Maria Island at a fastest dispersal rate of 3.5 km day^{-1} (McAllister et al 2015). The discrepancy between field observed dispersal and theoretical dispersal capacity derived from U_{opt} suggests factors other than physiology alone may constrain shark pup dispersal. Perhaps slowing dispersal is again part of the trade-off allowing for allocation of resources to growth rather than locomotion.

Diel behavioural strategies may also play a role in mediating dispersal. Increased activity levels (e.g. swimming during foraging or dispersal) bring increased predation risks (Lima 1998, Heithaus 2007). Perhaps therefore, increased nocturnal activity and daytime refuging occurs in pups of many shark species (Holland et al. 1992, Sims et al. 1993, Garla et al. 2006). Young *G. galeus* in Norfolk Bay adjacent to Pittwater are more active at night, retreating to the deep middle of the bay during the day and patrolling the bay's edges by night, when activity levels were considerably greater (Barnett & Semmens 2012). This may be a response to predation risks, e.g. from broadnose sevengill shark *Notorynchus cepedianus* that prey on young *G. galeus* in Pittwater and adjacent bays during warmer months (Barnett & Semmens 2012). Broadnose sevengill sharks also disperse north along the coast in late autumn–winter (Stehfest et al. 2014), i.e. concurrent with *G. galeus* pup dispersal, when they likely exert continued predatory pressure. If activities including foraging and dispersive swimming compete for time at night, this would limit dispersive capacity, though further study is required to test the hypothesis of behaviourally mediated dispersal.

Prioritisation of growth, low energy stores, and behavioural mediation may thus constrain dispersal capacity in shark pups until sufficient growth and energy storage occur or ambient conditions, e.g. water temperature, are favourable and reduce energetic costs. These limitations are compounded by the higher cost of transport for pups relative to adults; decreasing surface to volume ratios with increasing size contribute to COT decreasing with increasing mass by an exponent of ~0.3 in sharks (Schmidt-Nielsen 1984). Field observations of *G. galeus* pups support an incremental dispersal from pupping areas rather than direct, rapid dispersal. Mark-recapture of *G. galeus* pups in Port Phillip Bay showed pups began congregating in channels in March before taking circuitous routes towards the open sea, punctuated by periods of milling about before dispersal from the bay by July (Olsen 1954). In Pittwater, a similar trend was observed with acoustically tracked *G. galeus* pups beginning to move into lower reaches of the estuary during March–May and dispersing into adjacent coastal areas in May–June (McAllister et al. 2015). These movements are consistent with our findings of low energy stores in early autumn and the energetic benefits of delaying dispersal until water temperatures fall in late autumn and winter.

Neonate *G. galeus* (<45 cm: Stevens & West 1997) have previously been reported in South Australia by fishers who inferred local pupping areas, however these reports have also been attributed to pups dispersing rapidly from distant pupping areas around Tasmania or Bass Strait (Prince 1996; Riseley pers. comm.). Recent scientific records of neonates have also occurred in South Australia, far from recorded pupping areas. Neonates of 34 and 41 cm total length (i.e. ages of ~1 and 4 months: Stevens & West 1997) were recorded in January and February respectively (Rogers et al. 2017, McMillan et al. 2018b). Sites of capture (western Great Australian Bight and Marion Bay: Fig. 1) would have required pups to swim at least 1,700 and 840 km respectively from the nearest recorded pupping area in Port Phillip Bay. In the unlikely event that these neonates began dispersing immediately after birth (foregoing the usual months of post-natal growth in pupping areas to build swimming strength and energy stores), they would have needed to disperse at minimum speeds of ~60 and 7.5 km day^{-1} respectively to cover these distances; far in excess of the fastest recorded dispersal rate of 3.5 km day^{-1} (McAllister et al. 2015). Since these neonates were recorded in January and February, i.e. before the March–July movement of *G. galeus* pups to the open sea begins in known pupping areas (Olsen 1954, McAllister et al. 2015), it is most likely that they were born close to their capture sites in South Australia rather than migrants from Bass Strait or Tasmania. This is consistent with the fastest recorded dispersals of *G. galeus* pups from Bass Strait and Tasmania to South Australia requiring 18–24 months, by which time they are no longer neonates (Olsen 1954, Semmens unpublished data).

This study suggests a trade-off in shark pups that prioritises growth over dispersal and demonstrates the high costs of growth. For pups there were clear energetic benefits in delaying dispersal until late autumn or winter when routine energy demands and transport costs fall in response to falling ambient temperatures. This study also indicates that neonate *G. galeus* recorded in South Australia were likely born locally rather than migrants from distant pupping areas in south-eastern Australia. Future developments, e.g. miniaturisation of pop-up archival tags or expansion of acoustic receiver networks, may enable explicit information to be gathered about shark pup dispersal from pupping areas in terms of routes, behaviour (e.g. direct movement *v*. foraging), practical swimming speed, and destination that may have important ramifications for both this Conservation Dependent species and other elasmobranchs. More generally, the approach presented here may be adapted to other marine taxa that rely on dispersal of young between natal areas and adult populations to quantify dispersal capacity and address various conservation and management issues, e.g. timing, likely routes, and rate of dispersive movements.

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CHAPTER 5

Stay warm, travel cool: Partial female migration and cool-water migration pathways in an overfished shark

Khaleesi, a.k.a. shark S7, about to be released with a PSAT tag in heavy seas southwest of Kangaroo Island in October 2017.

Statement of Authorship

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Contribution to the Paper	Study concept and design, organise and carry out field work, tagging, data analysis, lead authorship of manuscript. Acting as corresponding author.		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Partial female migration and cool-water migration pathways in an overfished shark

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Knowledge about reproductive movements can be of important conservation value for over-exploited species that are vulnerable when moving between and within key reproductive habitats. Lack of knowledge persists around such movements in the overfished school shark *Galeorhinus galeus* in Australia. Management assumes all pregnant females migrate between adult aggregations in the Great Australian Bight, South Australia, and nursery areas around Bass Strait and Tasmania. We tracked 14 late-term pregnant females tagged in South Australia using satellite-linked pop-up archival tags to investigate extent, timing, and routes of migrations. We found partial migration, with some females ($n = 7$) remaining near aggregating areas throughout the pupping season, some migrating to known nursery areas ($n = 3$), and one migrating ~3 000 km to New Zealand. We conclude female movements and pupping habitats are less spatially constrained than assumed and propose females use cool-water routes along the shelf break to reduce energy costs of migration. Migrating females using these routes faced greater fishing pressure than sharks in inshore areas and were not protected by inshore shark fishing closures designed to protect them. This study demonstrates the complexity of reproductive movements that can occur in wide-ranging species and highlights the value of explicit movement data.

Keywords: behavioural plasticity, bioenergetics, fishing pressure, *Galeorhinus galeus*, large-scale movements, PSAT, soupfin shark, tope.

Introduction

Partial migration, whereby migratory behaviour varies producing migrants and residents within populations, has been reported in all major vertebrate taxa including birds (Lack, 1943; Lundberg, 1985; Adriaensen and Dhondt, 1990), mammals (Talbot and Talbot, 1963; Maddock, 1979; Ball *et al.*, 2001), and fishes (Jonsson and Jonsson, 1993; Wroblewski *et al.*, 1994; Papastamatiou *et al.*, 2013). The drive to migrate may arise through reproductive philopatry (Hendry *et al.*, 2003), genetic predisposition (Biebach, 1983), or individual choice weighing the benefits of migration against the costs of residency (Chapman *et al.*, 2011). Such decisions may be condition-dependent, e.g. fish in good condition can be more likely to migrate than those in poorer condition (Brodersen *et al.*, 2008). While varying participation and movements associated with partial migration can confer population-level resilience, it may also complicate conservation planning, e.g. through varying vulnerability to human impacts in different areas (Secor *et al.*, 2001; Parsons *et al.*, 2011).

Where species have been depleted by human interference, knowledge about reproductive movements can be of important conservation value, allowing for protection of critical habitats such as birthing or nesting areas (Myers *et al.*, 1987; Webster *et al.*, 2002; Martin *et al.*, 2007). Sanctuary or no-take zones are increasingly being used as a conservation tool to protect important habitats for fishes (Bohnsack and Ault, 1996; Beck *et al.*, 2001; Edgar *et al.*, 2014) including sharks (Carrier and Pratt, 1998; Escalle *et al.*, 2015; Speed *et al.*, 2018). Many marine vertebrates, however, have life-histories punctuated by migrations, e.g. between foraging and reproductive habitats or between areas associated with different stages of ontogenetic development (Johannes, 1978; Shillinger

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Figure 1. Pregnant female *G. galeus* aggregate in the Great Australian Bight (diamond, upper left) in austral spring and are assumed to migrate (dashed line) to nursery areas around Bass Strait and Tasmania (e.g. Port Phillip Bay, Westernport, Port Sorell, Georges Bay, Pittwater) to give birth in austral summer. Line along coast denotes shark fishing closure from Kangaroo Island (to ∼2 km from shore) to Bass Strait (to ∼6 km from shore along mainland) designed to protect migrating *G. galeus*. Shark sanctuary zones designed to protect *G. galeus* pupping activity in Tasmania are also marked. Shaded area is the continental shelf. Inset: map area (boxed) relative to Australia.

et al., 2008; Grüss *et al.*, 2011). The efficacy of no-take zones may therefore be reduced if animals are captured en route during migrations from other habitats (Gerber *et al.*, 2005; Shillinger *et al.*, 2008; Costa *et al.*, 2012). Furthermore, in species where movements are commonly greater than the distance between sub-populations, the incorporation of up-to-date and informative movement data into management models is essential to produce effective, spatially explicit management policies and assessments (Walker *et al.*, 2008; Goethel *et al.*, 2011; Braccini *et al.*, 2016).

The school shark *Galeorhinus galeus* (also: soupfin shark, tope) is broadly distributed in temperate waters globally and has been overfished throughout its range, e.g. in California, Great Britain, and Australia (Walker, 1998; Molfese *et al.*, 2014). Like many sharks, *G. galeus* shares life history traits inhibiting recovery from population depletion, e.g. slow growth (reaching 60 years and ∼175 cm in Australia), late maturity (8–10 years), long reproductive cycles (2–3 years), discrete reproductive seasons (pupping in November–January in Australia), and selective use of reproductive habitats (Olsen, 1954; Walker, 1999). In Australian waters, *G. galeus* is Conservation Dependent after over-exploitation until the 1990s, and has not recovered despite fisheries management and conservation efforts introduced since the early 2000s (AFMA, 2009; Huveneers *et al.*, 2013; McAllister *et al.*, 2018). No-take zones designed to protect *G. galeus* migrations and recruitment are in place along the coastal strip from central South Australia to Bass Strait (to ∼6 km from shore) and in known nursery areas (Figure 1). However, a national recovery plan highlighted an important knowledge gap around critical reproductive habitats and movements for the species (AFMA, 2009).

Although wide-ranging and formerly common, >90% of *G. galeus* pupping in Australia is estimated to occur in unknown areas (Stevens and West, 1997). In the 1950s, spring aggregations of pregnant females were found in the Great Australian Bight, South Australia, in the northwest of the species' range, while nursery areas used in summer were found in Tasmania and Bass Strait in the southeast (Olsen, 1954) (Figure 1). Obligate female migrations between these areas were assumed, an assumption that persists and has shaped management of the species (Walker *et al.*, 2008), despite evidence that pupping may also occur close to aggregating areas in South Australia (Prince, 1996; Rogers *et al.*, 2017; McMillan *et al.*, 2018). Given genetic evidence for a single stock throughout this range (Hernández *et al.*, 2015), pupping near the northwest range of the species would entail partial female migration and present different challenges for conservation and fisheries management than the current model of reproductive movement and habitat use.

Temperature is a key driver of movement and habitat use in ectothermic fishes (Brett, 1971; Bernatchez and Dodson, 1987; Tanaka *et al.*, 2000); sharks have been proposed to select areas of favourable water temperature to behaviourally thermoregulate (Carey *et al.*, 1990; Thums *et al.*, 2012; Andrzejaczek *et al.*, 2018), conserve energy (Sims *et al.*, 2006), and assist digestion (Papastamatiou *et al.*, 2015). There is potential that behaviour of female *G. galeus* is likewise driven by thermal constraints, e.g. aggregating in warm areas to promote growth and gestation, and using cool waters to lower metabolic costs during migrations. We used satellite-linked pop-up archival tags (PSATs) to investigate extent, timing, and routes of migrations of pregnant *G. galeus* from aggregating sites in the Great Australian Bight. We aimed to address the knowledge gap around pupping movements and areas outlined in the national recovery plan for *G. galeus* (AFMA, 2009), and seek to understand potential drivers behind the aggregating and migratory behaviours of female *G. galeus* that may also be applicable to other wide-ranging ectothermic species.

Methods

Tagging

Tagging was undertaken in two different locations at different times. First, inshore near the head of the Great Australian Bight (Figure 2a) in early December 2015 at the start of the pupping season targeting females immediately prior to pupping (capture depth: 33 m, $n = 8$). Second, offshore southwest of Kangaroo Island, South Australia (Figure 2b) in October 2017 prior to the pupping season targeting migrating females (capture depth: 110–143 m, $n = 5$). A further female was tagged inshore in the Great Australian Bight in November 2017 (capture depth: 40 m) (shark x3: Supplementary Table S1). In 2015, females were caught by angling, using 50 kg braid and wire trace to 10/0 circle hooks set on the bottom and baited with Australian herring *Arripis georgianus.* In 2017, females were caught aboard commercial longlining vessels using 7 mm sinking rope main lines with up to 1 500 ~40 cm long traces of 2 mm monofilament to 10/0 circle hooks at ~7 m intervals and baited with slimy mackerel *Scomber australasicus.*

Only lively females (i.e. exhibiting strong, active, or responsive movements) free of injury to major organs (e.g. the gills) were selected for pregnancy examination and tagging. Selected females were kept oxygenated by pumping seawater over the gills via a hose inserted in the mouth and a moist cloth was placed over the eyes to reduce stress. Rolling females onto their back on a moistened rubber mat induced a tonic state whereby sharks became calmer, facilitating inspection, and tagging. Pregnancy was determined by visual inspection based on the characteristic triangular shape of late-term gravid females and *in-utero* movements of embryos that could be seen or felt externally. In 2017, ultrasound (Easi-Scan, BCF Technology Ltd, Livingston, UK) was used to validate the visual inspection method. Total length was also recorded to the nearest cm. All procedures were carried out under a research permit (S-2015–162) issued by the University of Adelaide Animal Ethics Committee in accordance with the Australian code for the use and care of animals for scientific purposes (NHMRC, 2013).

Wildlife Computers MiniPAT tags (Wildlife Computers, Redmond, WA, USA) programmed for 120-day deployments to cover the pupping season were deployed, fixed to either plastic umbrella or titanium anchor darts via 12 cm plastic coated wire tethers. Tag darts were inserted adjacent to the base of the first dorsal fin using an applicator cleaned with alcohol swabs before and after applications. Care was taken to insert the applicator at a sufficiently shallow angle and remain lateral to the vertebral column to avoid injury to vital organs. Time on deck ranged from 1.5 to 3 min, depending largely on sea conditions and liveliness of sharks. Sharks were swum next to the vessel into the current for up to 30 s until they swam away. Release location and capture depth were then recorded.

Data retrieval and analysis

Tags were programmed to record swimming depth and temperature at 5-min intervals along with daily summaries of thermal mixed layer depth and temperature, and light-based geolocation estimates at dawn and dusk. Upon tag detachment, data were transmitted to the ARGOS satellite network in a randomized manner until remaining battery power was exhausted, such that any gaps in transmission were distributed throughout the dataset rather than concentrated in any one period. Data were retrieved from the ARGOS platform then processed and exported using the Wildlife Computers DAP Processor software v 3.0. Kernel density distributions of swimming depth, temperature, and thermal mixed layer depth were plotted in R (R Core Team, 2013) using the *sm* package (Bowman and Azzalini, 2014). Environmental data were retrieved from the Kangaroo Island Integrated Marine Observing System (IMOS) monitoring station near our 2017 offshore tagging area and compared between tagging years to assess whether environmental conditions varied between years, including sea surface temperature, current speed, current direction, and sub-surface water temperature.

Raw geolocation estimates were refined using the Wildlife Computers GPE3 hidden Markov model fitting location estimates derived from dawn-dusk light levels against known sea surface temperature (SST) and depth data to generate maximum likelihood positions. Model parameters included a swimming speed of $1\ \mathrm{m\ s^{-1}}$ (consistent with other pelagic sharks: Queiroz *et al.*, 2010) and reference data sets were NOAA OI SST V2 high resolution for SST and ETOPO1-Bedrock for bathymetry. Here, we present horizontal movement tracks only for individuals that moved >150 km from tagging sites, since GPE3 geolocation error can exceed actual movements in deployments covering short distances (Braun *et al.*, 2018; Hueter *et al.*, 2018). Pop-up locations are accurate to within 1.5 km (ARGOS, 2016). Resident vs. transient habitat use was explored using the Panoply software v 4.8.10 to extract residency distributions based on 0.25° grid squares from GPE3 outputs. Residency distributions were calculated using a hidden Markov model smoothing approach to state space modelling that uses the posterior distribution of an individual's state to estimate probabilities of behavioural shifts between residency and migration (Pedersen *et al.*, 2011). Movement tracks were plotted against *G. galeus* commercial catch data from shark longline, gillnet, and trawl vessels during tag deployment periods in the Great Australian Bight (December 2015–March 2016) and between Kangaroo Island–Bass Strait (October–November 2017) to relate shark movements to fishing pressure.

Results

Data from three deployments were excluded from analyses due to a mortality based on 3 days of inactivity after release (shark x1: possible wounding based on presence of a mako shark *Isurus oxyrinchus* immediately after release), a premature detachment after 6 days (shark x2), and a tag reporting failure (shark x3) (Supplementary Table S1). Of the remaining eleven tags, six tags (55%) remained deployed for the full scheduled 120 days and deployments spanned a total of 1 083 days (mean ± *SD*: 99 ± 30 days) (Supplementary Table S1). All females tagged in December 2015 ($n = 6$: inshore and early in the pupping season) remained in South Australia, while of those tagged in October 2017 ($n = 5$: offshore and >1 month prior to the pupping season), one remained in South Australia, three migrated to Bass Strait and Tasmania, and one migrated to New Zealand (Figure 2). Some females moved short distances, remaining resident near tagging sites (e.g. 6 km in 80 days [shark S6] or 12 km in 77 days [shark S1]: Supplementary Table S1), while others made long and rapid dispersive movements (e.g. 2 908 km in 120 days [shark S11]: Supplementary Table S1) (Figure 2). One female tagged in the Great Australian Bight in 2015 was captured by a commercial fisher after 80 days at liberty ~6 km from the tagging location prior to tag detachment (shark S6). Another shark from this group was captured by commercial fishers the following pupping season (January 2017) south of Avoid Bay, South Australia

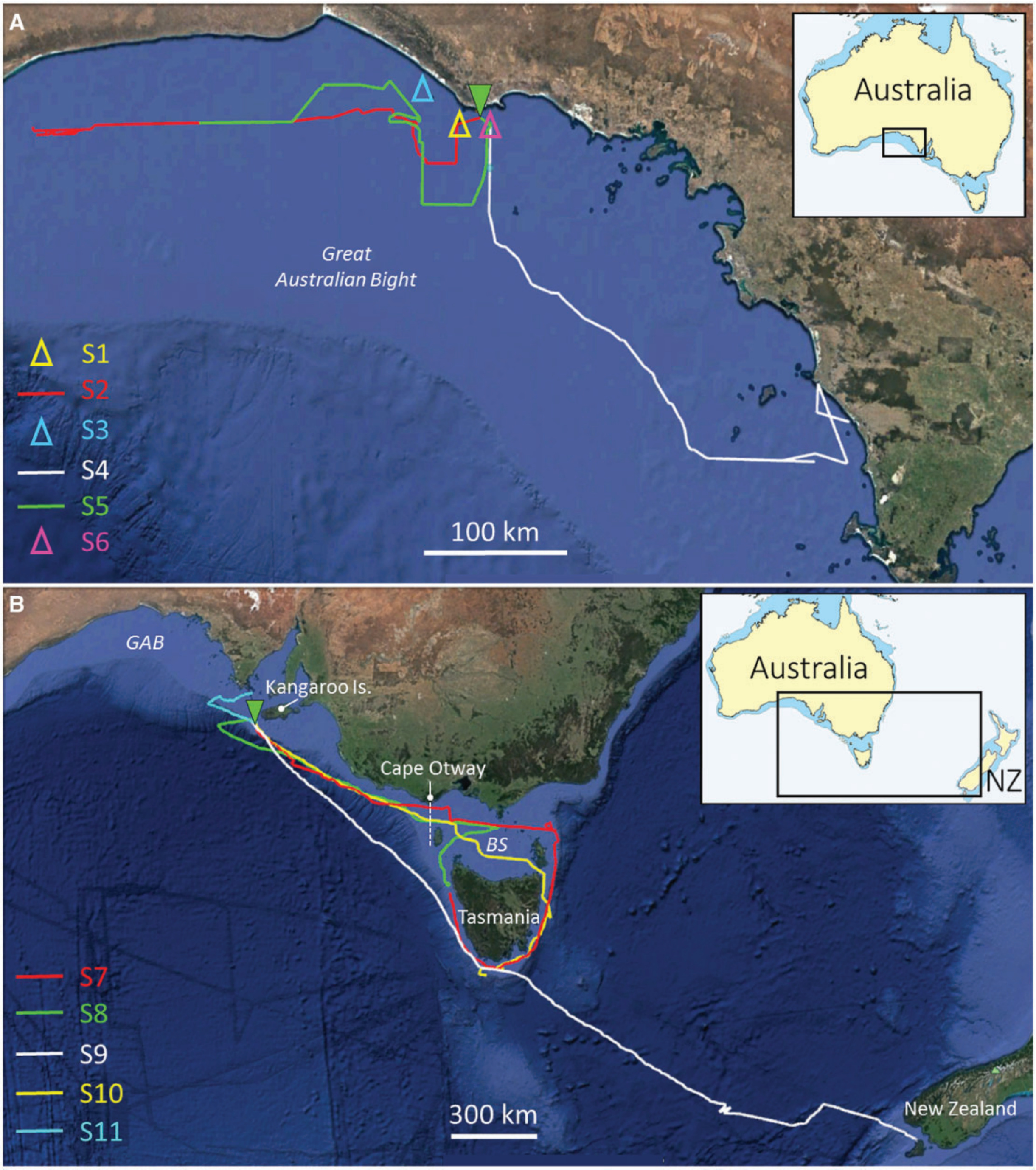

Figure 2. Movements of pregnant *G. galeus* tagged with PSATs in southern Australia in December 2015 (*n* = 6; a) and October 2017 (*n* = 5; b). Estimated movement tracks based on maximum likelihood locations are shown (lines) where sharks moved >150 km from tagging sites. Green inverse triangles = deployment sites. Terminal ends of tracks = pop-up locations (error: <1.5 km). Only pop-up or recapture locations are shown (hollow triangles) where sharks moved <150 km. Insets show study areas (boxed) relative to Australia. GAB, Great Australian Bight; BS, Bass Strait; NZ, New Zealand. Scale bars differ between panels.

~420 km from the tagging location, however it could not be identified as only the titanium anchor dart and tether remained *in situ* and total length was not recorded at recapture.

Residency distributions revealed strong plasticity in behaviour among sharks. Maximum probabilities of resident behaviour ranged from <0.1 to 0.9, where the highest probability of residence = 1 (Figure 3). Sharks tagged inshore in the Great Australian Bight in 2015 were more likely to exhibit semi-resident or resident behaviour (maximum residency probabilities: 0.2–0.9, mean: 0.4 ± 0.3) associated with their short movements, while sharks tagged off Kangaroo Island in 2017 were more transient (maximum residency probabilities: <0.1, mean: 0.04 ± 0.02)

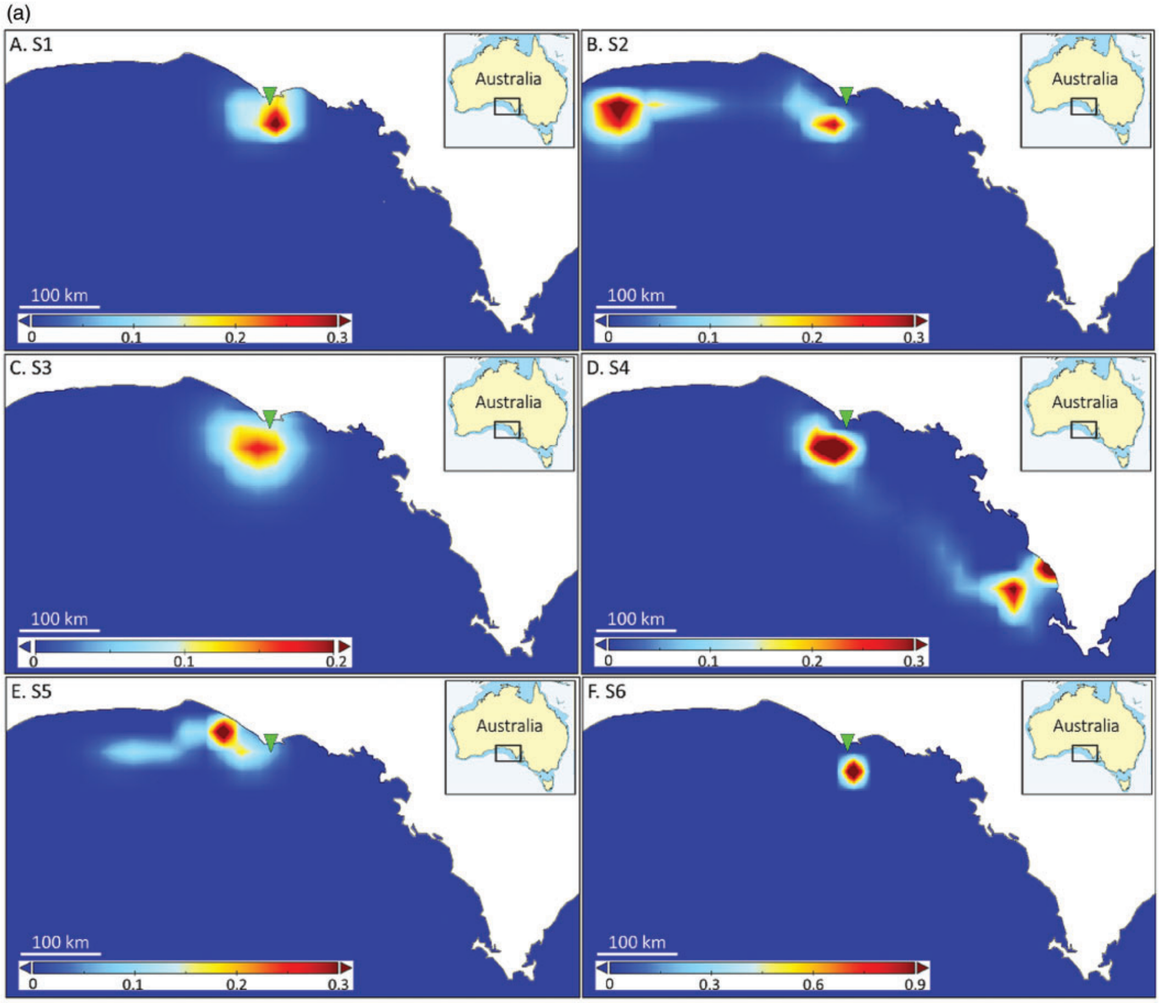

Figure 3. (a) Habitat use by pregnant *G. galeus* tagged in the Great Australian Bight in December 2015 at the start of the pupping season shown by residency distributions, i.e. probability of resident vs. transient behaviour based on 0.25° grid squares. Probability scales differ according to degree of residency in individual sharks (range: 0.2–0.9), maximum residency probability = 1. Inverse triangles = tagging locations. Scale bars differ among panels. Insets show study areas (boxed) relative to Australia. (b) Habitat use by pregnant *G. galeus* tagged offshore from Kangaroo Island in October 2017 prior to the pupping season shown by residency distributions, i.e. probability of resident vs transient behaviour based on 0.25° grid squares. Maximum residency probability = 1. Inverse triangles = tagging locations. Scale bars differ among panels. Insets show study areas (boxed) relative to Australia.

(Figure 3). Environmental conditions were broadly similar in both tagging periods. Sea surface temperature was similar throughout the study area during October (i.e. during our offshore tagging period) with no significant intrusion of the Leeuwin current from Western Australia in the Great Australian Bight (Supplementary Figure S1). Water temperature near the offshore tagging site off Kangaroo Island was also similar in this period (mean ± *SD*: 2015 = 14.5 ± 1.2°C, 2017 = 14.7 ± 0.9°C), as were current speed (2015 = 0.4 ± 0.3 m s^{-1}, 2017 = 0.4 ± 0.3 m s^{-1}), and current direction (2015 = 179 ± 11°, 2017 = 171 ± 19°). A paired *t*-test showed mean monthly water temperatures at this site were also similar throughout September–December (i.e. when pupping migrations are undertaken) in 2015 (mean ± *SD*: 14.7 ± 0.2°C) and 2017 (mean ± *SD*: 15 ± 0.1°C, $t_3 = -2.9$, $p = 0.06$).

Three females that migrated to Bass Strait followed close to the shelf break (depth: 115–200 m) with excursions onto the slope, rather than via the inshore no-take zone (depth: generally <50 m though <80 m near Portland and Cape Otway), reaching mean daily maximum depths of 170 m (Figure 2b, Supplementary Figure S2). Females following this migration pathway (all tagged southwest of Kangaroo Island in early October) passed Cape Otway and were into Bass Strait prior to the end of November (Figure 2b). In contrast, females that remained in the Great Australian Bight reached mean daily maximum depths of only 43 m and spent more time (67% of observations) in the thermal mixed layer than migrating females (55% of observations) (Figure 4). Temperatures encountered ranged from 8 to 22°C and differed between residents and migrants ($t_9 = -4.2$, $p < 0.01$), with migrating females generally experiencing colder temperatures (mean: 14°C, range: 8–18°C) than those that remained resident in the Great Australian Bight (mean: 18°C, range: 13–22°C) (Figure 4). Although migrants maintained highly transient behaviour throughout the tracking period with probability of resident behaviour < 0.1, they appeared to select slightly warmer waters

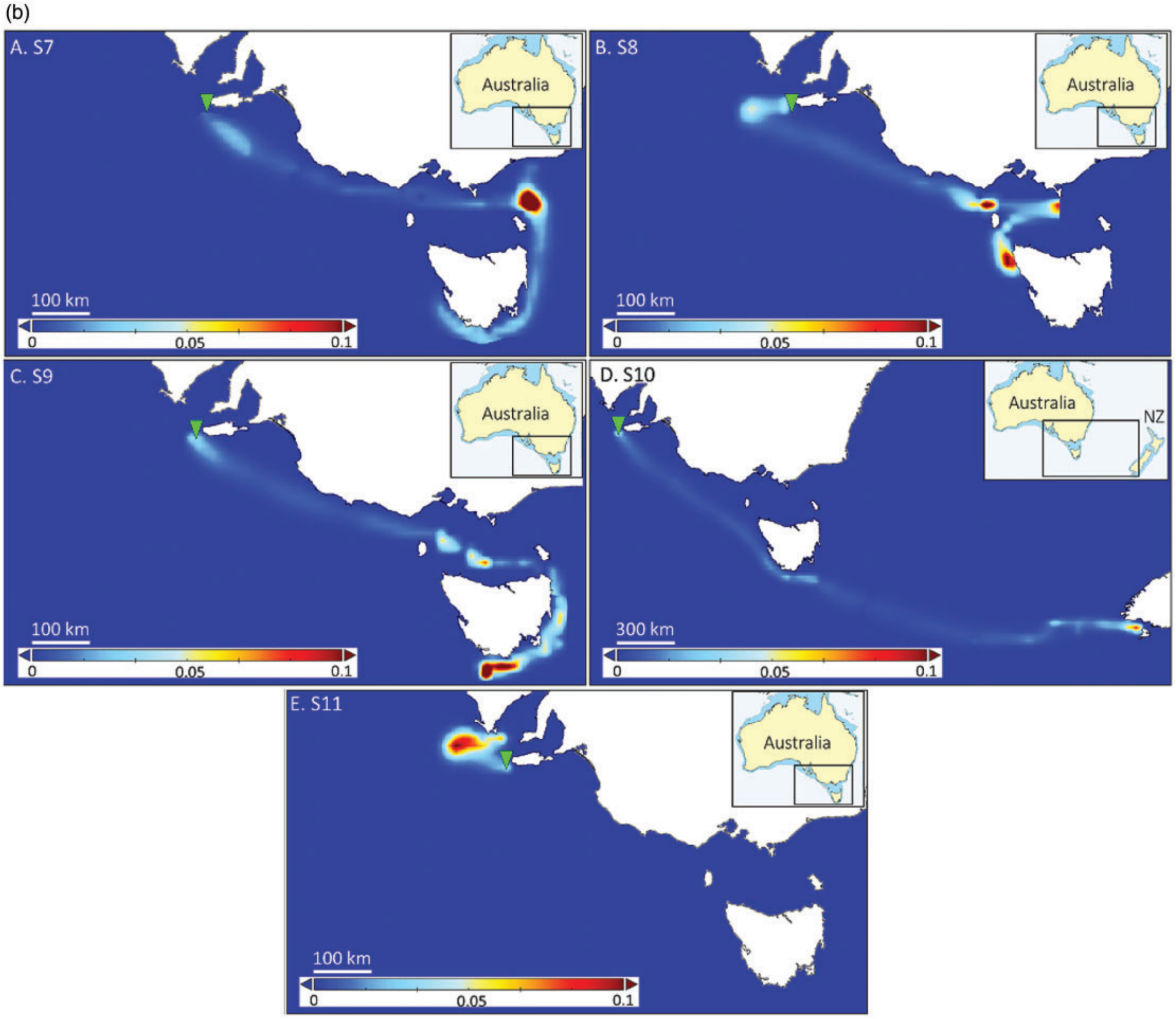

Figure 3. Continued.

during brief periods where residency probability was ≥ 0.05 (mean $\pm$ $SD = 15.3 \pm 1.5$°C) compared to periods where residency probability was < 0.05 (mean $\pm$ $SD = 14 \pm 1.7$°C), however a paired *t*-test found these differences were not statistically significant ($t_3 = -1.7$, $p = 0.19$). Migrating females moving along the outer shelf from Kangaroo Island to Bass Strait were exposed to greater fishing pressure than females that remained inshore (Figure 5). Vertical migrations were ubiquitous, continuing even during long-range movements >500 km (Supplementary Figure S2). The furthest moving female (shark S11 to New Zealand: 2 908 km) reached the maximum dive depth (536 m), and recorded the fastest swimming speed (>500 km: 24 km day^{-1}).

Discussion

We found partial female migration in Australian *G. galeus*, with some females remaining resident close to tagging locations (<15 km) over the pupping season in November–January and others migrating long distances to New Zealand (~3 000 km) (Figure 2). One female was also in western South Australia during two consecutive pupping seasons (tagged in December 2015 and recaptured January 2017). Pupping in South Australia (and thus partial female migration given the mixed nature of the stock) is also supported by recent evidence, such as capture of neonates there (Rogers *et al.*, 2017; McMillan *et al.*, 2018) and use of different pupping areas by South Australian and Bass Strait populations revealed by postnatal vertebral element signatures (McMillan *et al.*, 2018). Dispersive female *G. galeus* thus appear to migrate north from Tasmania and Bass Strait to overwinter in South Australia (Olsen, 1954; Punt *et al.*, 2000) before returning south to pup, while some pregnant females remain resident in South Australia throughout the pupping season. Behavioural plasticity among sharks tagged inshore at the start of the pupping season (December 2015) and those tagged offshore prior to the pupping season (October 2017) was marked by divergent modes of resident vs. transient habitat use. This was consistent with advice from experienced fishers who insist they encounter *G. galeus* in the same areas that differ in behaviour, colouration, and ectoparasite loads, which they relate to differences between resident and transient sharks.

Inshore shark fishing closures are in place along the coast to ~6 km offshore from Kangaroo Island running east along the mainland into Bass Strait to protect migrating *G. galeus*.

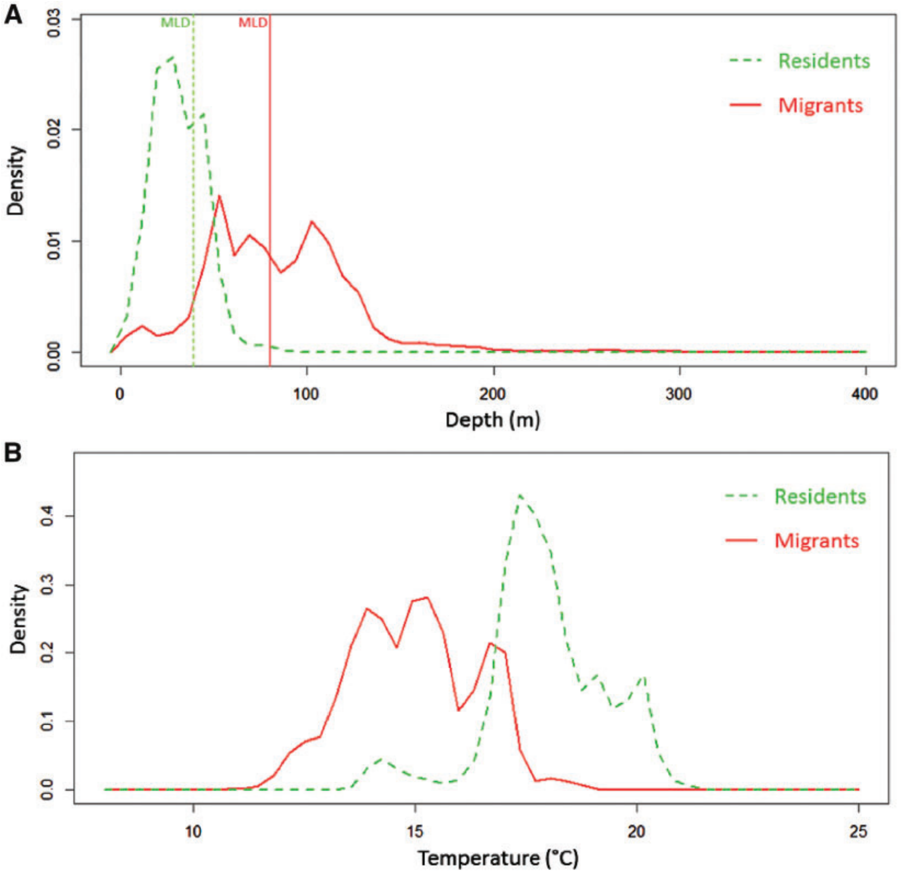

Figure 4. Density distributions showing time-at-depth (a) and time-at-temperature (b) for pregnant *G. galeus* that remained resident in the Great Australian Bight (dashed lines) and migrated to Bass Strait (solid lines). Vertical lines on upper panel (a) denote mean thermal mixed layer depth (MLD) for residents (dashed: 39 m) and migrants (solid: 80 m).

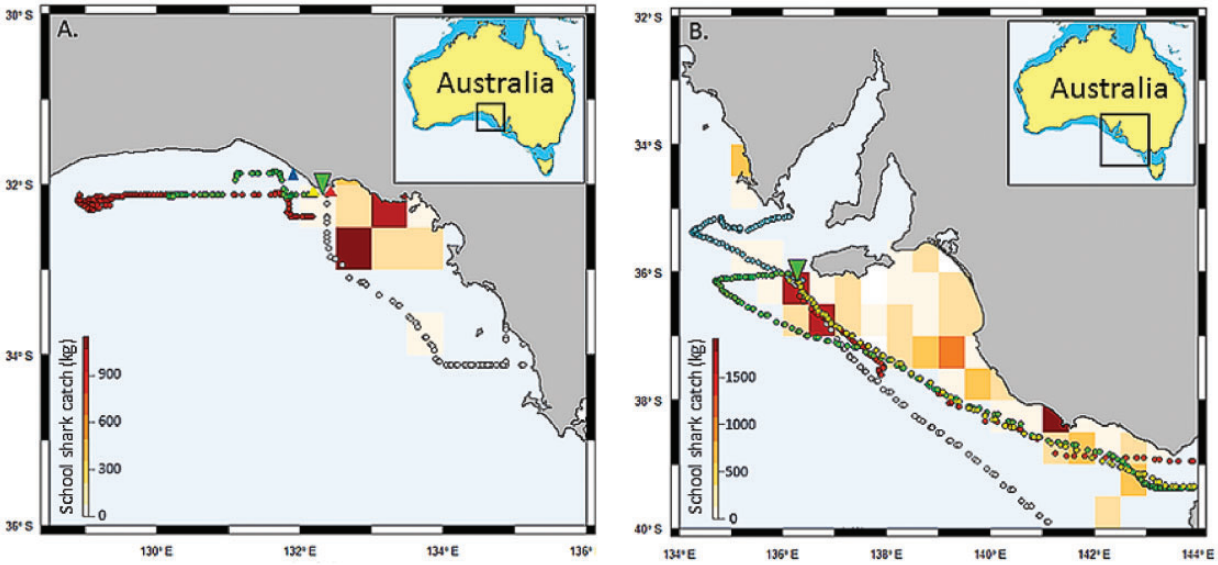

Figure 5. Movement tracks of tagged pregnant *G. galeus* relative to fishing pressure in the shark fishery off southern Australia. Density of *G. galeus* commercial catch (kg 0.25° grid square-1) is shown during periods of tag deployment from December 2015 to March 2016 in the Great Australian Bight (a) and from October to November 2017 on the migration pathway between Kangaroo Island and Bass Strait (b). Inverse triangles = deployment sites. Track IDs as for Figure 2. Catch data courtesy: Australian Fisheries Management Authority.

However, migrating females in this study favoured an offshore migration pathway close to the shelf break en route to Bass Strait, swimming at mean daily maximum depths of 170 m with excursions into deeper water on the continental shelf slope. While existing closures may benefit females moving close inshore during pupping runs, females migrating in deeper waters remain unprotected and exposed to fishing pressure. Migrating females faced greater fishing pressure from longline, gillnet and trawl vessels along this route than individuals remaining in shallower inshore areas (Figure 5). Given sharks' reliance on internal fertilization and thus limited fecundity, exposing mature female sharks to fishing pressure can impact heavily on population resilience (Ford, 1921; Mucientes *et al.*, 2009). The failure of current protections to cover migrating females may therefore limit the efficacy of terminal sanctuaries in south-eastern nursery areas (Gerber *et al.*, 2005; Costa *et al.*, 2012).

Migrating in deep, cool waters may reduce energetic costs of migration. Marine ectotherms can receive large energetic rewards, i.e. reduced energy costs, by moving to deep, cold waters (Steffensen, 2005; Seibel and Drazen, 2007). Metabolic demands of the related leopard shark *Triakis semifasciata*, decreased by a factor of 2.51 per 10°C decrease in temperature (Miklos *et al.*, 2003). Female *G. galeus* migrated in cool, deep water en route to Bass Strait and New Zealand (mean temperature: 14°C, range: 8–18°C). Migration along the shelf break may also be assisted by favourable currents. The eastward flowing South Australian current flows along the shelf break from the Great Australian Bight to the western edge of Bass Strait (Ridgway and Condie, 2004). The underlying Flinders current flows parallel at >400 m depth in the opposite direction pushing cool water up the shelf slope (Middleton and Bye, 2007). The shelf break may thus provide a convergence of favourable current direction and cool waters to lower metabolic costs. Despite using greater depths than residents, migrants maintained vertical foraging excursions into the warm thermal mixed layer (Supplementary Figure S2), where productivity is high and prey more abundant (McGowan and Hayward, 1978; Brainerd and Gregg, 1995). In contrast, sharks remaining in the Great Australian Bight stayed in warmer, inshore waters (Figure 4). Of six females tagged in the Great Australian Bight (mean temperature: 18°C, range: 13–22°C), only one (which moved the furthest: 334 km) swam in water <14°C (mean: 16°C), further suggesting that large-scale movements occur in cool waters potentially to reduce transport costs. Distribution of *G. galeus* on the shelf may be driven by interactions among shelf water masses (Jaureguizar *et al.*, 2018); future availability of detailed bottom temperature and other environmental data in the Great Australian Bight may allow any such relationships to be explored.

It is unclear whether female migrations are driven by philopatry, genetic predisposition, or condition-dependent choice. Philopatry could have important repercussions for conservation, e.g. disproportionate female mortality on certain migration routes could drive local population declines (Prince, 2005). Alternatively, if migration is a condition-dependent choice, numbers of migrants may vary over time. Environmental conditions (temperature, current speed, and current direction) were similar between tagging periods on the migration pathway and thus appear unlikely to have influenced resident vs. migratory behaviour. Prey abundance can drive shark movements (Sims, 2003; Hussey *et al.*, 2009; Speed *et al.*, 2010) and female *G. galeus* from southern latitudes are thought to pursue key prey species, e.g. jack mackerel *Trachurus declivis* as they move north into South Australia in winter with the highly productive subtropical convergence (Harris *et al.*, 1992; Punt *et al.*, 2000). However, while resource-driven migrations make sense for females from the southeast, they do not for females from South Australia where productivity remains comparatively high year round, enhanced by winter intrusions of the subtropical convergence and summer upwellings supporting vast shoals of sardines *Sardinops sagax* (Ward *et al.*, 2006), important prey for *G. galeus* (Ripley, 1946; Freer, 1992). The role of temperature as an ecological resource may also be important. Ectothermic fishes are known to use warm temperatures as a resource to maximize growth and fitness (Magnuson *et al.*, 1979; Brandt, 1993; Wirsing *et al.*, 2006). There is thus biological sense in mature females aggregating in the relatively warm waters of the Great Australian Bight over cooler months to maximize growth and fitness of themselves and their young, as has been suggested in other sharks (Hight and Lowe, 2007; Speed *et al.*, 2012), while building energy stores before dispersing to their respective pupping areas.

Partial migration is consistent with the current state of knowledge around *G. galeus* population dynamics in Australia. Demographic connectivity among populations has long been established by mark–recapture studies (Olsen, 1954; Brown *et al.*, 2000; Walker *et al.*, 2008) and genetic connectivity has been established more recently (Hernández *et al.*, 2015). However, the fact that new, apparently virgin populations were encountered as the fishery expanded westward after denuding previously fished populations (Olsen, 1959; Prince, 1996), is difficult to reconcile with a model of obligate female migration and a fully mixed stock. Partial migration explains both the established connectivity between populations and the capacity for populations to be locally over-exploited. Regional variations in resource availability over time may offer partially migratory species population-level resilience by benefiting migrants and residents at different times (Kerr *et al.*, 2009; Gillanders *et al.*, 2015). However, differences in movements and habitat use may also expose partially migratory populations to different threats, e.g. overharvesting or habitat degradation in different areas (Secor *et al.*, 2001; Parsons *et al.*, 2011). Where partial migration is occurring, it should therefore be identified and incorporated in conservation and management models. In addition, direct evidence of reproductive connectivity between Australia and New Zealand complements recent evidence of genetic connectivity (Hernández *et al.*, 2015; Bester-van der Merwe *et al.*, 2017), supporting a single panmictic stock. Consideration should therefore be given to trans-national management of the Australia–New Zealand school shark stock as a single management unit to best incorporate spatial modelling into fisheries management (Guan *et al.*, 2013; Secor, 2013; Braccini *et al.*, 2016).

Pregnant *G. galeus* in Australia thus undertake partial female migrations, with migrants using potentially predictable offshore migration pathways and timings. Pupping habitats are also likely less spatially confined than currently assumed, stretching from the Great Australian Bight to New Zealand rather than being concentrated in Bass Strait and Tasmania. This behaviour is analogous to that of many birds, dispersive contingents of which migrate to warmer climes from higher latitudes during winter while those from lower latitudes remain resident (Adriaensen and Dhondt, 1990; Berthold, 1991; Newton, 2010). These findings demonstrate the value of spatially explicit data from archival tags to refine information elicited from conventional mark–recapture studies. Our finding of partial migration may help clarify difficulties the current management model encounters in explaining serial depletion of the *G. galeus* stock in different areas, which appears incompatible with the assumption of obligate migration (R. Thomson, pers. comm.). In *K*-selected taxa with limited numbers of offspring, fulfilment of reproductive behaviours and movements by mature females is critical to population recovery and resilience. Spatially and temporally explicit movement information, as presented here, may thus assist conservation and fisheries managers in enabling fulfilment of key reproductive tasks by females of such taxa.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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Appendix V.A: Supplementary material

Partial female migration and cool-water migration pathways in an overfished shark

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Table S5.1. Sampling data for pregnant *G. galeus* tagged with PSATs in South Australia. ID = shark identification (sharks prefixed by x were excluded from analyses), TL = total length. Deploy days = duration of deployment in days. Distance = distance between deployment and pop-up locations. NA = not available.

ID	**TL cm**	**Deploy location**	**Depth m**	**Start date**	**End date**	**Pop-up trigger**	**Pop-up location**	**Deploy days**	**Distance km**
S1	156	32.054S 132.291E	33	4/12/2015	18/02/2016	Premature	32.054S 132.163E	77	12
S2	155	32.054S 132.291E	33	4/12/2015	2/04/2016	Scheduled	31.962S 128.980E	120	313
x1	141	32.054S 132.291E	33	4/12/2015	7/12/2015	Mortality	31.993S 132.550E	3	0
x2	155	32.054S 132.291E	33	4/12/2015	10/12/2015	Premature	32.021S 132.464E	6	21
S3	159	32.054S 132.291E	33	4/12/2015	2/04/2016	Scheduled	31.799S 131.881E	120	47
S4	161	32.054S 132.291E	33	4/12/2015	2/04/2016	Scheduled	33.681S 134.923E	120	334
S5	155	32.054S 132.291E	33	4/12/2015	4/03/2016	Premature	32.785S 128.700E	58	193
S6	157	32.054S 132.291E	33	4/12/2015	21/02/2016	Captured	NA	80	6
S7	165	36.499S 136.343E	143	7/10/2017	5/02/2018	Scheduled	41.391S 144.544E	120	894
S8	163	36.016S 136.316E	110	9/10/2017	6/02/2018	Scheduled	41.203S 144.422E	120	910
S9	151	36.066S 136.249E	123	9/10/2017	6/02/2018	Scheduled	46.763S 167.573E	120	2908
S10	170	36.094S 136.216E	130	9/10/2017	27/01/2017	Premature	44.039S 146.622E	111	1251
S11	154	36.089S 136.216E	128	9/10/2017	14/11/2017	Premature	35.062S 136.135E	37	114
x3	161	32.027S 132.805E	40	24/11/2017	NA	No report	NA	NA	NA

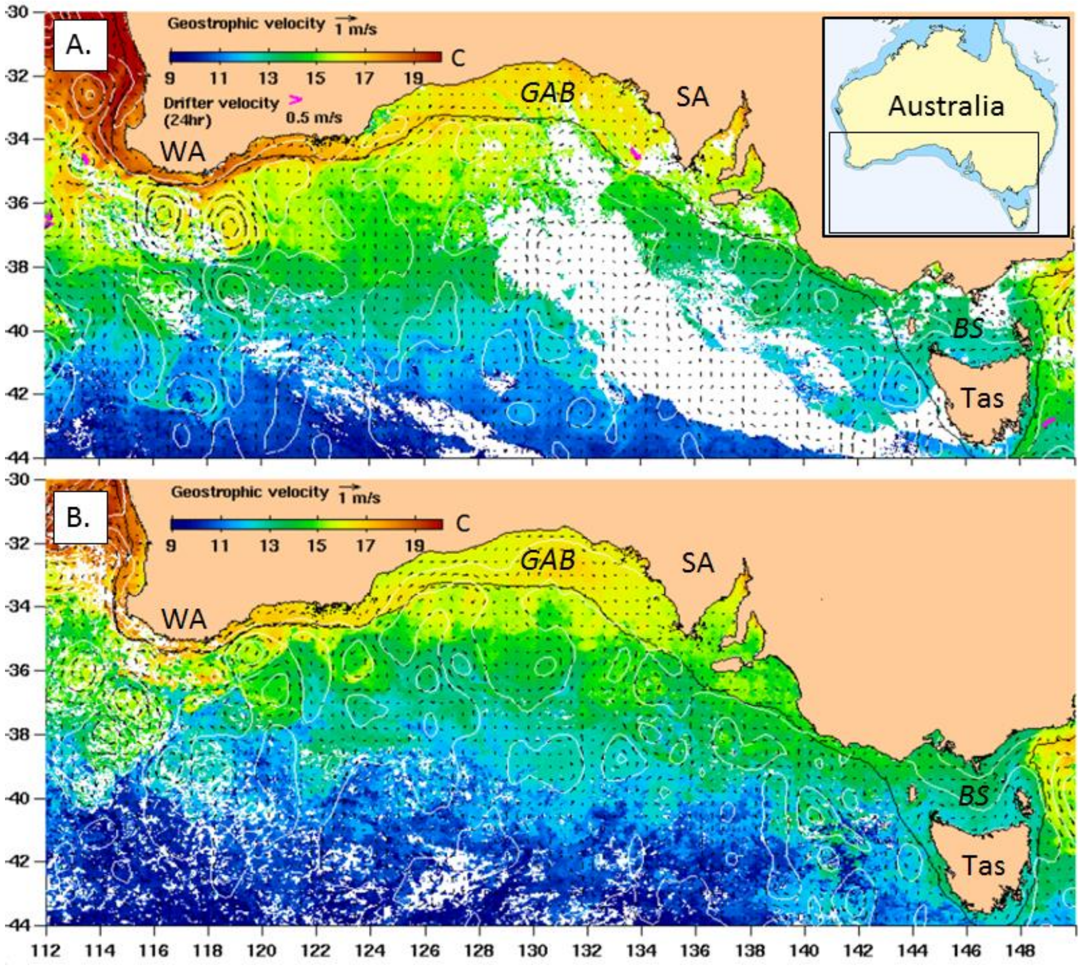

Fig. S5.1. 3-day composite sea surface temperature during our offshore tagging period (9th October) for both tagging years: 2015 (A) and 2017 (B). Intrusion of the Leeuwin current wrapping around the bottom of Western Australia (WA) into the Great Australian Bight (GAB), South Australia (SA), was weak and similar at this time in both years. BS = Bass Strait. Tas = Tasmania. Inset = map area (boxed) relative to Australia (IMOS, 2018).

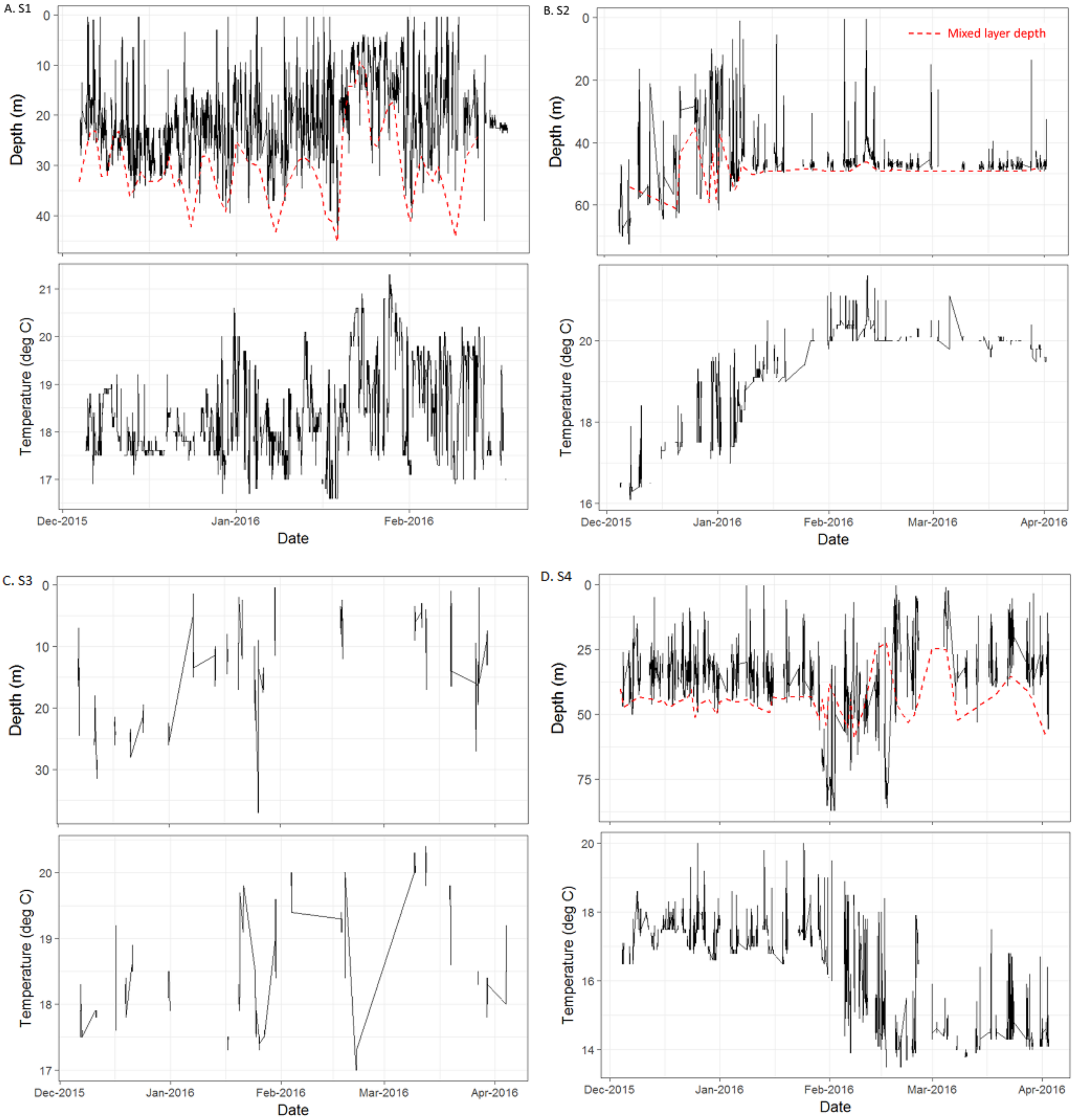

Fig. S5.2. Time series of depth (upper panels) and temperature (lower panels) for pregnant *G. galeus* tagged in southern Australia in December 2015 (A–F) and October 2017 (G–K). Interpolated depth of the thermal mixed layer is given on depth panels (red dashed line). Axes differ among panels. Continued next page.

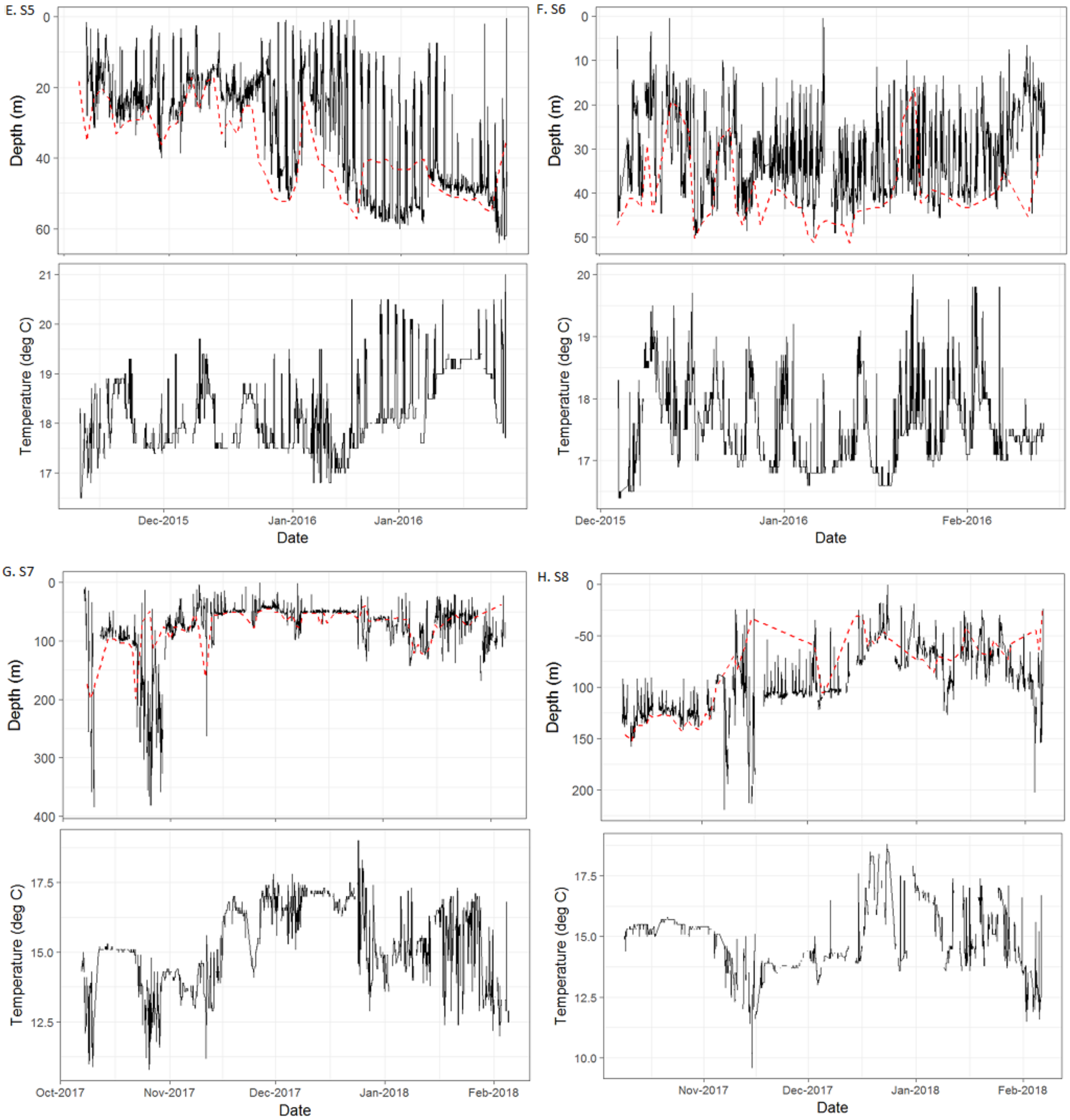

Fig. S5.2. cont. Time series of depth (upper panels) and temperature (lower panels) for pregnant *G. galeus* tagged in southern Australia in December 2015 (A–F) and October 2017 (G–K). Interpolated depth of the thermal mixed layer is given on depth panels (red dashed line). Axes differ among panels. Continued next page.

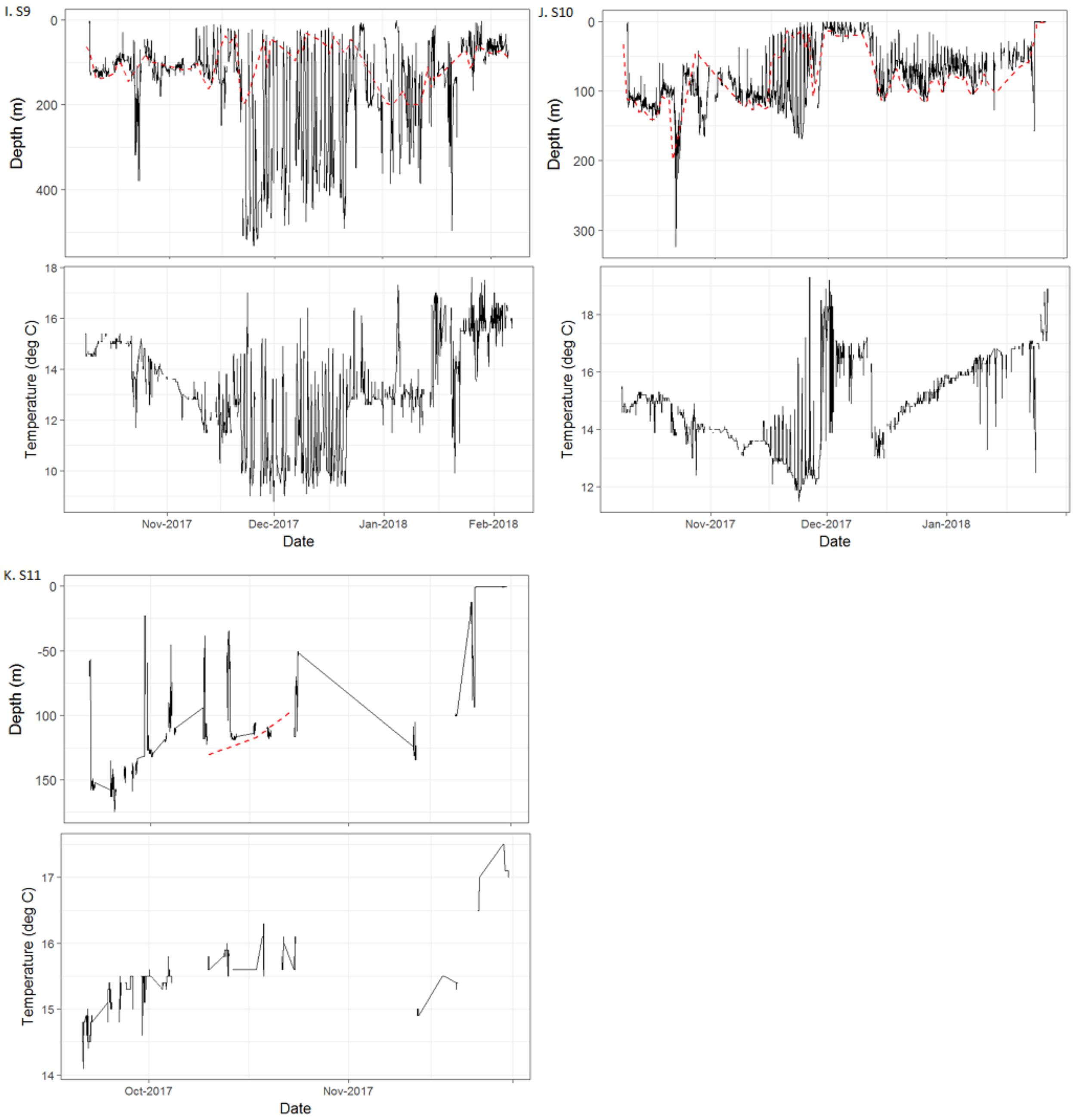

Fig. S5.2. cont. Time series of depth (upper panels) and temperature (lower panels) for pregnant *G. galeus* tagged in southern Australia in December 2015 (A–F) and October 2017 (G–K). Interpolated depth of the thermal mixed layer is given on depth panels (red dashed line). Axes differ among panels.

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CHAPTER 6

General discussion

Returning to port at Fowlers Bay near the head of the Great Australian Bight, South Australia.

General discussion

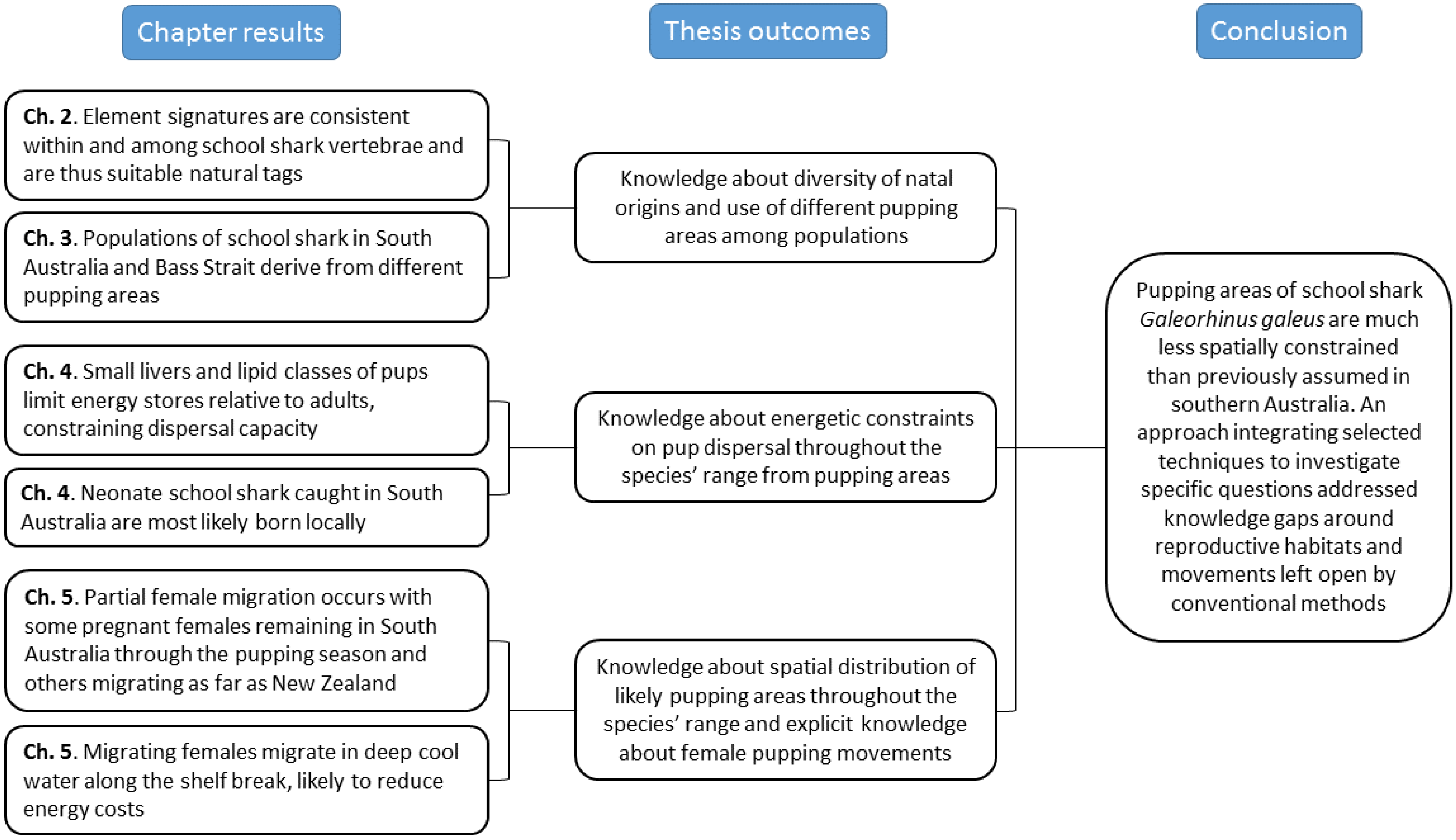
Knowledge about shark pupping habitats and origins of recruitment into adult stocks can inform management policies, e.g. area protections (Williams & Schaap 1992, Bonfil 1999, Speed et al. 2010). Conventional methods such as mark-recapture programs and genetic investigations may establish connectivity among populations, but may not answer key questions about the spatial distribution of pupping habitats, timings/routes of movements to them, and variability in origins of different populations. An integrated approach combining novel techniques with traditional ones applied in innovative ways is therefore required. Throughout this thesis, I employed several techniques including novel methods and applications to provide new data describing the diversity of population-level origins and the spatial distribution of pupping areas of the overfished school shark *Galeorhinus galeus* in southern Australia. Specifically:

(i) Populations of school shark in South Australia and Bass Strait derive primarily from different pupping areas rather than from commonly used areas, at least in some years. This was shown using natural vertebral chemistry tags stored since birth, i.e. element signatures from water and diet in pupping areas (Chapters 2 and 3) (Fig. 6.1);

(ii) By combining bioenergetic approaches, I was able to assess constraints on pup dispersal from pupping areas (Chapter 4). These constraints strongly suggest neonate school shark caught in South Australia are likely born locally rather than migrants from distant pupping areas as assumed (Fig. 6.1); and

(iii) Pop-up satellite archival tags tracked movements of late-term pregnant females (Chapter 5). This revealed pupping habitats are far less spatially constrained than assumed, likely stretching from the Great Australian Bight to New Zealand rather than being concentrated in Bass Strait and eastern Tasmania (Fig. 6.1).

In this concluding chapter, I discuss the main findings, future study directions, and conservation implications arising from my research.



Ch. 2. Element signatures are consistent within and among school shark vertebrae and are thus suitable natural tags

Ch. 3. Populations of school shark in South Australia and Bass Strait derive from different pupping areas

Knowledge about diversity of natal origins and use of different pupping areas among populations

Ch. 4. Small livers and lipid classes of pups limit energy stores relative to adults, constraining dispersal capacity

Ch. 4. Neonate school shark caught in South Australia are most likely born locally

Knowledge about energetic constraints on pup dispersal throughout the species' range from pupping areas

Ch. 5. Partial female migration occurs with some pregnant females remaining in South Australia through the pupping season and others migrating as far as New Zealand

Ch. 5. Migrating females migrate in deep cool water along the shelf break, likely to reduce energy costs

Knowledge about spatial distribution of likely pupping areas throughout the species' range and explicit knowledge about female pupping movements

Pupping areas of school shark *Galeorhinus galeus* are much less spatially constrained than previously assumed in southern Australia. An approach integrating selected techniques to investigate specific questions addressed knowledge gaps around reproductive habitats and movements left open by conventional methods

Fig. 6.1. Schematic representation of the chapter results, thesis outcomes, and conclusion of this thesis.

Microchemistry to distinguish origins of shark populations

The potential for natural tags in the form of trace element signatures to trace sharks back to their natal areas has been suggested since the 1990s (Stevens & West 1997) and used in practice more recently (Tillett et al. 2011, Lewis et al. 2016, Smith et al. 2016). However, such an approach requires knowledge of natal areas to relate vertebral element signatures of sharks in wider populations back to neonate signatures of known origin (i.e. sampled *in situ*). I used this technique in a novel way to address a specific question: can I discern if school shark throughout southern Australia derive from common pupping areas when most pupping occurs in unknown areas (Prince 1996, Stevens & West 1997) and populations are likely mixed due to wide-ranging movements by some individuals (Olsen 1984, Brown et al. 2000)? In this context, it was not my aim to link individuals back to known pupping areas and determine specific natal origins, since these origins remain mostly unknown. Rather, my aim was to assess whether population-level natal origins at the broad scale were similar among two major regions of the school shark fishery in Australia: South Australia and Bass Strait. That is, do sharks in both regions derive from common pupping areas, as an assumption of obligate female migration and uniform population mixing would suggest? Although not concrete evidence in isolation, use of different pupping areas by these populations would be a prerequisite for pupping occurring in South Australia in addition to traditionally identified sites in Bass Strait and Tasmania.

Ground-truthing experiments using LA ICP-MS (laser ablation inductively coupled-mass spectrometry) showed element signatures in school shark vertebral centra were not affected by routine bleach preparation (<40 mins) and were consistent both among related time-resolved portions of the same vertebra and among adjacent vertebrae for a suite of trace elements including Ba, Sr, Mg, Mn, Li, and Pb (Chapter 2). Zinc was an unreliable marker, differing in concentration among vertebrae, probably due to its mode of incorporation into vertebrae (entrapment in interstitial spaces in the cartilagenous matrix rather than substitution for Ca: Tang et al. 2009). Vertebral uptake of Zn also varied with somatic growth in another elasmobranch, the round stingray *Urobatis halleri*, further suggesting Zn is not a reliable elemental marker (Smith et al. 2013). Sodium concentrations were affected by routine bleach preparation, as might be expected given bleach has Na as a component. For these reasons, Zn and Na were excluded from vertebral chemistry analyses.

Differences in post-natal element signatures between South Australian and Bass Strait populations occurred in two out of three cohorts (1997 and 1998) and were driven primarily by Mg and Mn (Chapter 3). Uptake of these elements has been found to be temperature-dependent in elasmobranchs (Smith et al. 2013), with Mg decreasing and Mn increasing with increasing temperature. The lower Mg and higher Mn of sharks caught in South Australia were thus consistent with water temperature there being typically warmer than Bass Strait during the summer pupping season. In the birth year of the cohort whose signatures were similar (1996), an atypically weak intrusion of the Leeuwin current drove cooler temperatures in South Australia more similar to those in Bass Strait than the other two years (Fig. S3.2) and may explain the similarity of signatures in this year, rather than use of common pupping areas. Despite some school shark pupping areas in Australia occurring in estuaries, vertebral Sr (a useful salinity tracer in sharks: Tillett et al. 2011) was similar between populations suggesting relatively homogeneous marine origins may predominate in both regions. This is consistent with use of marine pupping areas throughout *G. galeus* global range, including in New Zealand (Hurst et al. 2000), Argentina (Bovcon et al. 2018), California (Ripley 1946), and South Africa (Freer 1992).

There appeared to be some mixing between populations, as would be expected in a species known to undertake large-scale movements (Olsen 1954, Walker et al. 2008). However, region of capture could be predicted for 75% of sharks based on post-natal signatures. This was consistent with mark-recapture studies in New Zealand where 76% of individuals moved <500 km (Hurst et al. 1999) and in Australia where most individuals of the size classes analysed here (<140 cm TL) also moved <500 km (Brown et al. 2000). Vertebral chemistry analysis of post-natal element signatures thus provided a useful tool to determine use of different pupping areas by populations at the broad spatial scale investigated here.

Energetic constraints on shark pup dispersal

Bioenergetic analyses have been used to investigate swimming performance (Graham et al. 1990, Lowe 1996, Whitney et al. 2016) and energy budgets (Gruber 1982, Lowe 2002, Sims et al. 2006) in sharks. I integrated these techniques to answer a specific question: could neonate school shark caught in South Australia feasibly be migrants from distant recorded pupping areas in Bass Strait and Tasmania rather than evidence for local pupping areas, as has been asserted (Prince 1996, Riseley pers. comm.). In addition, this study provided a unique opportunity to investigate physiological constraints on shark pup dispersal more generally. Dispersal capacity must theoretically be constrained either by swimming speed or energy stores. Both of these parameters may in turn be influenced by environmental conditions, e.g. currents may hinder or assist dispersal (Brodersen et al. 2008, Chapman et al. 2011) or ambient temperatures may affect metabolic costs (Carlson & Parsons 1999, Miklos et al. 2003).

High costs of growth and low energy reserves in pups relative to adults suggested a trade-off prioritising growth over energy storage. Newborn shark pups are at their most vulnerable life stage and growth is imperative to reduce predation risks and increase competitive advantages to maximise chances of survival (Morrissey & Gruber 1993, Heupel et al. 2007). Energetic constraints including small livers (where most energy is stored in sharks: Sargent et al. 1972, Zammit & Newsholme 1979), small amounts of lipids (that transport energy to muscles), and high concentrations of growth related structural lipids at the expense of energy storage lipids likely constrain pup dispersal capacity. These constraints may explain why field observed dispersal speeds (<3.5 km day^{-1}) are much slower than the theoretical speed (~41 km day^{-1}) that assumes continuous swimming at optimal swimming speed for pups (0.6 m s^{-1}) and accounts for mean current flow on the coastal dispersal route. This disparity suggests pup dispersal is punctuated by bouts of foraging and resting. Behavioural mediation may also slow dispersal; acoustic tracking shows young school shark are most active at night, tending to seek refuge during daytime possibly to avoid predation risks (Barnett & Semmens 2012). After birth in the austral summer, there were clear energetic benefits for pups to delay dispersal until water temperatures on the coastal dispersal route fell in late autumn and winter, nearly halving costs of dispersal despite increased growth. This was consistent with timing of school shark dispersal from known pupping areas that occurs during austral autumn and winter (Olsen 1954, McAllister et al. 2015). Timing and rate of dispersal thus provided strong evidence that neonate school shark present in South Australia in summer are born locally, rather than migrants from distant pupping areas in Bass Strait or Tasmania.

Satellite archival tagging of pregnant school shark

With mounting evidence in support of school shark pupping occurring in South Australia, conclusive evidence was sought by tracking movements of late-term pregnant females. Pop-up satellite archival tags (PSATs) were used given the bentho-pelagic behaviour of school shark. These tags have provided information suggesting reproductive movements in other sharks, e.g. porbeagle shark, *Lamna nasus* (Campana et al. 2010), oceanic whitetip, *Carcharhinus longimanus* (Howey-Jordan et al. 2013), and blue shark, *Prionace glauca* (Vandeperre et al. 2014). Unlike these studies, I targeted only late-term pregnant females, rather than incidentally tagging mature females among other age classes and sexes. Post-deployment tag retention in this study was longer (mean deployment: 84 days) than other studies using PSATs on school shark, that had mean deployments of 13 days (Cuevas et al. 2014) and 24 days (Rogers et al. 2017) respectively.

My results revealed partial female migration in school shark with some females remaining close to tagging sites in South Australia over the summer pupping season and some migrating long distances as far as New Zealand (Chapter 5). These results were consistent with anecdotal- (Prince 1996) and recent scientific evidence (Rogers et al. 2017, McMillan et al. 2018) of pupping occurring in South Australia. These results were also consistent with established demographic- (Hurst et al. 1999) and genetic connectivity (Hernández et al. 2015, Bester-van der Merwe et al. 2017) between Australia and New Zealand, where school shark pups are widely found in coastal areas (Hurst et al. 2000). Pupping habitats thus occur over a much wider area of the species' range than previously assumed. This evidence of late-term pregnant school shark remaining in South Australia over the pupping season clarified a long-running debate about school shark movements and habitat use. In doing so, this study demonstrated the benefits of explicit movement data in answering questions previously unresolved by conventional mark-recapture and genetic studies. I also found that migrating females moved along direct routes in cool, deep waters near the shelf break, rather than circuitous routes along coastal beaches where shark fishing closures exist to protect them. Finally, I proposed that bioenergetics drive female school shark behaviour; aggregating in warm waters in South Australia over winter to promote growth and gestation with migrants then using cool-water migration pathways to minimise energetic costs of migration to pupping areas.

A note on school shark pupping areas in South Australia

Prior to this study, there were no scientific records of neonate school shark in South Australia even after seven decades of research on the species in Australia since the 1940s. The three and a half years of this study have led to publication of two such recordings (Rogers et al.

2017, McMillan et al. 2018). Previously, a lack of awareness about the significance of school shark pupping areas prevailed. Many fishers were not aware that school shark were assumed to pup elsewhere. Additionally, there were few studies that searched for evidence of pupping areas in South Australia. Both of these shortcomings are in spite of ongoing recognition of the importance of such knowledge, particularly since the school shark stock collapsed from overfishing in the 1990s (Prince 1996, Stevens & West 1997), which was reiterated in the Commonwealth school shark stock rebuilding strategy of 2008 (AFMA 2009).

The scientific community has relied almost entirely on the work of Olsen (1954) in this regard, with no significant pupping areas recorded since the 1950s. Since Olsen's initial efforts working closely with fishers, studies have either resampled his pupping areas or surveyed sites in the same general area of Bass Strait and Tasmania (Prince 1996, Stevens & West 1997). Surveying of potential pupping areas in South Australia has been limited in sample size and the temporal and spatial extent may not have focused on the appropriate areas. In the 1980s, 15 fine-mesh net sets were made in South Australia compared to 98 in Bass Strait and Tasmania, however all sets in South Australia were in April–October and mostly at depths >30 m, when pupping occurs in November–January mostly at depths <30 m (data reported in: Prince 1996). In the 1990s, 7 net sets were made opportunistically in South Australia by a volunteer fisher provided with a net compared to >1,300 scientifically observed and conducted net sets and longline shots in Bass Strait and Tasmania (Stevens & West 1997). In 2007–2008 during industry-based fixed-station surveys, gillnets of 10 cm mesh size were deployed in South Australia that were likely suitable to catch pups, however site selection and sampling times were designed to survey commercial fishing grounds and catches of small school shark were rare throughout all of southern Australia (Braccini et al. 2009). These combined sampling efforts also failed to record pups of the co-distributed gummy shark *Mustelus antarcticus* in South Australia, despite their widespread occurrence there (Lenanton et al. 1990, Baker 2015), again suggesting the limited number of replicates may have been an issue.

There has been increasing recognition that cooperation and consultation at the interface between science and stakeholders can improve ecological management outcomes, in particular through integrating local- and scientific knowledge (Gilchrist et al. 2005, Lundquist & Granek 2005, Reed 2008). In the 1950s, Olsen relied on advice from experienced local fishers to locate school shark pupping areas in Bass Strait and Tasmania, however the shark fishery had only recently expanded into South Australia and Olsen could not find fishers with local knowledge of pupping areas (Olsen 1954, Prince 1996). During a workshop between scientists and stakeholders to discuss school shark pupping areas in 1994 (Prince 1996), fishers provided anecdotal evidence of pupping occurring in South Australia including:

- Runs of late-term pregnant females at sheltered inshore locations that fishers targeted reliably during the summer pupping season and considered to be recurrently used pupping areas;
- The fouling of nets with birth sacs after females had left these areas, which fishers related to having narrowly missed pupping females by a matter of hours or days; and
- Captures of newborn school shark.

Follow-up sampling was not undertaken to investigate these claims. Rigorous scientific sampling would improve the state of knowledge around school shark pupping areas in Australia and be of aid to managers. The necessary structured sampling effort was beyond the resources of the present study.

During the present study I sought further advice from retired and active fishers, both commercial and recreational, for information about school shark pups in South Australian waters. These included professional shark fishers, charter operators, prawn trawlers, as well as boat- and shore-based line fishers. Numerous anecdotal reports of school shark pups <45 cm were obtained (Fig. 6.2). However, in the current climate where marine parks and fisheries closures have recently been implemented it was difficult to elicit cooperation, since many interviewees were reluctant to share information they suggested could be used to instigate area closures. To effectively combine local- and scientific knowledge, stakeholder involvement should be fostered by a philosophy of trust and equity among all parties (Failing et al. 2007, Reed 2008). In the absence of a program of dedicated fisheries-independent coastal research trawls as are conducted e.g. in New Zealand and Norway, local knowledge will likely be a necessary component of locating pupping areas. Going forward, it will therefore be important for researchers to build trust and confidence with stakeholders if they wish to access local knowledge of the type on which Olsen depended to locate school shark pupping areas.

I conducted limited survey trials of potential pupping areas in 2017 and 2018 using baited remote underwater video (BRUVs) and longlines. Due to vessel restrictions, I initially targeted semi-enclosed embayments in Venus Bay and inner Coffin Bay on the west coast of South Australia; physically similar habitats to pupping areas in Bass Strait and Tasmania and home to pupping areas of co-occurring gummy shark. However, I found no evidence of school shark in these systems, despite presence of gummy shark. High summer temperatures in these areas may be a factor and may also explain why school shark seldom occur in the South Australian gulfs. Elevated salinity caused by high evaporation in these inverse estuaries does not appear likely to limit use of these areas, since school shark are more physiologically tolerant to hypersalinity than gummy shark (Tunnah et al. 2016) that occur in these areas (Baker 2015).

Based on the advice of local fishers, I propose the following sites as candidates for targeted surveys to locate potential school shark pupping areas in South Australia: the Head of the Bight west to Bunda Cliffs, St Mary Bay, Cape Radstock–Anxious Bay, the ‘sole’ of Yorke Peninsula, and the Coorong coast, all of which have supported multiple independent and corroborating reports of neonate captures (Fig. 6.2).

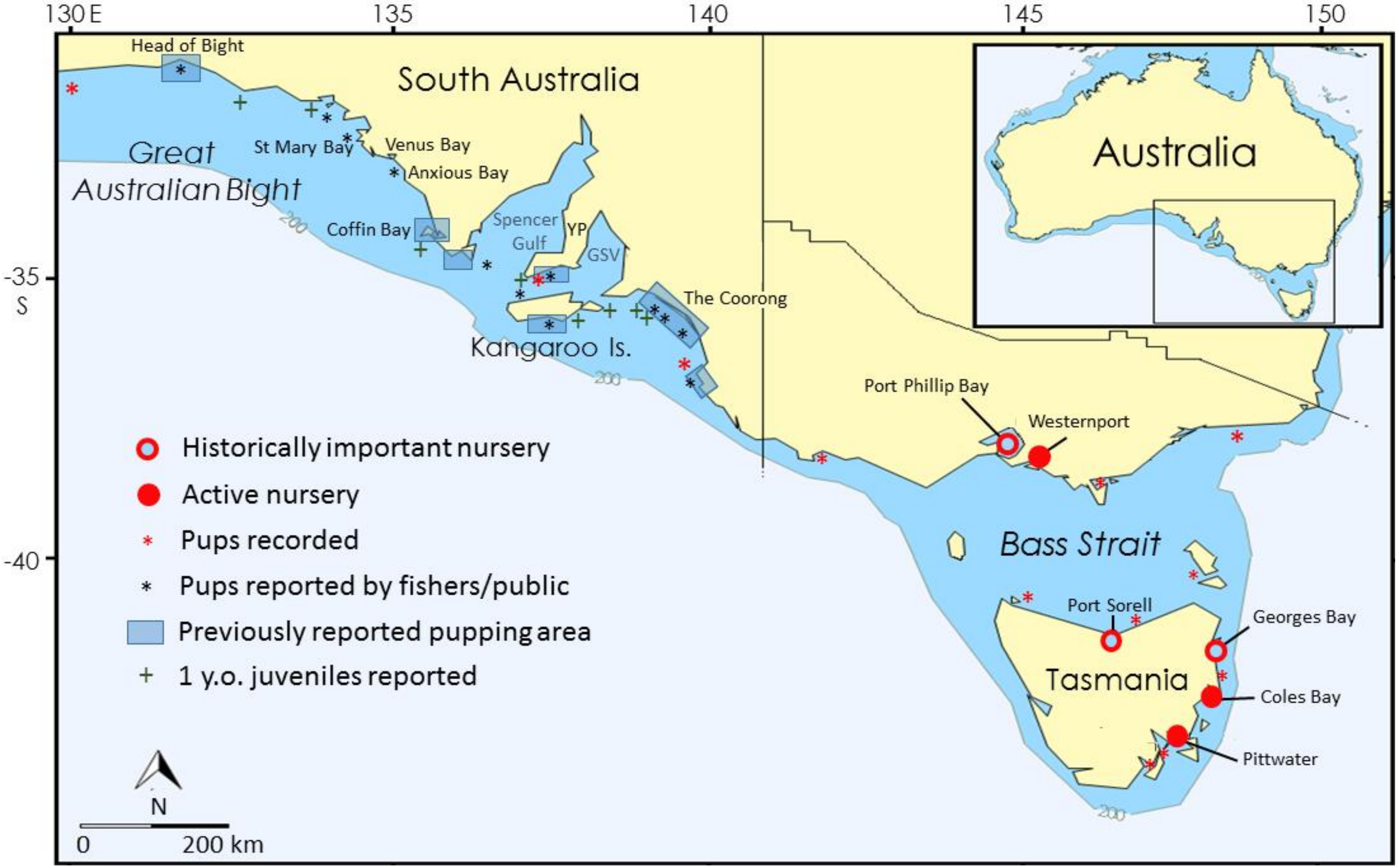


Fig. 6.2. Sites of school shark pupping areas and pup captures (<45 cm total length) in southern Australia. Previously reported pupping areas in South Australia (shaded boxes) were nominated by fishers at the school shark pupping workshop in 1994 (Prince 1996). Red asterisks denote scientifically recorded pup captures. Black asterisks denote sites reported by fishers to the author during the present study where pups have been caught in South Australia. YP = Yorke Peninsula. GSV = Gulf St Vincent. Inset shows map area (boxed) relative to Australia.

Future directions for research

The program of annual survey trawls conducted in New Zealand has led to documentation and mapping of the occurrence of school shark pups (Hurst et al. 2000) that far exceeds the knowledge of pupping areas in Australia. Such a program would be of obvious benefit in Australia, not just in relation to school shark, but to map important recruitment sources for many species. In the absence of such a program, targeted surveys of potential pupping areas is advisable to locate, protect, and monitor important sources of recruitment to the commercially exploited stock. The experience gained in the present study suggests efficient allocation of resources to such surveys could benefit from the following:

- Fine-mesh gillnets (e.g. 5 cm mesh size: Stevens & West 1997) are preferable to longlines in the coastal areas of South Australia due to the high abundance of organisms, e.g. crabs, that strip baited hooks rapidly and thoroughly. Gillnets may increase incidental bycatch (Stevens & West 1997), however mortality could be mitigated by short set times to regularly check for and release bycatch.
- While stereo BRUVs appeared an attractive option, their principal restriction may be that BRUVs are limited to daytime use, while sharks (particularly small ones) are typically more active at night. It was also sometimes difficult to identify small sharks in poor visibility using BRUVs.
- As previously stated (Prince 1996), depths at which pups were reported in the present study were typically at 10–30 m and not more than 50 m.
- Effort should be concentrated in well-mixed coastal areas, rather than semi-enclosed systems with narrow entrances. In South Australia, high temperatures in such systems may make these areas unattractive habitats for school shark who appear to prefer coastal and marine habitats there.

The importance of traditionally identified pupping areas, i.e. their relative contribution to the school shark stock, remains unclear. The present study suggests pupping in coastal areas probably supports the bulk of the stock (Chapter 3) as has been previously suggested (Prince 1996, Stevens & West 1997). However, conditions in estuarine pupping areas may be particularly favourable to recruitment, leading to higher densities of pups than in marine pupping areas. It would be useful to study specific natal origins of school shark to assess relative contributions of recorded pupping areas and establish their importance. Vertebral chemistry analysis could achieve this by sampling element signatures of pups of known origin, i.e. sampled *in situ* in known pupping areas, to determine baseline post-natal signatures. If baseline signatures differ among sites, school shark from the wider population would be sampled in following years, e.g. from commercial catches throughout the fishery. Post-natal signatures of sharks of appropriate age, i.e. born in the year baseline signatures were obtained, could then be compared to baseline signatures to determine relative contributions of pupping areas and any apparent patterns in spatial distribution of recruits from these areas throughout the stock.

In terms of conservation and management, it would also be of merit to determine whether migrations in school shark are based on philopatry, i.e. natal homing in females. If philopatry occurs, sub-populations depending on recruitment from females using particular migratory routes or pupping areas may be more at risk from fishing pressure or habitat degradation than others. If philopatry does not occur, migratory patterns and behaviour may be less predictable and vary over time. Philopatry could be investigated by targeting mature females adjacent to known pupping areas during the pupping season with long-life acoustic transmitters. Pupping areas such as Pittwater and Port Phillip Bay are already equipped with acoustic receiver arrays that would facilitate this work. Since females would be expected to return every 2–3 years if natal homing occurs, transmitters with a battery life covering 2 or 3 such cycles should be sufficient to elucidate evidence for philopatric behaviour. A number of such females could also be double-tagged with PSATs to determine timing and routes of female return movements to aggregating areas (e.g. South Australia) after pupping.

Conservation implications

The recovery of marine predator populations that are susceptible to fishing pressure has been suggested to depend on a combination of reductions in fishing mortality, reduction of bycatch, and protection of critical life history stages (Myers & Worm 2005, Speed et al. 2010). In the case of school shark in Australian waters, measures have been taken to reduce targeted fishing pressure (e.g. catch quotas) and protect some life history stages (e.g. fishing closures in pupping areas). However, further measures may be available as tools to promote recovery, e.g. reducing bycatch and protecting reproductive age classes.

While focus has been given to protecting neonate and juvenile school shark in pupping areas (Williams & Schaap 1992, Stevens & West 1997), protection of other life history stages is also important to facilitate population stability or recovery (Brewster-Geisz & Miller 2000, Kinney & Simpfendorfer 2009). School shark recovery depends fundamentally on the success of reproductive movements and tasks by mature females that produce future generations. This study has shown that female reproductive movements are less uniform than previously assumed. Serial depletion of the school shark stock that has occurred in different areas and times (Olsen 1959, Prince 1996) is likely explained by partial migration, with contributions to sub-populations from different natal areas as described in this study. There is anecdotal evidence that school shark are presently more abundant in western South Australia than in Bass Strait (T. Willis pers. comm.). This may be because the movement of migratory females from Bass Strait and Tasmania to aggregating areas in South Australia and back to their pupping areas exposes them to a gauntlet of fishing pressure throughout the entire range of these movements, whereas females remaining resident in South Australia are subjected to less fishing effort (Fig. 5.5) and are thus more likely to complete reproductive tasks. Dynamic spatial management that employs shifting temporary closures is an effective tool to reduce longline bycatch while minimising costs to fisheries (Hobday & Hartmann 2006, Grantham et al. 2008). In the present context, seasonal closures along female migration pathways, e.g. along the shelf break between Kangaroo Island and Bass Strait in October–November, may assist female reproductive movements and recovery.

The removal of mature females can be particularly detrimental to shark populations due to internal fertilisation limiting fecundity (Ford 1921, Mucientes et al. 2009). Unfortunately, the habits of mature female school shark leave them particularly exposed to fishing pressure, e.g. segregating from males and aggregating inshore in large schools or undertaking predictable movements. This is of particular relevance in South Australia, where mature females from across the species' range gather seasonally (Olsen 1954, Brown et al. 2000). They are also an attractive target for fishers, since their large size and catchability returns high catches for effort. At least 2 out of 14 mature females tagged during this brief study were caught by fishers. The change to longline gear from gillnets in the South Australian fishery to limit interactions with marine mammals (Steer et al. 2018) also disproportionately imperils mature female sharks. Longlines of baited hooks are inherently non-selective by age class in contrast to gillnets, whose mesh size can be regulated to target sharks of certain sizes. There is a strong argument that a size-selective fishery counter-intuitively targeting juvenile and sub-adult age classes while avoiding reproductive adults is the most sustainable model for fishing sharks like school shark with *K*-selected life history traits and selective habitat use (Simpfendorfer 1999, Prince 2005, McAuley et al. 2007). Development of effective measures to ward marine mammals away from gillnets thus allowing the introduction of a size-selective fishery in South Australia could therefore greatly benefit school shark recovery by alleviating catches of mature females. In the meantime, while longlines continue to be used, there is strong evidence that bait selection can minimise shark bycatch in longline fisheries (Erickson & Berkeley 2008, Coelho et al. 2012). Given that school shark diet comprises almost entirely teleosts and cephalopods, while the fishery target species gummy shark prey heavily on crustaceans, use of crustacean baits or processed baits derived from crustaceans, rather than teleost bait as presently used, could reduce incidental bycatch of school shark.

Conclusions

Given the evidence accumulated herein, I suggest that school shark reproductive behaviour is driven by biological and bioenergetic imperatives that do not fit the previously assumed model of obligate female migrations and uniform movements across the Australian stock. I propose:

- Movement of female school shark from across their range into warmer waters (e.g. South Australia) during the winter gestation period helps maximise growth and development of themselves and their offspring. Dispersal in spring to their respective pupping areas uses direct, cool-water migration pathways to lower energetic costs of migration, rather than routes meandering along the coast.
- Pupping areas occur throughout the range of the species, stretching from the Great Australian Bight to New Zealand taking advantage of suitable habitats, and are not restricted to estuaries and semi-enclosed embayments; coastal bays, beaches, reefs, and islands are likely used as pupping areas in South Australia.
- Pupping in late spring and summer when water temperatures are high helps promote rapid growth and development of neonates. Dispersal from pupping areas in late autumn and winter capitalises on falling water temperatures on dispersal routes to lower the energetic costs of migration into the wider school shark population.

- Although large-scale movements are not uncommon and mixing among populations occurs, this appears to be the exception rather than the rule in immature age classes. That is, there is a minority of dispersive individuals who have migrated from other areas and a majority of individuals with similar origins.

Thus, school shark pupping is less spatially constrained than previously assumed and neonates caught in South Australia should be accepted as evidence for pupping there. Female reproductive movements and habitat use are correspondingly less uniform than assumed, with partial female migration and pupping occurring from the Great Australian Bight to New Zealand. The integrated approach used here to assess spatial distribution of pupping areas and uniformity of origins among populations has addressed knowledge gaps left open by the conventional methods previously employed.

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