Fishery Demographics, Biology and Habitat Use of Hairtail *(Trichiurus lepturus)* in South-eastern Australia

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B. Sc
Master of Philosophy (Science) Thesis
School of Science and Health
Western Sydney University
2020

Preface

The contents contained within this thesis are my own work with guidance from Dr Sandra Diamond (Western Sydney University), Dr John Stewart (Fisheries NSW), Dr Ashley Fowler (Fisheries NSW) and Professor Basant Maheshwari (Western Sydney University). The design of the research presented was personally conceptualised with the guidance of my supervisors.

This thesis contains 5 chapters. Chapter 1 is a general introduction to the thesis and chapter 5 is a general discussion of the thesis. Chapters 2, 3 and 4 were prepared as stand-alone journal manuscripts (unpublished). For this reason, there will be some repetition in the content. To prevent unnecessary duplication a single reference list will be provided.

This Thesis is dedicated to my life partner Anne-Marie Hegarty for her endless love and support.

Acknowledgments

I would like to thank the Recreational Fishing trust for providing funding for the otolith chemistry component of this project.

I thank Donna and Stewart from the online fishing forum Fish Raider They were enthusiastic to help facilitate my valuable data collection from their hairtail fishing competition as well as providing me with the length data from previous years and enabling my communication with a community of recreational fishers.

I would like to thank NSW Department of Fisheries compliance officers, Ben Audet and Travis Morgan for answering approximately 1000 questions about recreational fishing for hairtail. Volunteering their time to provide the additional data and their knowledge and insights added a valuable component to the thesis.

I thank Fiona Richards, Amanda Bielanowicz and Michele Biles from HMRI for processing histology samples.

I would like to thank my supervisors from the bottom of my heart for their proactive guidance, support and contributions to this thesis.

My primary supervisor Sandra Diamond introduced me to the field of resource sustainability and fisheries science. Sandy encouraged my interest in the field with enthusiasm and passion for the subject. Sandra regularly ensured I was focused and on track with my research objectives and time management.

My supervisor John Stewart employed me as a technician with fisheries NSW, which was instrumental in my personal development in the field of fisheries research. John went above and beyond in his supervisory role of my project, investing bulk time and energy to coach me through all aspects of my technical and academic development, He has been a consistent support, each and every week.

Acknowledgments

Despite his official addition to my supervisory panel being much later than it should have been, my supervisor Ashley Fowler has been a consistent support from beginning to end on all aspects this project. Ash offered countless hours to provide clear and concise direction and assistance on literature reviews, experimental designs, technical work, data collection, data analysis and writing. Ash would make time to help with this project at the drop of a hat and was sure to read all documents I submitted to him thoroughly and promptly provide very helpful feedback and suggestions.

Basant Maheshwari has been a great help in guiding the final stages of my thesis and assistance with university protocol and communication.

I would like to thank the research technicians at NSW Fisheries for collecting Length frequency data and biological samples from fish Coops (often in the middle of the night). I would also like to thank all of my colleagues at NSW DPI Fisheries, for an overall fun, friendly and encouraging workplace. In particular Anne-Marie Hegarty for regularly checking in on me and sharing lots of laughs and banana muffins with me, Caitlin Poppy Young for being the sunshine to my storm, Julian Hughes for his academic support and for using his great humour to lift not just my spirits but the spirits of everyone else around him. Also Thank you to Anne-Marie, Caitlin and Julian for providing me with helpful material from their own research and always being available to offer their expertise, support and encouragement. Antony Gould for hours of technical training and contributions, Holly Gunton for academic comradery, support and advice, Cameron Doak for looking out for me, Veronica Silberschneider for her empowerment, Geoff Liggins, Marcus Miller, Giles Ballinger, Amy Smoothey and Vic Peddemors for being legends and Alice Pidd and Nick Meadows for being two of the greats.

I would lastly like to thank my Mum Caroline Boyd for her huge heart, warm hugs and generosity, my Dad Jean-Francois Clain for his inspiring perseverance and his encouragement by always telling me I have the best job ever and my brother Patrick Clain for being my best friend, for putting a smile on my face every single time I speak to him and for being my little wise and calming Buddha.

Statement of Authentication

Author: Chantelle Monique Clain
Degree: Master of Philosophy (Science)
Date: 15/04/2019

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

[redacted]

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Abbreviations

Ba	Barium
BW	Body weight
Ca	Calcium
DPI	Department of Primary Industries
EAC	East Australian Current
FAO	Food and Agriculture Organization
LA-ICP-MS	Laser ablation - inductively coupled plasma - mass spectrometer
Mg	Magnesium
Mn	Manganese
NRFS	National Recreational and Indigenous Fishing
NSW	New South Wales
OW	Otolith weight
PL	Pre-anal length
Sr	Strontium
TAC	Total allowable catch
TL	Total length
UN	United Nations

Abstract

Largehead hairtail *(Trichiurus lepturus)* is an important part of the global fisheries catch, the species is consistently placed in the top ten marine species landed worldwide, but there is a lack of understanding regarding population demography, productivity, and vulnerability of *T. lepturus* in New South Wales (NSW). In this thesis, the spatial and temporal dynamics of the fisheries yield and the length composition of the local commercial and recreational fisheries for *T. lepturus* were investigated. Fisheries were characterised by considerable inter-annual variation. The size range of observed fish was 13–78 cm pre-anal length (PL). The length composition varied spatially and temporally and was influenced by gear selectivity. Difficulties in sampling a sporadically occurring fishery such as the *T. lepturus* recreational fishery in NSW may cause significant underestimates of the contribution of these fisheries to the total catch during some years. The population demographics of *T. lepturus*, including the maximum length, longevity, length at maturity, spawning period, sex ratios, length-weight relationship and growth were also investigated. There were significant differences in the length distributions between sexes and the largest individuals in the collection, (56–78 cm PL) were all female. The lengths at which 50% of females and males reached sexual maturity (L_{50}) were 40.15 cm PL and 40.65 cm PL respectively. The mean gonado-somatic index (GSI) for females peaked in June at 1.9% and elevated GSIs suggested a spawning period from approximately June to September, the Austral winter/early spring. Male GSIs and macroscopic maturity staging displayed similar patterns, in support of the estimated spawning period. Females dominated the sample collections, with a F:M sex ratio of 1: 0.4 and this pattern was consistent during both the spawning and non-spawning period. Females were also more prevalent than males in both estuarine and coastal habitats. There was significant variation in the sex ratios among catch events. The life history traits of *T. lepturus*, including a larger maximum body length, longer lifespan, larger body size at maturity, slower individual growth rate and shorter spawning period all indicated that the population of *T. lepturus* in south-eastern Australia could be less productive than populations in other global regions. Lastly, an evaluation was made of the utility of otolith microchemistry to investigate habitat use and movements of *T. lepturus* between estuarine and coastal habitats. Ratios of Sr, Ba, Mg, and Mn to Ca at the otolith edge were analysed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Sr and Ba displayed significant positive relationships with ontogeny (using body length as a proxy), while Mn showed a significant negative relationship. Concentrations of Mg and Mn but not Sr or Ba differed significantly among months, but no inter-annual variability was detected in any of the four elements. There were no significant differences found in elements between the coastal and estuarine habitats, although there were significant differences between the two estuaries located ~83 km apart. Core-to-edge transects showed a pattern of substantial Ba elevation before the formation of the first annual increment in 21 out of 30 individuals. In summary, this project has revealed that population productivity in south-eastern Australia is lower than populations in other global regions, and connectivity with distant populations may be low, meaning the *T. lepturus* population in south-east Australia is vulnerable to increasing natural and anthropogenic pressures. The results indicate the need for ongoing monitoring and further investigation into *T. lepturus* population demographics in south-eastern Australia.

Chapter 1: General Introduction

1.1 Increase in anthropogenic pressures on fish populations

The rapid increase in human populations has contributed to an increase in the detrimental impacts on global fish populations, including climate change, habitat degradation and an increase in fishing pressure (FAO, 2018). Climate change is predicted to have negative impacts on the overall primary production of the oceans, which is predicted to decline by 6% by 2100 on a global scale and 11% in the tropics (Kwiatkowski et al., 2017). Fish populations depend on primary production and consequently, climate change is impacting negatively on global fish biomass and biodiversity (FAO 2018). The impacts of climate change, through processes like ocean warming and acidification, vary geographically (FAO, 2018) and are causing changes in the global population distribution of fishes (Poloczanska et al., 2013). Populations are tending to shift poleward and to deeper water (Pinsky et al., 2013, Poloczanska et al., 2013). The impacts of climate change are predicted to result in a substantial decrease in biodiversity of marine species in the tropics, which will coincide with an increase in biodiversity in regions outside the tropics (Barange et al., 2014, Molinos et al., 2016). The global shift of species is highly dependent on how reliant a species is on specific habitats, they might rely on one habitat for their entire life history or for one aspect such as feeding, spawning or protection from predators (Brown et al., 2018).

The rapid increase in human populations is also causing an increase in habitat degradation due to a range of human activities, with cumulative impacts (Vasconcelos et al., 2007). Some examples of human activities that can contribute to the degradation of estuarine and coastal habitats are destructive fishing methods (bottom trawling) (Hiddink et al., 2006), and agricultural and industrial pollution entering coastal waterways from terrestrial run-off that cause detrimental blooms of algae (Le Luherne et al., 2016). These processes lead to ecological disruptions, with ultimately negative impacts on fish populations (Brown et al., 2018).

The rapid increase in the human population is also causing a major increase in the global demand for seafood (FAO 2018). In response to this increased demand, fishing technology has made rapid advancements, including larger and faster boats, improved fishing gear, improved methods for preserving catches on board, and improved sonars and tracking equipment for target species (Ding et al., 2017, Hughes, 2012). The improved capacity to track and catch target species is becomes a concern if overfishing is allowed to occur, resulting in unsustainable fisheries and unbalanced food webs within ecosystems (Scheffer et al., 2005) Global fishing pressure increased markedly from 1950, when worldwide catch was 16.8 million t from wild fisheries for all species excluding anchoveta (*Engraulis ringens*), to 1988, when worldwide catch reached 78 million t (FAO 2016). The yield remained steady from 2003-2009 before a further increase began in 2010. The increase was gradual each year up until 2014, when global catch peaked at 78.4 million t (FAO 2016). A global trend has been documented of a decline in the trophic level of the species targeted by fisheries. The mean trophic level has shown a gradual transition from long lived, high trophic level species, towards short lived, low trophic level species (Del Toro, 2001). Climate change, habitat degradation and fishing pressure all have cumulative and detrimental effects on fish populations, therefore it is becoming increasingly important to closely monitor and adequately manage populations to ensure their sustainability.

1.2 Population productivity

Population productivity relates to the vital rates of birth, growth and survival. If vital rates are high, then the population has a high level of turnover and a high production to biomass ratio (P/B) (Randall et al., 2013). Populations of exploited fish stocks with high productivity are generally less vulnerable to natural and anthropogenic pressure and more resilient to stressors and can typically be sustainably exploited at higher rates (Coulson et al., 2012). It would be insufficient to only assess the productivity of a population at one point in time because the vital rates of a population can be influenced by natural and anthropogenic pressure over time (Brander, 2013). In relation to fish populations, the vital rates directly reflect the life history traits of each population including longevity, length at maturity, spawning period, spawning strategies and length-weight relationships; therefore, the life history traits and strategies of a population ultimately contribute to its overall productivity (Randall et al., 2013).

Quantifying sex ratios and investigating the spatial and temporal patterns of these ratios can contribute to an understanding of life history traits and spawning strategies (Martins and Haimovici, 2000). Length-weight relationships can be used to quantify the ‘condition’ of individuals, based on the assumption that heavier fish of a particular length are in a better physiological condition (Chakravarty et al., 2012). Such condition factors can also be a useful index of individual growth; the heavier an individual is at a given length the more efficient the production of biomass is in the population. Therefore, the average condition of the individuals that make up a population can relate to population productivity (Tabassum et al., 2013). Individual growth rate is a vital rate that directly relates to population productivity but understanding how growth differs between sexes is also important. Depending on the species, comparing growth between the sexes can be important for ensuring one sex is not overfished relative to the other. Understanding how growth differs between sexes can also provide insights into the patterns of reproductive investment and feeding activity between sexes.

1.3 Fisheries assessment

An understanding of fishing pressure, life history traits, and strategies of exploited fish populations is necessary to inform the management strategies implemented for exploited stocks, ecosystems and critical habitat conservation (Hilborn and Walters, 1992, Randall et al., 2013). Without this information, management strategies for sustainable fishing and habitat conservation, such as total allowable catch (TAC) and species size limits cannot be accurately determined (Castilla and Defeo, 2005, Coulson et al., 2012). It is important to implement accurate and effective fisheries management for the sustainable harvest of fish (Hilborn and Walters, 1992) and local ecosystem and habitat conservation (Mapstone et al., 2004).

One of the most common research strategies implemented to gain an understanding of fishery characteristics and population life history traits of commercially exploited species is fishery dependent sampling using commercial fisher logbooks, and length frequency data, measured from commercial fishery catches. The strategy is useful because populations are often too large and hard to reach, or of insufficient financial value, to warrant a representative independent sampling program (Stewart et al., 2015). Hence, fishery-dependent sampling is implemented, which can provide relatively comprehensive insights into the life history traits of populations which are exploited by a large and fully developed fleet. Fishery dependent sampling is also useful for characterising the fishing pressure on a population, including how the fishery may influence the demography of a population. For example, if a recreational fishery focuses on large individuals, it can affect the spawning potential of the stock, or change the gene pool by selecting against fast-growing individuals or individuals with the potential to grow to large sizes. There are draw-backs to fishery dependent sampling, including the potential for fishery characteristics to bias conclusions on population life history traits (Hilborn and Walters, 1992).

It is difficult to attain comprehensive logbooks or length frequency data for recreational fisheries and therefore regional surveys are usually the first research strategies implemented to quantify the demographic traits of recreational fisheries. Generally, the surveys consider the broad recreational fishery, across all species, using techniques including: telephone-diary surveys, traffic and boat hire reports, access-point creel surveys; roving creel surveys; and telephone-diary surveys (Henry and Lyle, 2003, Steffe and Murphy, 2011, West et al., 2015).

1.4 Migration strategies and habitat use

Investigating the migration strategies and the habitat use of marine species is essential for understanding the productivity and resilience of marine populations, the maintenance of critical habitats (Crook et al., 2008, Milton et al., 2008), the protection of vulnerable life-stages from over-fishing, and for commercial and recreational fishery management (Fortunato et al., 2017, Walsh and Gillanders, 2018). In regards to critical habitats, if a population is dependent on a particular habitat, it is important to identify and protect these habitats from degradation to avoid population decline (Mercier et al., 2012). Marine fish species will often depend on both coastal and estuarine habitats. Estuaries are semi-enclosed bodies of water, where oceanic water and freshwater mix. Estuaries are typically characterised by a salinity gradient, decreasing with increasing distance from the coast, but are also characterised by salinity and temperature fluctuations. Tides, rainfall and fluvial flow rates all drive fluctuations in the salinity and the temperature of estuarine habitats. In addition, conditions can vary among estuaries, for example, temperature can vary depending on the volume to surface area ratio of the system (Del Toro, 2001). Estuaries are relied on as a nursery habitat for numerous species for the shelter they provide and increase survival rates of individuals in their early life stages (Potter et al., 2015). In older life stages, individuals of coastal species may move offshore, often in pursuit of increased prey availability (Del Toro, 2001).

The pollution and degradation of estuarine nurseries can reduce contribution of juvenile fish to adult populations and substantially impact recruitment and population dynamics (Ashford et al., 2005, Gillanders, 2005). In contrast, there are many species that cannot cope with the environmental fluctuations inherent in estuaries, therefore, estuaries can be highly productive, but they typically have relatively low biodiversity (Del Toro, 2001). The blue crab *(Callinectes sapidus)* cannot cope with the low/fluctuating salinity of estuarine habitats in their larval stage and therefore they spawn offshore but return to the estuaries for shelter and their preferred prey items (Del Toro, 2001).

Research on the migration strategies, movement patterns and the habitat preferences of fish can also uncover information on trophodynamics (predator-prey relationships) and the pathways of nutrient and energy transfer. For example, movement patterns and ecological interactions between hairtail (*Trichiurus lepturus)* and anchovy (*Engraulidae)* in India demonstrated that two species spawn in different regions, and subsequently migrate to the same area as they mature, where *E. japonicas* is a major prey species for large *T. lepturus* (Kim et al., 2005). Advancing technology and techniques for tracing individual migration are increasingly showing that the migration of individuals within some populations is more complex than was first thought (Chapman et al., 2011, Fowler et al., 2016, Tsukamoto et al., 2002). The extent of different migration strategies within populations can influence the resilience of a population. If the individuals within a population vary their migration strategies, they mitigate their risks (Chapman et al., 2011). For example, if a proportion of a population skips an annual migration, they could avoid a targeted fishing event and avoid the heavy mortality inflicted on the migrating individuals (Fowler et al., 2016). Consequently, the resilience of the overall population to perturbation is improved, which improves survival rates and the reproductive success of the population. Understanding the extent of individual migration strategies within populations is important for effective fisheries and ecosystem management, to ensure that reproductive strategies are understood and mortality sources for the entire population are considered. There are numerous benefits for understanding the migration strategies and habitat use of populations, including the contribution to an overall understanding of critical habitats, the population productivity and the understanding of population vulnerability to external pressure (Fowler et al., 2016, Kim et al., 2005). Knowledge of migration patterns and the spatial distribution of fish populations allows for evidence based management for sustainable fisheries, ecosystems and habitats (Mapstone et al., 2004, Randall et al., 2013)

1.5 Largehead hairtail (*Trichiurus lepturus*)

Largehead hairtail *(Trichiurus lepturus)*, also known as cutlassfish and ribbonfish, is a cosmopolitan species, occurring in both tropical and temperate waters (Bryan and Gill, 2007, CHIOU et al., 2006, Ghosh et al., 2009, Prathiba Rohit, 2015). *T. lepturus* is consistently placed in the top ten marine species landed worldwide based on catch weight, which makes the species an important global commodity (FAO 2018).

There is a global trend of increased fishery landings for *T. lepturus* (Figure 1). The average annual world catch between 2005 and 2014 was reported at 1 315 337 t (FAO, 2018a). *T. lepturus* is harvested in high quantities by China (FAO, 2018b), Korea (Kim et al., 2005), Japan (Hirose et al., 2017, Watari et al., 2017) and India (Ghosh et al., 2009), and harvested in lower quantities by the United States (Bryan and Gill, 2007); Brazil (Martins and Haimovici, 1997, Vilar et al., 2011) and Australia (Stewart et al., 2015). China has consistently taken the highest proportion of global landings, typically between 60-80% of the global yield (FAO, 2018b, Shih et al., 2011). A number of commercial fishing methods are used, including trawl, handline, gillnet, haul net, mesh net, light attractant purse seine, and (Stewart et al., 2015, Yan et al., 2011). Cosmopolitan species are likely to be subject to a broad range of natural and anthropogenic impacts across their geographic range, including varied climate, fishing methods and varying levels of fishing pressure (Hughes et al., 2017, Stewart et al., 2018, Whitfield et al., 2012). At various global locations, there are reports of over-exploitation of *T. lepturus*, leading to local depletion, including the Bungo Channel of Japan, Veraval, India (Ghosh et al., 2009) and Maharashtra, India (Khan, 2006). Over-exploitation has also been reported from China and Korea (Kim et al., 2005, Zhang et al., 2018).

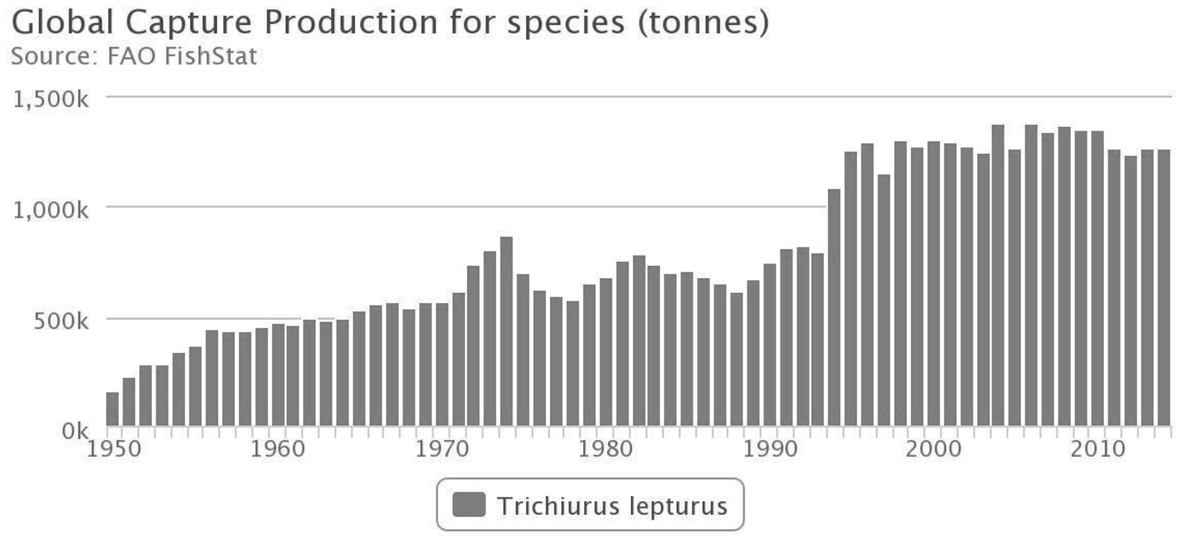


Figure 1. Global capture production for *Trichiurus lepturus* (t). Source: FAO FishStat 2019

In contrast to the reports of heavy exploitation in the north-western region of the Pacific Ocean, the commercial fishery for *T. lepturus* in south-eastern Australia has a generally low yield, which is sporadic in time and space (Stewart et al., 2015). The recreational fishery for *T. lepturus* in New South Wales (NSW), south-eastern Australia, holds great value for a subset of anglers, some for cultural or sporting motivations and some for subsistence fishing (Kirkwood 2016). In NSW, *T. lepturus* has a stock status of ‘undefined’ according to the NSW Department of Primary Industries - Fisheries, due to a limited understanding of the population demographics in south-eastern Australia (Stewart et al., 2015).

There is little agreement among global literature on the maximum body length, length at maturity, length-weight relationship, spawning period and spawning strategies of *T. lepturus* populations. There is general agreement that the species is relatively short lived and fast growing (Del Toro, 2001) however there is a wide range of parameters reported from different global regions. A Von Bertanlanfy analysis - a function used to model the mean length of an individual at age (Von Bertalanffy, 1957) - has been carried out in various global regions and the reported asymptotic average length – L_∞ (cm) ranges from 43 PL to 87 PL and the reported body growth rate coefficient – K (per yr) ranges from 0.110 to 0.612. Females are often reported to spawn more than once in a reproductive season and Kwok and Ni (1999) have reported group-synchronous spawning behavior. Numerous studies have reported that sex ratios favour females, especially in larger length classes (Al-Nahdi et al., 2009, Ghosh et al., 2014, Kwok and Ni, 1999). In southern Brazil, females dominated sex ratios, dependent on the location and season. Global *T. lepturus* populations have been reported to have flexible reproductive strategies, depending on latitude (Al-Nahdi et al., 2009) and Martins and Haimovici (2000) suggested a further need for more published data on the reproductive traits of the species at lower latitudes.

T. lepturus is a relatively short lived and fast growing species (Del Toro, 2001), indicating that it potentially has a high relative productivity and low vulnerability to local population declines. However, we have hypothesized the demographics of the south-eastern Australian population differs from populations in other global regions due to the wide geographic distribution, the potential taxonomic variation among global regions (Shih et al., 2011) and the relatively low yield of Australian fisheries, compared with the high yield of fisheries in the North-West Pacific.

1.6 Objectives

The overall aims of this thesis were to examine the demography of the *T. lepturus* population in New South Wales (NSW), Australia, and characterise the fisheries exploiting the species in the region, to provide insights into the productivity, resilience and potential threats to population persistence. The objectives were to: (1) describe the spatial and temporal dynamics of the fishery yield and the length composition of *T. lepturus* in south-eastern Australia (Chapter 2); (2) describe the population demographics of *T. lepturus* on the south-eastern coast of Australia, which relate to the population productivity (Chapter 3); and (3) evaluate the utility of otolith chemistry for reconstructing estuarine-coastal movements, to gain understanding on the extent of different migration strategies within the population (Chapter 4).

Chapter 2: Challenges and Insights for Assessing Sporadic, Multi-Sector Fisheries: A Case Study of Largehead Hairtail (*Trichiurus lepturus)* in South-Eastern Australia

Abstract

The spatial and temporal dynamics of the fishery yield and length composition of *T. lepturus* in New South Wales (NSW), Australia were quantified, as well as the market price, to characterise the commercial and recreational fisheries exploiting this under-studied population. The average annual commercial fishery landings ± standard error for *T. lepturus* between 1986 and 2015 were 34.7 (± 18.3) t and the fishery was characterised by episodically high and low catch periods, with annual landings peaking at more than 160 t. Most of the commercial landings were taken by handline fishing in estuarine sites and trawl fishing in coastal sites, within the latitudinal range of 31°S to 33°S. Landings occurred year-round, but were on average greatest between January and June, and there was considerable inter-annual variation. Trends in catch rates for handline fishing (kg per day) were similar to the landings trends, with three distinct periods of high catch rates between 1989/90 to 1992/93, 1997/98 to 2000/01 and more recently between 2010/11 to 2013/14. Catch rates by trawling (kg per day) were relatively low and stable, except for a distinct peak in 1989/90. The average market price between 2013 and 2017 for whole fish was relatively high at AUD 19.98/kg for line-caught individuals and AUD 9.25/kg for trawl-caught individuals. The length range of all *T. lepturus* observed from a commercial fisheries port monitoring program run in south-eastern Australia between 2011 and 2016 was 13–78 cm and the length composition was shown to vary between two latitudinal zones separated by ~83 km, between an estuarine and coastal habitat, and was also influenced by gear selectivity. Biological insights can sometimes be drawn from variations in length composition, however, for *T. lepturus* in NSW, there was a difference observed in length composition between handline-caught and trawl-caught samples and needed to be considered to avoid sampling bias. Opportunistically gathered information from a high-intensity recreational fishing event for *T. lepturus* was used to illustrate the difficulties in sampling a sporadically occurring fishery. The recreational harvest during a 75-day period, from mid-February to mid-April 2016, was estimated at 21 t. This outweighed the commercial annual yield by weight, averaged over the previous 5 years (2010

– 2015) at 13.6 (± 2.8) t. During this time, the recreational yield was substantially greater than the commercial yield.

2.1 Introduction

2.1.1 Data used in fisheries assessments

The assessment and monitoring of small-scale, data poor fisheries with a sporadic yield is challenging and may require a different approach compared with larger industrial fisheries (Vincent et al., 2007). If there is a large difference in the scale of the fishery, there are often challenges to obtaining representative, fishery-dependent data, and so there needs to be a different approach to the strategies of monitoring and forming solutions for management (Castilla and Defeo, 2005). The low total yield often renders such fisheries low priority, limiting the resources that can be allocated for monitoring and assessment under a cost-recovery model, therefore these fisheries are often relatively data-poor. These challenges are compounded when sporadic fisheries are multi-gear and multi-sector and if a substantial component of the yield is taken by recreational anglers (Coleman, 2004), or if each sector is exploiting different stock components. Due to these challenges, such fisheries are typically poorly monitored or not monitored at all. Populations with low abundance and limited data and resources for monitoring can be more vulnerable to local population decline (Dowling, 2008) and a greater potential for population crashes and local extinction (Salas et al., 2007, Sale et al., 2005)

The use of fishery yield data, fishing effort, and catch rates to make inferences about the productivity and resilience of exploited fishery populations forms the basis for the assessment and monitoring of fisheries (Hilborn and Walters, 1992). One of the primary aims of fishery assessment and monitoring is to form an understanding of population size and how the population responds to external pressures, therefore, it is important to assess these external pressures, including the spatial and temporal dynamics of the fishery yield (Hilborn and Walters, 1992).

Length frequency data is often used to quantify life history parameters relating to population productivity and resilience (e.g. recruitment pulses, mortality and growth rates) (Hilborn and Walters, 1992). If length frequency data obtained through fishery dependent sampling from a multi-gear fishery is the only practical option for quantifying the life history parameters of a population, then the effects of gear selectivity and the spatial patterns of fishing effort on length composition needs to be characterised (Hilborn and Walters, 1992). Components of the fishery such as gear selectivity and spatial extent of fishing effort can bias length composition data, meaning the fishery-dependent sample might not be representative of the true length composition of the population. The gears used for fishing are designed to be selective of target species based on their demographic traits (Flood et al., 2014) therefore, the type of gears used for obtaining samples need to be considered when using demographic data, including length frequency data, to make inferences on population demographics (Hilborn and Walters, 1992, Otway and Craig, 1993). Gear selectivity can bias length composition directly by the size of mesh or hooks and indirectly, if particular size demographics of the population are inclined to avoid certain gear types (Hilborn and Walters, 1992). Length composition can also vary temporally, spatially, or by habitat, and is typically based on ontogenetic differences in habitat use and movement patterns. If length distribution data are compared between fishing gears or between locations and habitats, then bias can be characterised and accounted for in any case where length distribution data is utilised for inferring any parameters of the population (Griffiths and Fay, 2015, Hilborn and Walters, 1992).

It is important to include the impact of the recreational fishery when assessing and monitoring populations that are exploited by multi-sector fisheries to account for each contribution to overall mortality. Global participation in recreational fishing has been reported at 11.5% (Cooke and Cowx, 2004). In some regions, recreational fishing yield exceeds the commercial yield (Allen et al., 2006, Coleman et al., 2004) and for some species, recreational fishing can be the primary contributor to species mortality (McPhee et al., 2002). Full quantitative stock assessment models will often have the flexibility to incorporate a variety of data from multiple fishing fleets (Griffiths and Fay, 2015). It is not common that recreational fisheries will have extensive sampling data, such as logbook data or age frequencies, however, sometimes it is feasible to use survey methods to obtain data such as fishery yield in numbers or by weight, and to incorporate these data into the species assessment models (Griffiths and Fay, 2015). As well as contributing to the overall mortality on a species, recreational fisheries may impact different length or age components of populations compared to the commercial fleets, due to differences in gear and spatial and temporal distribution of fishing effort (Griffiths and Fay, 2015).

Standard regional survey designs to sample recreational fisheries typically survey the general population of recreational anglers (Griffiths et al., 2013). Using a standard survey design to sample for a sporadic caught species with generally low yields and occasional high catch events is likely to overlook substantial catch events (Green et al., 2012). A sampling design targeted to the species and, more specifically, targeted to the high catch events is likely to be more effective (Dixon et al., 2005, Guisan et al., 2006). If the high catch events are consistent in time and space, then it may be feasible to implement intensive sampling in response to the fishing effort for a species exceeding a predetermined threshold (Moore et al., 2015). Examples of thresholds could include the number of fishers at a particular wharf, or number of mentions of the target species on social media (Moore et al., 2015). An effective strategy for Intensive, response-based sampling would be an on-site census of all retained catch of the species by recreational anglers within a specified region (Cox, 2000). If a threshold based on fishing effort was implemented, background monitoring would be necessary throughout the year at designated fishing access points and could be based on anecdotal evidence, media articles and informal interviews with local fishers, (Rhodes and Sadovy, 2002).

2.1.2 *Trichiurus lepturus* in south-eastern Australia

Largehead hairtail *(Trichiurus lepturus)* is a marine species with a global distribution (Bryan and Gill, 2007, CHIOU et al., 2006, Ghosh et al., 2009, Prathiba Rohit, 2015), and is consistently placed in the top ten marine species landed worldwide by the United Nations (UN) Food and Agriculture Organization (FAO) based on catch weight (FAO 2018). In contrast, the fisheries for *T. lepturus* in south-east Australia have a generally low yield and the yield is sporadic in time and space (Stewart et al., 2015). The fishery for *T. lepturus* in Australia is relatively small-scale and has limited data available for population assessment. The majority of the fishery yield in Australia occurs in both the estuarine and coastal habitats of the south-eastern state of New South Wales (NSW) (Stewart et al., 2015).

The commercial fishery for *T. lepturus* in NSW is a multi-gear fishery. The majority of landings are taken by handline gear and there are also substantial catches by prawn and fish trawl (Stewart et al., 2015). The commercial handline method in NSW is usually undertaken on small commercial vessels where fishers use either a handline, rod and reel, or electric reel from a stationary or slow-moving vessel (DPI, 2006). The method generally targets schooling pelagic or demersal species in shallow waters, to 30 m depth, with electric reels used up to

500 m depths (DPI, 2006). The trawl method in NSW involves a large net that is towed through the water behind the fishing vessel. Demersal fish trawl gear has a relatively larger mesh size compared with prawn trawls (Flood et al., 2014). Other gears that contribute a small proportion to the NSW landings include mesh nets, haul nets, droplines and demersal traps.

The fishery for *T. lepturus* in NSW is also a multi-sector fishery, exploited by both commercial and recreational fishers (Stewart et al., 2015). The overall trend for participation in recreational fishing in Australia is above the global average, reported at 19.5%, compared with 11.5% globally (Cooke and Cowx, 2004, Henry and Lyle, 2003). However, landings of *T. lepturus* by recreational anglers are not well understood. Although the recreational fishery is thought to have a varied yield among and across years, the fishery holds great significance for a subset of anglers (Kirkwood, 2016). Three newspaper articles, all published in the autumn months of 2016, 2015 and 2013, report that dozens of anglers arrive at a key angling location during the autumn months and that many have travelled a considerable distance to target *T. lepturus*. These articles indicate that that *T. lepturus* is heavily targeted, by recreational anglers, usually in predictable locations, and that during such times substantial harvests can be made (Kirkwood, 2016).

In Australia, recreational anglers are not required to record or report their catch data and so data collection methods for the recreational fishery are often cost and labour-intensive. There have been several recreational angling surveys conducted in south-eastern Australia aimed at estimating the recreational harvest of fisheries across numerous species. The surveys have used varying methodologies including access-point creel surveys, roving creel surveys, and random stratified telephone-diary surveys. They have been conducted at various spatial and temporal scales, from individual waterways through to a nation-wide scale (Henry and Lyle, 2003, Steffe and Murphy, 2011, West et al., 2015). However, none of the historic surveys were able to capture the recreational catch and effort during a high yield event, so the current estimates of recreational fishing impacts on *T. lepturus* in NSW are severely underestimated in some years.

The impacts of fishing on the *T. lepturus* population in Australia are not well understood, which has resulted in a stock status of ‘Undefined’ according to the NSW Department of Primary Industries - Fisheries (Stewart et al., 2015). There are no specific management regulations for the commercial harvest of *T. lepturus*, no regulated minimum legal length or total allowable catch limits and the only regulation on the recreational fishery is a bag limit of 10 (Stewart et al., 2015). To ensure that the fishery for *T. lepturus* in south-east Australia is sustainable, it is important to characterise the fishery to understand how it might influence population demographics, and to determine how representative fishery-dependent sampling is for inferring population demographics.

2.1.3 Objectives

The specific objectives for this study were to: (1) document the spatial and temporal variation in commercial fishery yield (landings by weight); (2) Characterise any bias in the length distributions taken by the fishery between habitats, latitudinal zones, commercial gear types and fishery sectors and; (3) to provide a snapshot of the recreational fishery yield for *T. lepturus* during one high-yield fishing event in south-east Australia and to demonstrate the potential relative impacts of the recreational fishery yield compared with the commercial fishery yield in one high-catch time-period.

2.2 Materials and Methods

2.2.1 Commercial fishery catch records

The spatial and temporal patterns of the NSW commercial fishery for *T. lepturus* were investigated using data from mandatory commercial fishing catch and effort logbooks administered by the NSW Department of Primary Industries - Fisheries between 1986 and 2015. The logbook program requires commercial fishers in NSW to report each commercial fishing event, including the date, where the catch was taken (fishery reporting location), the method used, and the weight of landed catch (kg).

The coast of NSW is divided up into ten coastal fishing zones at a one degree latitudinal scale and fishers are required to either specify the estuary or the coastal zone of each fishing event. Most coastal fisheries are limited to three nautical miles offshore, however some fishers have permits that extend to the 4000-metre depth contour, typically between 60 and 80 nautical miles offshore.

The weight of commercial catch for *T. lepturus* over time by fishing gears, by latitude and by habitat were examined. To test the null hypothesis that the commercial yield for *T. lepturus* will not differ among months, a one-factor analyses of variance (ANOVA) was used to compare the average commercial catch among months and the data were log transformed in order to meet the assumptions of normality and homoscedasticity. Catch rate trends (kg/day) across years were then examined to determine if catch rate trends related to trends in fishery yield.

An investigation was made on the Sydney Fish Market AUD sale reports for all *T. lepturus* commercially caught in NSW and sold between 2013 and 2017. These reports were made available by the NSW Department of Primary Industries – Fisheries and were investigated by length class and fishing method.

2.2.2 Commercial fishery length frequencies

The lengths of *T. lepturus* landed by the commercial fishery were estimated through a commercial fisheries port-monitoring program conducted between 2010 and 2016. The port-monitoring program is a fishery-dependent data collection program run by the NSW Department of Primary Industries – Fisheries. Resource Assessment Unit. The program generates estimates of the length and in some cases the age structure of the catch landed by the NSW commercial fishing fleet to monitor commercial catches and assess stock status. Length samples are used to examine trends in length of the fished population through time, for the purpose of informing stock assessment. The port-monitoring program is stratified spatially (estuarine and coastal fisheries and by degrees of latitude) and temporally (monthly) to ensure complete coverage of the extent of the fishery, and samples are weighted proportionally by reported catch to ensure they are representative of NSW fishery catch. Pre-anal length (PL), from the anterior extent of the lower jaw to the anal vent, was measured to the nearest centimetre. Pooled NSW length distributions were first examined among years. Then, to test the null hypothesis that length will not differ between the two predominant commercial fishing methods for *T. lepturus* in NSW, length distributions were compared between *T. lepturus* caught by commercial line fishing and *T. lepturus* caught by commercial trawl fishing. Next, to test the null hypothesis that length will not differ between regions separated by ~83 km, length distributions were compared between two latitudinal zones (32°S-33°S) and (33°S-34°S) (Figure 2). Lastly, to test the null hypothesis that length will not differ between habitats, length distributions were compared between the Hunter estuarine site and the Hunter coastal site (Figure 2). Comparisons were made using Kolmogorov-

Smirnoff (KS) tests, a non-parametric test of the significance of the greatest difference in their respective cumulative distributions (Zar, 1999).

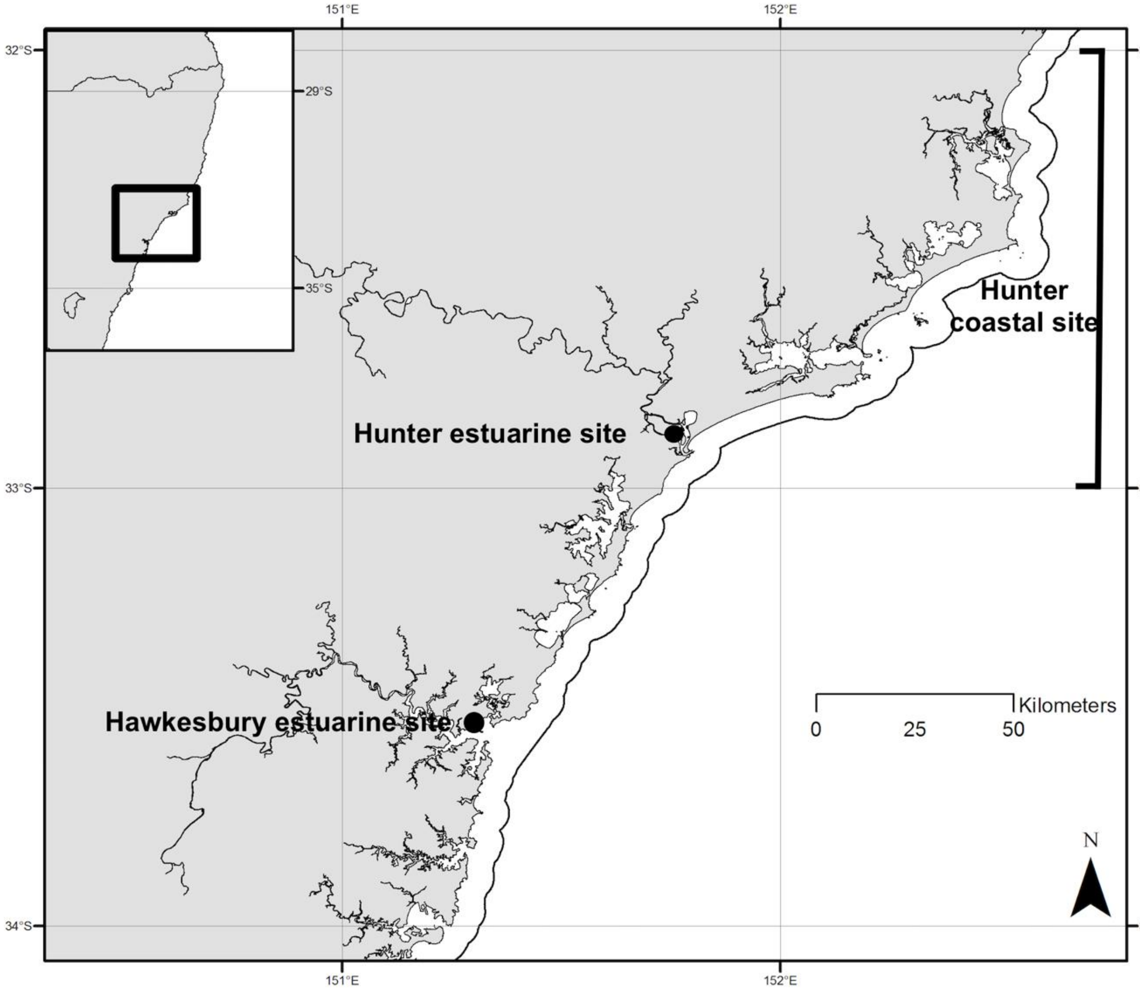


Figure 2. Map of the NSW Coast, showing the two latitudinal zones (32°S-33°S and 33°S-34°S) from which spatial length distribution comparisons were made and the Hunter and Hawkesbury estuarine site and the Hunter coastal site from which length distribution comparisons were made between habitat types. The coastal site extends to 3 nautical miles from the coast, marked by a black line.

2.2.3 Recreational fishery length frequencies

An estimate of the length composition of *T. lepturus* landed by the recreational fishery was made using records collected by an annual fishing competition, the HBA's Learning Centre's Hairtail Social'. The competition was held in the Hawkesbury estuarine site located in NSW at 33.63°S, 151.16°E (Figure 2), from 2014 to 2016 inclusive. All retained individuals were brought to the measuring station and were measured by the competition organisers as total length (TL) to the nearest centimetre, from the tip of the bottom jaw to the tip of the tail, and these were converted to pre-anal length (PL) using the TL to PL relationship (TL = 2.254 x PL +

17.322) developed in the current project (see Chapter 3). The null hypothesis that the lengths of *T. lepturus* landed by recreational and commercial line fishing did not differ significantly was tested by comparing the recreational competition length data with commercial length data. The length distributions were compared using a KS test.

2.2.4 Recreational fishery yield

Data collected during four separate recreational fishing surveys conducted over a 20-year period were used to determine the recreational catch of *T. lepturus* in NSW. The National Recreational and Indigenous Fishing Survey (NRFS) was the first Australia-wide survey to collect information on the catch and effort of recreational fishers (Henry and Lyle, 2003). The Survey was carried out during 2000-2001 as a joint venture between the commonwealth and state Governments and was the first recreational survey to assess the entire recreational fishery in NSW. This survey primarily used telephone/diary techniques to gather information along with on-site survey techniques for information on the indigenous fisheries A pre-existing survey technique established by the Bureau of Tourism was used for information on recreational fishing by interstate and overseas tourists. Since the NRFS, there have been more surveys that assess sections of the recreational fishery including Steffe et al. (2005a), which is an assessment of the recreational fishery in one NSW estuary before and after a recreational fishing haven was established. The survey used an on-site, roving creel survey for shore-based fishers and an on-site, access-point creel survey for boat-based fishers from 1999-2000 and again from 2003-04. Datasets were supplemented with traffic and boat hire records. Steffe et al. (2005b) involved an assessment of the recreational fishery in a NSW estuary before and after a recreational fishing haven was established, using a boat-based roving survey design from 2007-2008. Steffe and Murphy (2011) involved an assessment of the daytime catch and effort of the recreational fishery in the greater Sydney region of NSW, using random, stratified sampling and access-point creel surveys between 2007 and 2009.

Observations and information gathered by NSW fishery compliance officers during a high-catch recreational angling event for *T. lepturus* were used in this study to provide an approximation of the recreational fishery catch and effort during the timespan and location of the event.

To test the null hypothesis that recreational yield does not have the potential to contribute a substantial proportion to the overall fishing pressure on *T. lepturus* in NSW, a snapshot of the recreational yield during the event was estimated. The event occurred in one small area (a community board walk) at the lower Hunter River, NSW (32.92°S, 151.79°E) (Figure 2). The event occurred over approximately 75 days, from mid-February to mid-April 2016. An estimation of angler yield was made from the observations of catch and effort recorded by the officers during the event. Observations included the duration of the high-catch event, the general number of fishers observed targeting *T. lepturus* during the event, the length of fish retained and the assumption that each angler retained the bag limit of fish. Compliance officers attended the event two to three times per day, every day, for the duration of the event including regular patrols during the day and night. The compliance officers inspected the catch of each angler participating in recreational fishing at the board walk on every patrol. Based on the two to three patrols made each day, the officers estimated that 80 anglers participated at the board walk each day and would continue fishing until they had reached their bag limit of 10 fish per day. The average total length of retained *T. lepturus* was estimated at ~1m by compliance officers base on their daily observations. The total length estimated by officers was consistent with total lengths observed in biological sample of 30 fish confiscated from one angler who had exceeded the bag limit. The average weight per individual was converted from the average length observed, using a determined length-weight relationship developed in the current project (see Chapter 3).

An approximation of total landed weight during the event was made by multiplying the bag limit by the assumed number of fishers per day by the number of days that the fishing event was observed.

2.3 Results

2.3.1 Commercial fishery yield

The average annual commercial fishery landings for *T. lepturus* between 1986 and 2015 were 34.7 (± 18.3) t and the fishery was characterised by episodically high and low catch periods, with annual landings peaking at more than 160 t. Most of the commercial yield was taken by handline from estuarine sites, followed by trawl methods from coastal sites (Table 1), however this pattern varied among years, with commercial catch in some years dominated by estuarine trawl or ocean handline (Figure 3). Landings occurred in both estuarine and ocean waters and were characterised by the intermittently high and low catch periods in both habitats (Figure 3). The bulk of fishery landings occurred over a relatively small latitudinal range (31°S to 33°S) although a pulse of high landings was observed further north during 1990/91 in latitude 29°S to 31°S (Figure 4).

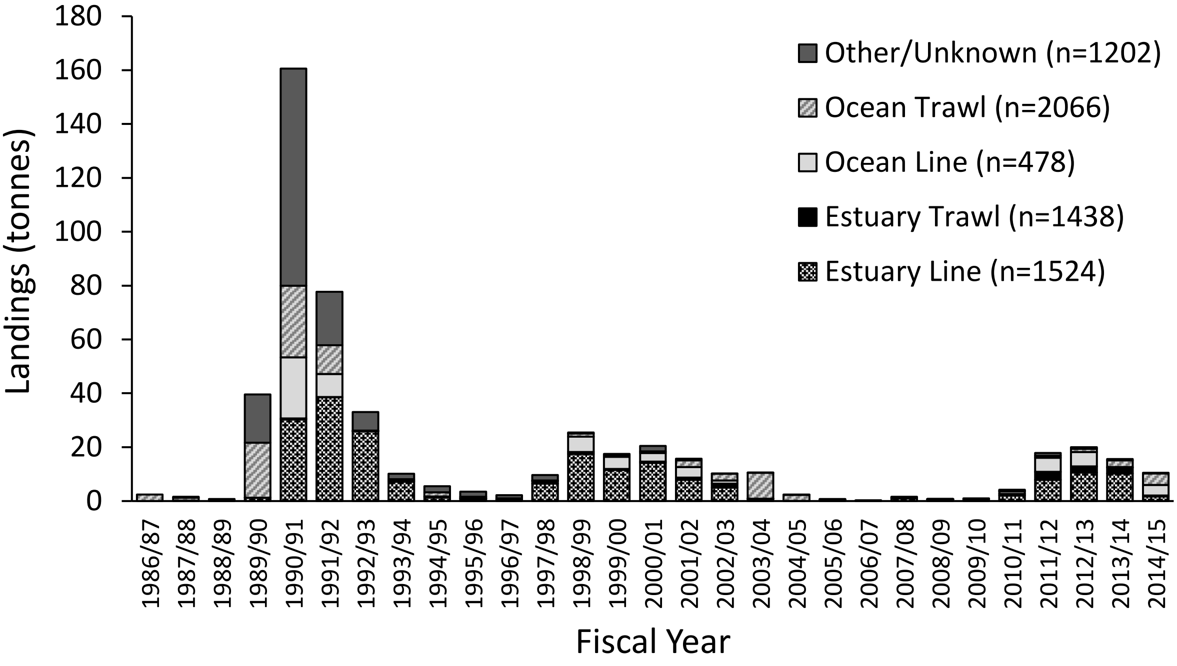


Figure 3. Commercial landings of *Trichiurus lepturus* in New South Wales by fishing method and location from 1989 to 2015, n is the number of recorded catches from mandatory logbooks.

Table 1. Commercial landings of *Trichiurus lepturus* in New South Wales by weight between 1989 and 2015

Habitat	Commercial fishing method	Proportion of landings
Estuary	Handline	40%
	Estuarine prawn trawl	4%
	Estuary prawn trawl (squid)	2%
	Mesh net, top set bottom set or splashing	1%
Coast	Fish trawl	34%
	Handline	15%
	Ocean prawn trawl	2%
Other/unknown	Other/unknown	2%
Total		100%

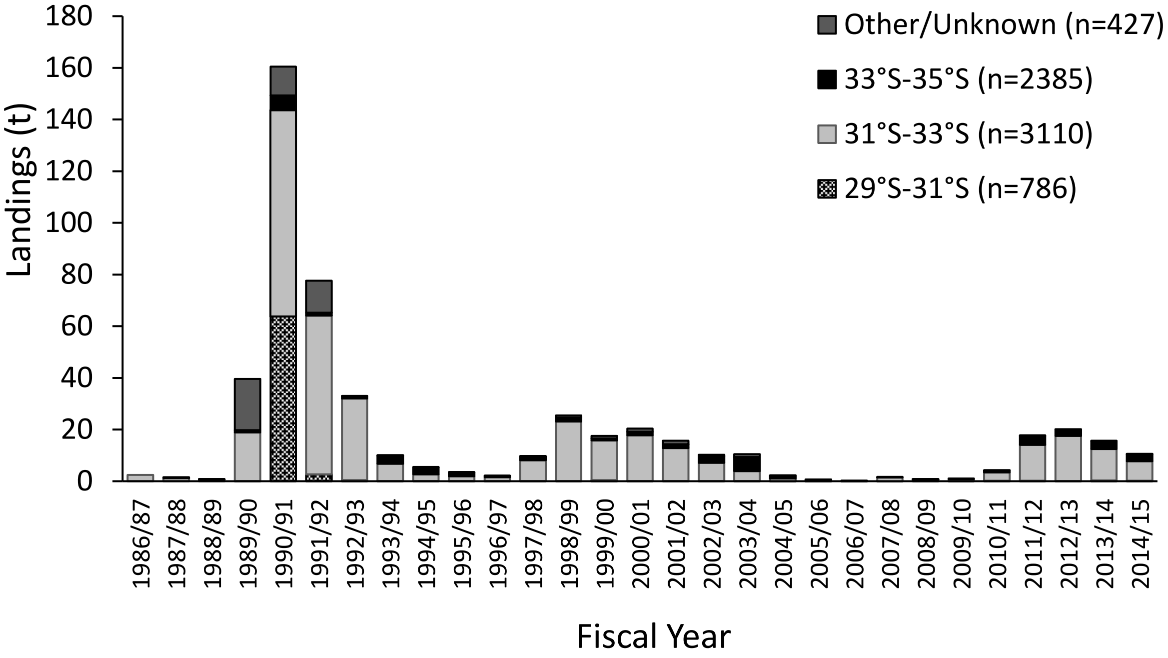


Figure 4. Commercial landings of *Trichiurus lepturus* in New South Wales by latitude, n is the number of recorded catches.

Landings of *T. lepturus* occur year-round, but are on average greatest between January and June, during the late summer to early winter months; however, there is considerable inter-annual variation (Figure 5). A significant difference was found among months (Figure 5, One-factor ANOVA: $F_{11} = 2.92$, $p < 0.001$).

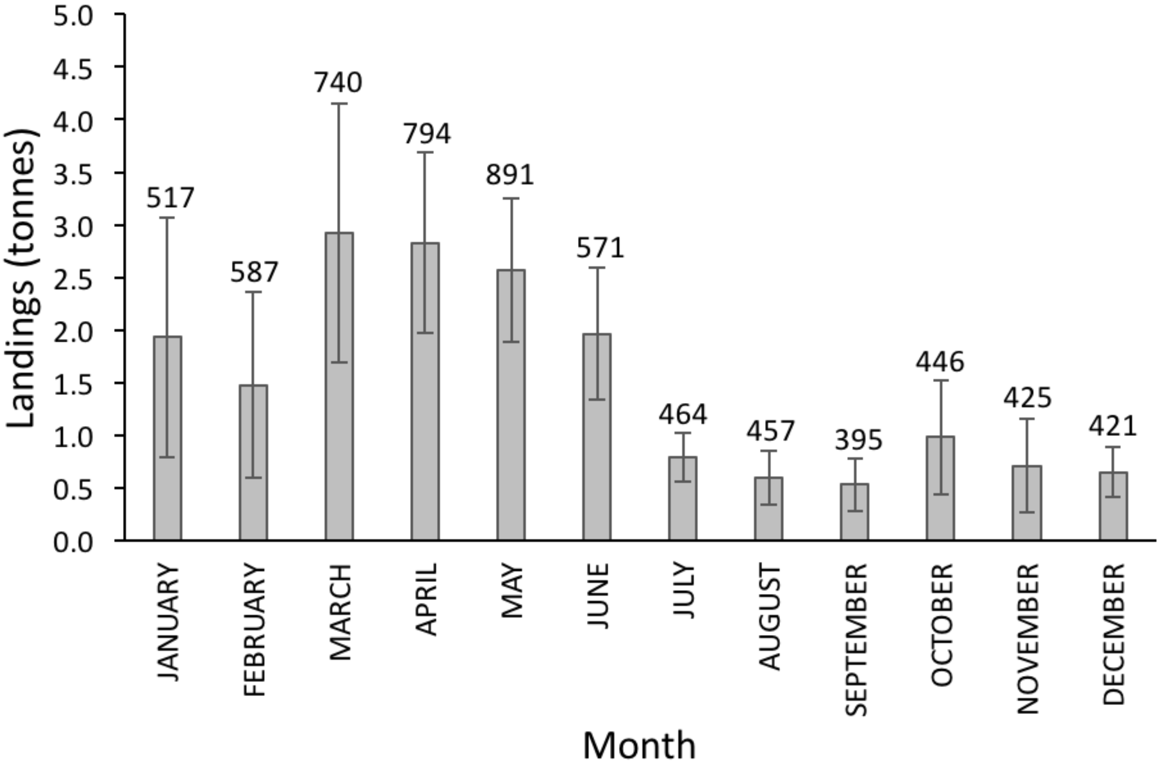


Figure 5. Reported average (± S.E.) commercial landings of *Trichiurus lepturus* by month (1986 to 2015). The numbers displayed above the bars represent sample sizes (number of reported catches from mandatory logbooks).

Trends in catch rates for line fishing (kg per day) were similar to the landings trends, with three distinct periods of high catch rates between 1989/90 to 1992/93, 1997/98 to 2000/01 and more recently between 2010/11 to 2013/14 (Figure 6.a). In contrast, catch rates by trawling (kg per day) were relatively low and stable, apart from a distinct peak in 1989/90 (Figure 6.b).

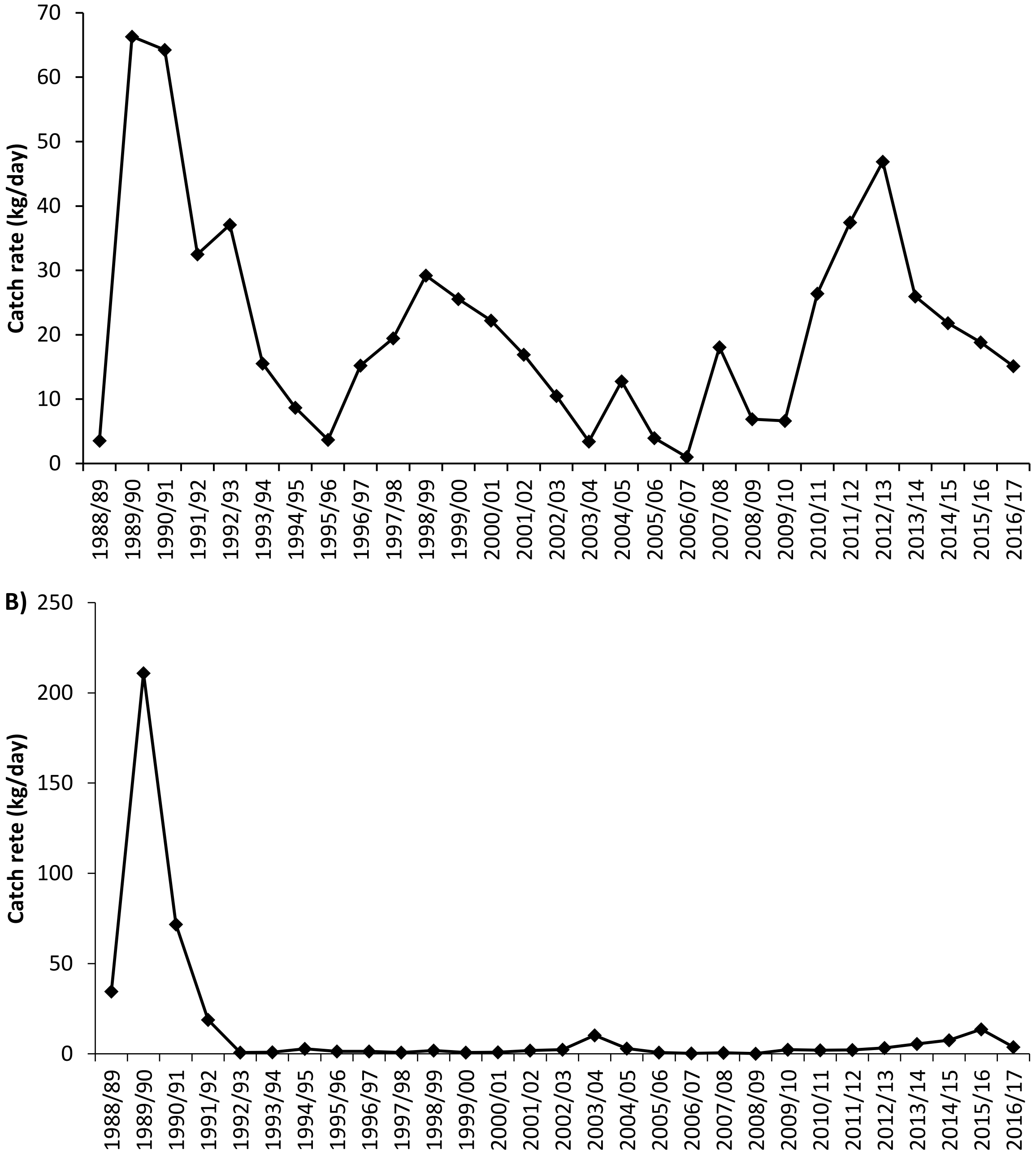


Figure 6. Catch rates of *Trichiurus lepturus* by line fishing (A) and trawl fishing (B) in NSW.

The market price of commercially-caught *T. lepturus* averaged AUD$14.08 (±0.14)/kg at the Sydney Fish Market between 2013 and 2017. Average price differed between handline-caught and trawled catches. Whole fish caught by handline fishing averaged AUD$19.98 (±0.32)//kg and full fish caught by trawl fishing averaged AUD$9.25 (±0.15)/kg (Figure 7). Average price was similar between the size grades, with AUD$14.74 (±0.42)/kg for medium and $15.28 (±0.23)/kg for large grades. There was a significant interaction between fishing method and fish size (Figure 7, Two-factor ANOVA, Method x Size, $F_{1,732}$ = 5.35, p= 0.021), however subsequent pair-wise tests did not support this, with price for both medium sized and large size *T. lepturus* caught by commercial line fishers being higher than the price for both medium and large size *T. lepturus* caught by trawl (Tukey's HSD, all p< 0.05). No differences were found between medium and large fish within each fishing method (Tukey's HSD, all p> 0.05).

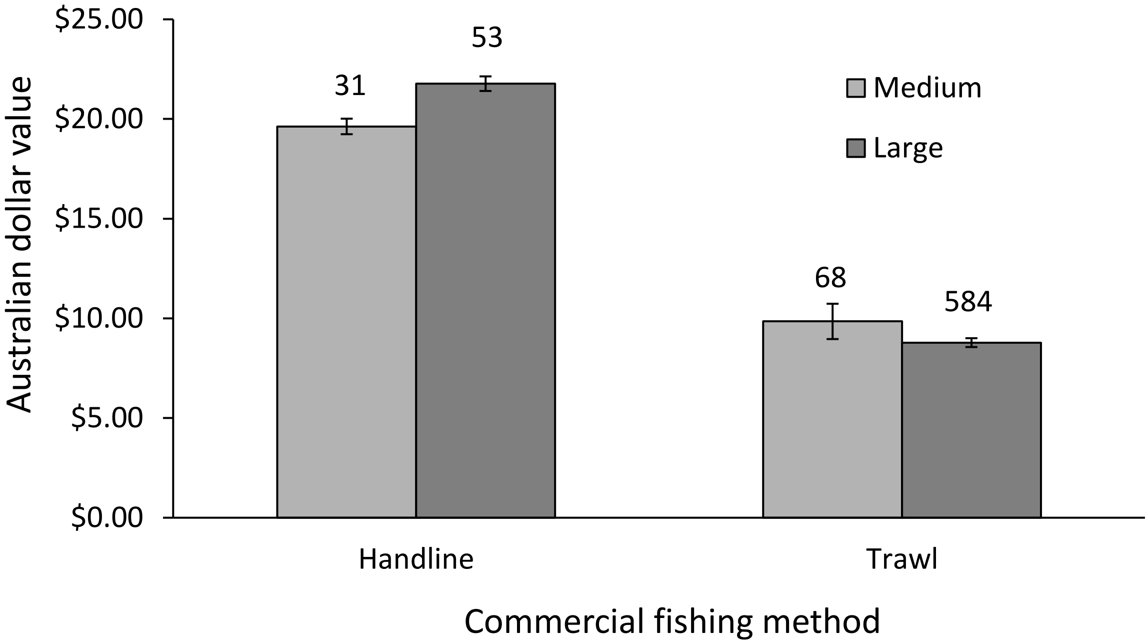


Figure 7. Average Sydney Fish Market auction prices (AUD) for *Trichiurus lepturus* by fishing method and the size grade assigned to catch by the Sydney Fish Markets, 2013 to 2017. The numbers displayed above the bars represent sample sizes (number of reported sales).

2.3.2 Commercial fishery length frequencies

The lengths *of T. lepturus* landed by the NSW commercial fishing fleet between 2010/11 and 2015/16 ranged between 13 and 77 PL, with the peak mode at either 25 or 30 cm each year (Figure 8). The longest *T. lepturus* sampled was 78 cm PL, with relatively few fish greater than 50 cm PL observed each year.

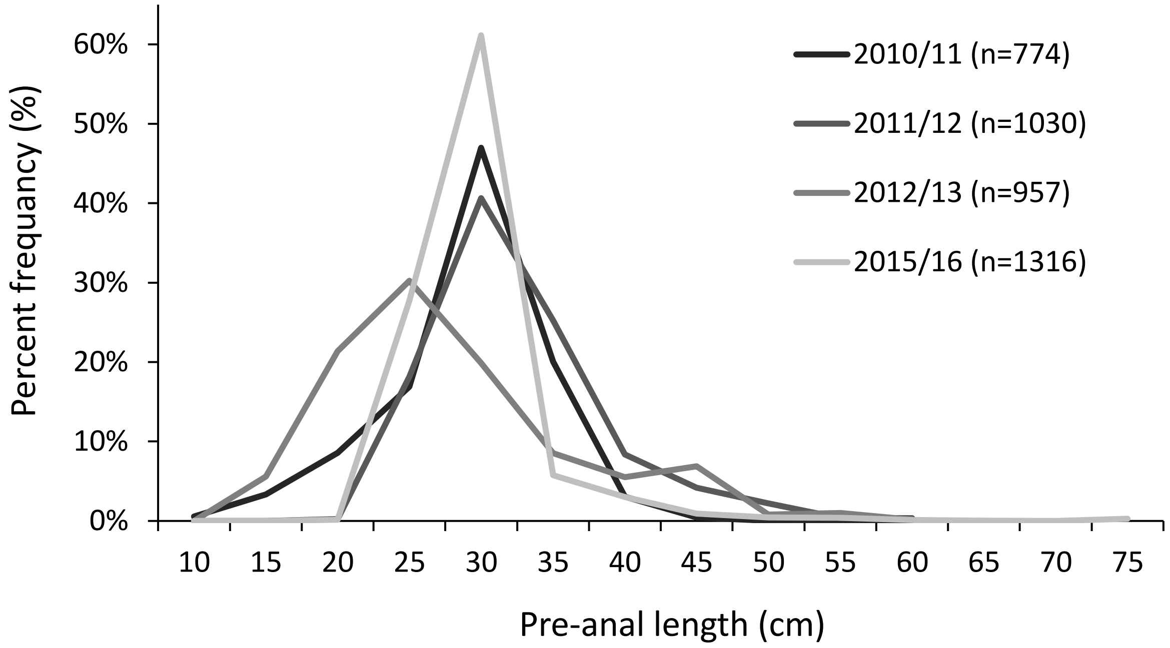


Figure 8. Length composition of the NSW commercial landings of *Trichiurus lepturus* from 2010/11 to 2015/16.

There was a significant length difference between *T. lepturus* landed by commercial line fishing and commercial trawl fishing (Figure 9, KS test, D = 0.24, P < 0.001). The two methods retained fish across similar length ranges; however, the line-caught fish were on average slightly smaller (line average: 32.28 ± 3.63, trawl average: 33.38 ± 5.11).

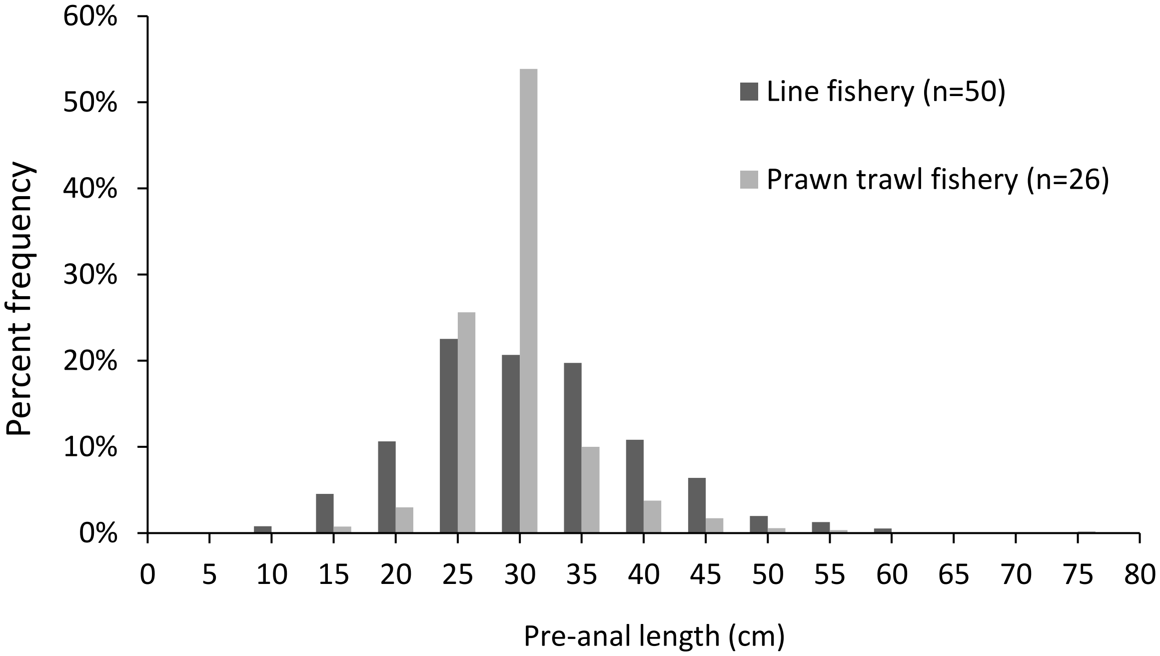


Figure 9. Comparison of *Trichiurus lepturus* lengths from the commercial line fishery (3097 individual lengths) and the prawn trawl fishery (696 individual lengths) in NSW. n refers to the number of catches. Data were collected from 2010 to 2016.

There was a significant length difference in *T. lepturus* landed by the line fishery between the Hunter estuarine site and the Hunter coastal site (Figure 10, KS test, D = 0.16, P < 0.001). The majority of *T. lepturus* caught from both habitats were approximately 30 cm, however individuals from the estuarine site had a higher mode and had less variation from the mode and individuals from the coastal site had a larger length range, meaning the coastal fishery landed more individuals in small and large length classes.

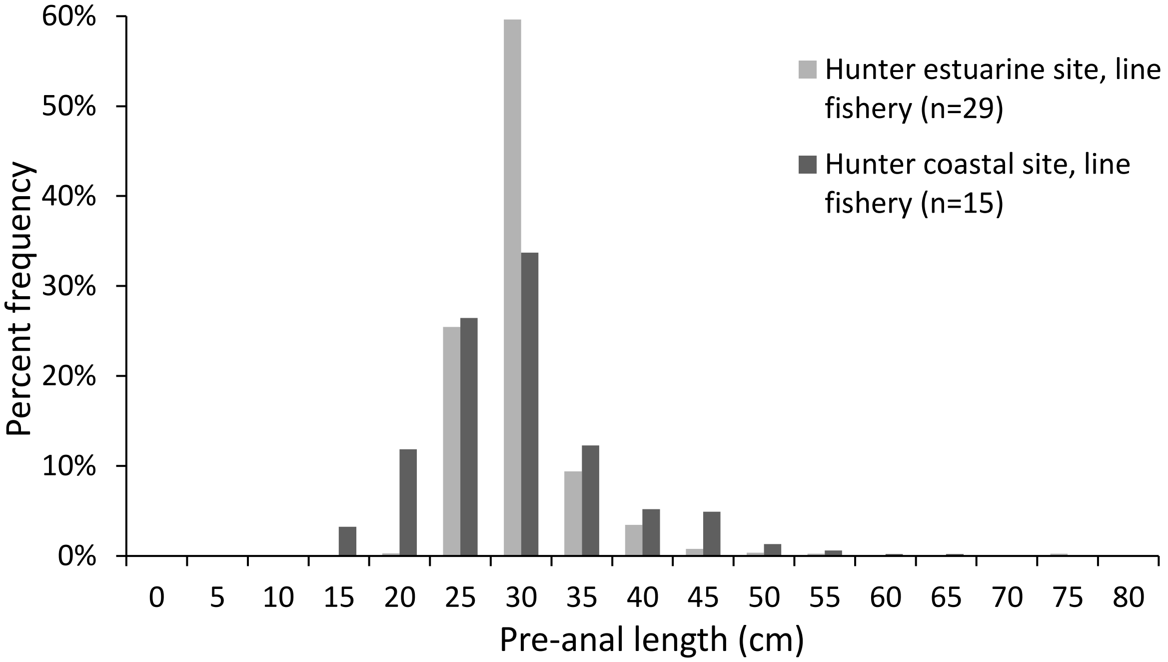


Figure 10. Comparison of *Trichiurus lepturus* lengths from the line fishery, between the Hunter estuarine site (1401 individual lengths) and the Hunter coastal site (1151 individual lengths) in NSW, n is the number of catches. Data collected from 2010 to 2016.

There was a significant difference between the length frequency distribution for *T. lepturus* landed by the commercial fishery in the Hunter region and the Hawkesbury region (Figure 11, K-S test, D = 0.14, P < 0.001). The two locations yielded fish across similar length ranges (Hunter average: 32.29 ± 3.67, Hawkesbury average: 32.81 ± 4.47). The yield from the Hunter region had a larger length range, with a higher proportion of small and large individuals. The Hawkesbury fishery had a higher mode and had less variation from the mode.

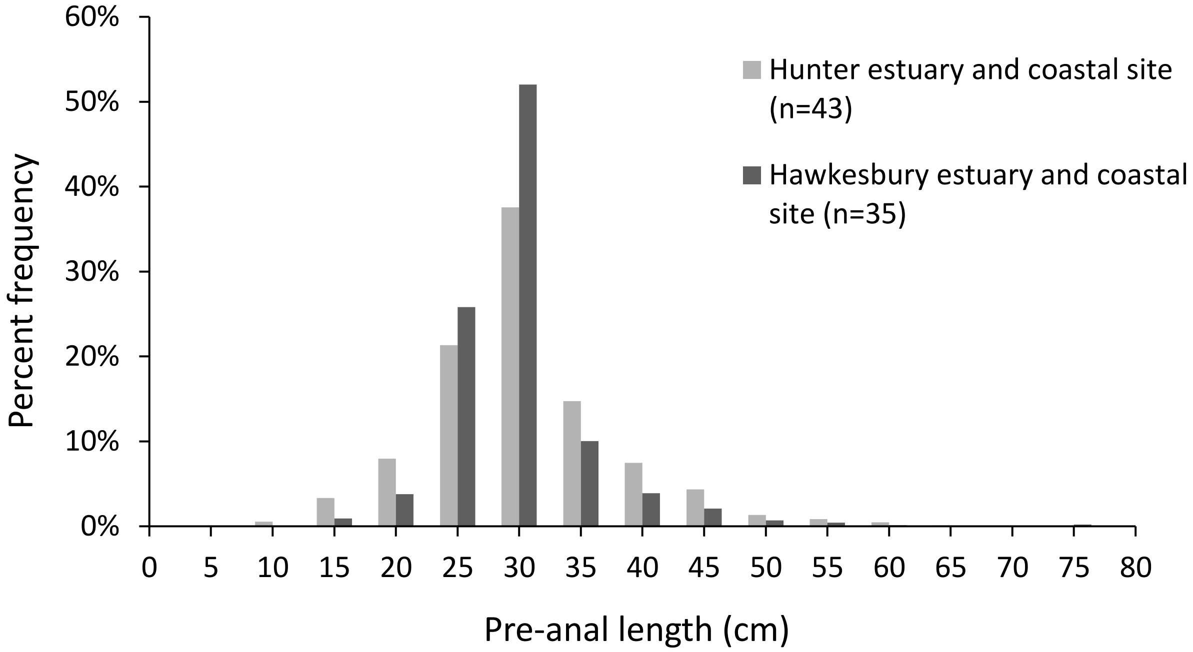


Figure 11. Comparison of *Trichiurus lepturus* length frequency distributions from the Hunter region (n = 2633 individual lengths) and the Hawkesbury region (1089 individual lengths) of NSW, n is the number of recorded catches. Data collected from 2010 to 2016.

2.3.3 Commercial fishery length frequencies

The length distribution of *T. lepturus* from the 'HBA Learning Centre’s Hairtail Social’ recreational fishing competition differed significantly to those from commercial landings pooled across 2010 to 2016 (Figure 12, K-S test, D = 0.82, P < 0.001). The recreationally caught individuals were all larger than 30cm PL and larger on average than commercial fish (recreational average: 40.66 ± 0.74, commercial average: 32.31 ± 3.73). The mode of the commercial fishery distribution was 30cm PL; however, the commercial distribution had a broad length range from 15 to 60 cm PL.

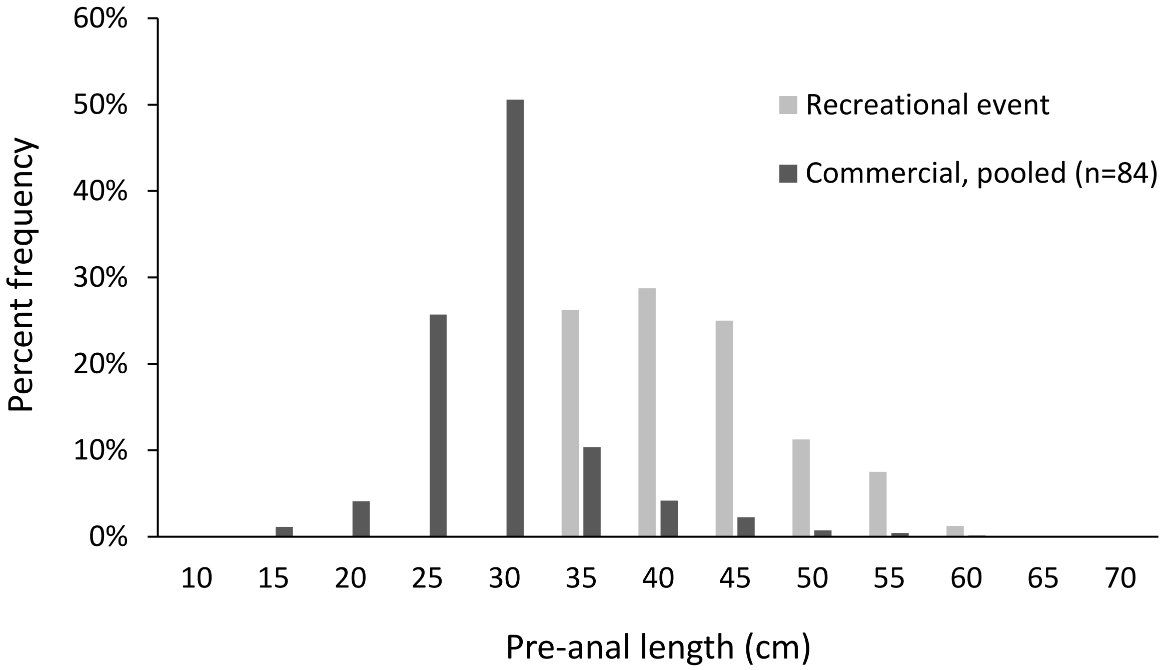


Figure 12. Length frequency distribution of the pooled commercial landings of *Trichiurus lepturus* landed by the commercial fishery in NSW from 2010 to 2016 (4077 individual lengths) and one sample of *Trichiurus lepturus* landed at the recreational fishing competition 'HBA Learning Centre's Hairtail Social' (84 individual lengths) from 2014 to 2016.

2.3.4 Recreational fishery yield

The data available from the four historical recreational fishing surveys in NSW indicated that no *T. lepturus* were recorded in three of the surveys while the fourth survey, a random stratified sampling and access-point creel survey implemented for the greater Sydney region of NSW, between 2007 and 2009 (Steffe and Murphy, 2011), recorded a total harvest of 306 fish during the two years the survey took place.

The estimated yield by weight of *T. lepturus* landed by recreational anglers in the lower Hunter River between mid-February and mid-April 2016 was 21 t (Table 2). The commercial annual average yield by weight for the previous 5 years (2010 – 2015) was 13.6 (± 2.8) t.

Table 2. Summary of the estimated harvest of *Trichiurus lepturus* retained by recreational anglers in the lower Hunter River between mid-February and mid-April 2016.

Event duration	75 days
Estimated anglers per day	~80
Assumed number of fish caught per person per day (bag limit)	10
Assumed average length of fish	100 cm TL
Approximate weight of each fish of 100 cm	0.360kg
Estimate of harvest by numbers	60,000 individuals
Estimate of harvest by weight	~21

2.4 Discussion

This study identified substantial variation in the NSW commercial fishery yield for *T. lepturus* through time. This variability may arise from sporadic aggregation, migration or recruitment of *T. lepturus* in south-east Australia. The variability in catch means it is unlikely that catch rate data will be representative of relative population abundance. Differences were found in the length distributions between commercial and recreational sectors indicating there were different population components exploited by the two sectors and commercial data suggested there may be spatial length-structuring in the population. These findings highlight the potential for sampling bias in length distribution data collected for *T. lepturus* in NSW. Length bias therefore needs to be accounted for if the length distribution data is utilised to quantify life history parameters. Data from this study supported the hypothesis that the recreational fishery for *T. lepturus* was being underestimated by the standard recreational survey designs in south-east Australia.

2.4.1 Temporal and spatial variability in catches

The observed trends for commercial fishing catch rates (kg per day) were like the observed trends in commercial yield, and line catch rates tracked with the same pattern through time, with three distinct periods of high catch rates. Trawling catch rates were low and stable apart from a distinct peak, which occurred at the same point in time of the distinct rise in landing in the fiscal year of 1989/90. The differences observed in catch rates between line fishing and trawl fishing are likely due to increased targeting of species for line fishing compared with the more incidental nature of *T. lepturus* catch by trawl fishing. This conclusion is supported by observations made by commercial Research Observers from NSW DPI - Fisheries on trawl vessels off the NSW coast and information provided by Peter Robinson, a commercial line fisher. The observed difference in commercial price value also supports this conclusion, as *T. lepturus* landed by the line fishery compared with the trawl fishery was shown to have a significantly higher price value at market, irrespective of the size grade. The relatively high price value for line-caught fish is a likely a further explanation for commercial line fishers to target *T. lepturus*. The periods of higher catches may have been influenced by one or more of the following mechanisms: (1) better fishing conditions (visibility, wind etc.); (2) increased, opportunistic targeting of *T. lepturus*; (3) improved *T. lepturus* recruitment or; (4) changes in *T. lepturus* behavior, feeding habits or migration (Vincent et al., 2007).

The similarities between trends in catch rate and trends in commercial fishery yield provides evidence in support of true population changes in abundance, especially when both metrics incline together and then also decline together again. The decline eliminates the potential for ‘gear creep’ to influence trends, where increasing catches and catch rates might be because fishers are improving gear/techniques (Hughes et al., 2004). This pattern also minimises the likelihood that catch trends are influenced by better fishing conditions because the fishing effort does not appear to greatly influence overall catch.

It is possible that fluctuations in opportunistic targeting could have influenced the variability in catch, especially due to evidence of aggregation in the *T. lepturus* population of NSW. For catch rates to form a useful index of abundance, catchability (ratio of the catch per unit effort to the abundance) must be constant (Rose and Kulka, 1999, Vincent et al., 2007), because changes in catchability or fisher behaviour can obscure real patterns of abundance (Gibson-Reinemer et al., 2016, Harley et al., 2001, Vincent et al., 2007). Species aggregation can affect population catchability, and there is some evidence that *T. lepturus* populations do aggregate in south-east Australia. For example, one small-scale commercial fisher indicated that *T. lepturus* will often school in specific locations of an estuary, identifiable using sonar, presenting an opportunity to land the species in large numbers (personal communication with Peter Robinson – Commercial line fisher). Information provided from fisheries compliance officers of the NSW DPI Fisheries in the current study indicated the presence of *T. lepturus* in high abundance in the Hunter estuarine system. An annual aggregation of *T. lepturus* can also be observed through a recreational angling competition which is held in the same location in the Hawkesbury estuarine system within the study region, where recreational anglers have consistently landed *T. lepturus* each year from 2014 to 2016. For aggregating species, there is the potential for catch rates to reflect fish density or abundance at particular locations or seasons rather than population (stock) abundance and there is the risk that elevated catch rates might be misinterpreted to reflect high stock abundance (hyperstability) when the reality is that the population (stock) is in decline (Rose and Kulka, 1999). Therefore, the catch rate parameters (kg per day) in this study should not be used in isolation for inferring the relative abundance of *T. lepturus* populations in south-east Australia.

The generally higher commercial fishery yield between January and June could also be explained by the aggregation of *T. lepturus* in NSW. There are two aggregations reported from different estuaries in NSW, as having a level of predictable by NSW DPI – Fisheries compliance officers and commercial fishers. They both coincide with the increase in commercial yield. Together this evidence suggests that some proportion of the *T. lepturus* population moves inshore at the start of summer and are likely to remain until mid-winter. The variability in commercial yield across years and months could also be explained by the aggregation of *T. lepturus* in NSW. While the reports from NSW DPI – Fisheries Observers, compliance officers and commercial fishers all report there is some level of predictability for *T. lepturus* aggregations, they also report that, in general the aggregations are unpredictable and patchy in time and space. These claims are supported by current media article and suggest there is variability in the abundance of *T. lepturus* populations in NSW.

The variability in commercial yield could be explained by environmental variability causing irregular recruitment to the local fishery of NSW. Environmental variability could explain the higher fishery yield for *T. lepturus* between January and June in south-eastern Australia. This time of year is generally associated with higher environmental productivity in south-eastern Australia, primarily due to the East Australian Current (EAC) (Suthers et al., 2011). The EAC is a poleward moving current that transports warm water from the Coral Sea in Queensland down the east coast of Australia into the Tasman Sea (Oke and Middleton, 2000). The EAC strengthens during the late summer and autumn months (December to May), then eases during the winter. When the EAC is at its strongest there is more nutrient rich upwelling occurring in the study region. (Hughes, 2012, Suthers et al., 2011). Environmental variability could also contribute to the variability across years. The majority of *T. lepturus* landed by the

NSW fishery are landed within the latitudinal range of 32°S to 35°S. This region is directly south of Seal Rocks (32.44°S), a section of coastline on the east coast of Australia that protrudes outward into the Pacific Ocean. At this location, the EAC will intermittently move away from the coast and start to move offshore and therefore, the current does not pass through the inshore waters of the high catch region (31°S to 35°S) and instead, the occurrence will generate cold or warm core eddies that are then sent along the coast of these regions (Suthers et al., 2011). During the years when the EAC is forming warm-core eddies and the eddies are circulating and transporting cold, particle-rich, productive waters to the study area. The increase in localised productivity is likely to draw shoals of bait fish to the region. Bait fish have been identified as major prey items for *T. lepturus* (Martins et al., 2005) therefore, the presence of shoals could draw *T. lepturus* to the region. If there are greater numbers of *T. lepturus* occurring in the immediate fishing grounds they are more vulnerable to fishing pressure, hence variable fishery yield (Young et al., 2001). Eddies formed by the EAC also entrain fish larvae and transport the larvae along the south-east coast of Australia (Mullaney and Suthers, 2013). The eddies allow the irregular immigration of fish larvae spawned in northern tropical waters, to populations in the temperate regions of NSW, for many of the region's fish species (Mullaney et al., 2014). It is possible *T. lepturus* populations in south-east Australia are supplemented with variable immigration of *T. lepturus* larvae from the tropical seas in the north, in turn causing year-to-year variability in commercial fishery yield.

The variability in commercial yield could also be explained by flexibility in life history traits and strategies causing irregular recruitment to the local fishery of NSW. Species with flexible life history traits are often efficient in regions where temperature anomalies and sudden temperature changes are common due to processes including variable ocean currents, cold and warm core eddies, convergence systems and coastal upwelling (Martins and Haimovici, 1997). *T. lepturus* have been reported as highly adaptable to temperature changes (Martins and Haimovici, 1997), to have non-specialised diets (Martins et al., 2005) and reported to display flexible spawning strategies that vary between years and vary with large-scale geographic segregation (Al-Nahdi et al., 2009). It has also been suggested that *T. lepturus* make large-scale movements participate in the spawning aggregation of a different spawning stock. The flexible nature of their life history could explain their irregular fishery landings due to irregular recruitment to the local fishery of NSW.

The variability in *T. lepturus* yield due to variable recruitment could also associated with global distribution and the south-east Australian population being at the lowest end of the global latitudinal range of the species. Therefore, the variability in yield could be related to the shared environmental characteristics, including latitudinal range, between the regions of south-eastern Australia and southern Brazil. Other shared characteristics include water temperature, temperature gradients and large variations in the abiotic characteristics of the environment and the environmental productivity (Bakhoum, 2007). T*. lepturus* is predominantly tropical to sub-tropical (Bakhoum, 2007) and the region with the highest fishery yield is the North-West Pacific Ocean, between 15°N – 65°N (FAO 2018). The global distribution of *T. lepturus* extends to the southern, temperate waters of Australia and Southern Brazil. In Brazil, they are a part of the fishery from the Cape of Santa Marta Grande (28°36’S) to Chui (34°45’S). Similar to Australia, the Southern Brazilian commercial fishery yield for *T. lepturus* is at a smaller scale, relative to the large industrial fisheries of the North-West Pacific, including China, Japan and Korea In Southern Brazil. (Martins and Haimovici, 1997). Also similar to Australia, Brazilian fisheries record periods of high yield for *T. lepturus* interspersed with lower catches (Martins and Haimovici, 1997, Vilar et al., 2011). Reports from Brazil suggest there are larger catches of *T. lepturus* with occurrences of temperature anomalies due to convergence systems between cold Antarctic currents and warm tropical currents (Martins and Haimovici, 1997). The shared environmental characteristics between southern Brazil and south-eastern Australia have also been credited for similar episodically high catches of yellowfin tuna (*Thunnus albacares*) in both regions (Martins and Haimovici, 1997, Young et al., 2001). It is possible the similarities observed regarding the variability in yield of *T. lepturus* between south-east Australia and Southern Brazil could also be related to shared environmental characteristics between these regions. The characteristics from both regions are associated with temperature anomalies, which increase particle aggregation/environmental productivity, attracting prey species and potentially drawing *T. lepturus* to the region. The variability associated with these anomalies could explain the variability in fishery yield for both regions due to variable recruitment of adults to the local fishery.

2.4.2 Length Distribution – Differences by gear, fishery sector, habitat and latitude

There was a significant difference observed in the length distribution of *T. lepturus* landed by commercial line fishing and commercial trawl fishing, however the difference between length distributions did not appear substantial. The finding therefore indicates that the two commercial fishing methods are not likely to be exploiting different components of the population. Significant differences were also found in the length distributions between commercial and recreational sectors indicating there were different population components exploited by the two sectors. The fish landed at the 2016 'HBA learning Hairtail Social' competition in the Hawkesbury River were all larger than 30 cm PL and, on average, significantly larger than the overall commercial catch for NSW, which is likely attributable to gear selectivity. Recreational anglers select their hook and bait size to target large *T. lepturus*, whereas the commercial fishery uses a larger variety of fishing gear including line and trawl. The smaller length classes observed in the commercial fishery were therefore likely to be under-represented in recreational catches due to selection for large individuals. Commercial Observers from NSW DPI Fisheries on trawl vessels off the NSW coast have observed catches of small *T. lepturus* and subsequently, return of this fraction of the catch. The individuals are returned due to the fragility of small *T. lepturus* and the damage that occurs when trawled, which is likely to dramatically reduce their price value at market.

The differences in length distributions observed between the two latitudinal zones, separated by ~83 km, and between the Hunter estuarine habitat and the Hunter coastal habitat, suggest there may be spatial length-structuring in the population. However, the spatial comparisons between latitudinal zones made in this study, may have been confounded by fishing gear, given that trawl fishing was predominant in the Hawkesbury estuarine and Hunter coastal sites and line fishing was predominant in the Hunter estuarine site. For the comparison between the Hunter coastal and Hunter estuarine sites, gear was standardised by sub setting the data to only similar gear types, therefore the confounding factor of fishing gear was controlled. For the spatial comparison between latitudinal zones, the limitations in the sampling did not allow for spatial comparisons to be limited to one gear type and therefore, gear may have been a confounding factor. Potential sampling bias in the length distribution data collected for *T. lepturus* in NSW has been highlighted with these findings and the bias needs to be accounted for if the length distribution data is utilised to quantify life history parameters relating to population productivity and resilience.

2.4.3 Recreational fisheries yield

T. lepturus in South-eastern Australia has been identified as an important recreational species (Stewart et al., 2015), however, an estimation of the recreational yield for *T. lepturus* in NSW using the four recreational fishing surveys, carried out in the region in the last 20 years was not possible. Regardless of the differing methodologies implemented among each of the four studies examined in the current work, the surveys did not detect *T. lepturus* as a species landed in large quantities by recreational fishers (Henry and Lyle, 2003, Steffe and Murphy, 2011, Steffe et al., 2005a, West et al., 2015). The near absence of *T. lepturus* from recreational surveys could give a false indication that the recreational fishery for *T. lepturus* in NSW is inconsequential. However, in this study the approximate harvest of *T. lepturus* by recreational anglers in the lower Hunter River over an approximately 3-month period, was a catch weight of around 21 t. This outweighed the commercial annual yield by weight averaged over the previous 5 years (2010 – 2015) at 13.6 (± 2.8) t. This approximation provided an indication on the scale of recreational landings and therefore, supported the hypothesis that the recreational fishery for *T. lepturus* was being underestimated by the standard recreational survey designs in south-east Australia. The survey demonstrated the species was exploited by the recreational fishery in significant quantities and the sector potentially makes up a large proportion of the overall landing of the species in NSW, at least during some years. While the information used for this approximation was very general, not intended for this purpose, and relied on several assumptions, it did provide a snapshot of the recreational yield at one location and one point in time, and a reasonable look at the potential relative magnitudes of the commercial and recreational fisheries in some years. However, since the information was gathered in response to a fishing anomaly (the high-yield fishing event), these results cannot be extrapolated to infer generally higher yield from recreational angling relative to commercial fishing of *T. lepturus* in NSW. If yield and length-frequency data from the recreational fishery are left out of fishery assessments, it can result in biased and inaccurate estimates. If fishery and ecosystem management is informed with inaccurate fishery estimates, the strategies implemented by management for sustainable populations and ecosystems could be insufficient and result in the local depletion of populations (Hilborn and

Walters, 1992), or have detrimental impacts on the health of ecosystems (Mapstone et al., 2004). For example, in Australia in 2009, a commercial TAC was implemented for blue eyed trevalla using only the commercial catch. When the recreational catch data was included in the assessment, the commercial TAC was subsequently reduced by 10% (Griffiths and Fay, 2015), to account for the increased total mortality on the population. The importance of incorporating recreational fishery data into fishery assessments and the type of sampling design used to assess a recreational fishery varies among species and regions (Griffiths and Fay, 2015). It is therefore important to characterise multi-sector fisheries before assessment and monitoring strategies are implemented. Bases on the results from the current study, further research on the recreational angling for *T. lepturus* in NSW is recommended to determine its contribution to population mortality.

The recreational angling ‘high yield fishing event’ observed in this study in the lower Hunter River over an approximately 3-month period, was a clear indication of species aggregation. This study did not provide the scope for elucidating the biological or environmental function for the aggregation. Fish populations might aggregate for shelter, to avoid predators, to migrate, to feed or to reproduce (Domieir, 1997, de Mitcheson, 2011). The 30 individuals collected from this event were assigned to the macroscopic maturity stage 2 (see Chapter 3) and were not considered mature, therefore the specific aggregation was not likely related to spawning. Successful fishing may suggest the purpose of the aggregation was feeding related. There may have been warm-core eddies transporting cold, particle-rich, productive waters to the study area, drawing in shoals of bait fish and consequently drawing in *T. lepturus*.

This chapter highlights the potential value of a specialised sampling technique for localised high-catch events, where sampling is targeted to the recreational anglers participating in the event and the yield they obtain. Because the use of standard survey designs has been ineffective for sampling the recreational fishery for *T. lepturus* in NSW by overlooking substantial catch events, specialised sampling could be considered as an ongoing strategy for monitoring the recreational fishery for *T. lepturus* in NSW. The monitoring strategy could be feasible for *T. lepturus* because anecdotal evidence collected from recreational anglers, compliance officers from the NSW Department of Fisheries and community members, indicate that the recreational, high-catch events for *T. lepturus* in NSW, will often reoccur in the same location at predictable times of the year. If high-catch events are consistent, it can be feasible to monitor for pre-determined thresholds at specified locations and times of year. If thresholds such as the number of fishers at a particular wharf, or number of mentions of the target species on social media are met then enumerators can be implemented to collect data on the catch by weight of *T. lepturus* taken by recreational fishers, at set times throughout the day or night (Moore et al., 2015). These types of strategies are recommended for *T. lepturus* in NSW because If recreational angling is not closely monitored then substantial harvests could continue to go unnoticed and could contribute to an unsustainable level of fishing mortality.

The engagement of local stakeholders in the process would provide the added benefit of generating trust and a sense of community ownership of any potential management strategies (Griffiths and Fay, 2015, Griffiths et al., 2013). If local fishers are involved in the formulation of the strategy, they are more likely to feel a moral obligation to comply with rules and encourage compliance to others in the community (Pomeroy and Douvere, 2008). An alternative sampling design would be cluster sampling design to collect representative data for species that demonstrate spatial and temporal heterogeneity (Gallucci and Hariharan, 2009). The aim for this type of design is to maximise the observations of a species that would otherwise go undetected. The methodology differs from traditional methods by sampling representative ports as usual with standard port-monitoring programs, then adjusting the spatial range of the sampling design to focus on the vicinity where observations have been made (Gallucci and Hariharan, 2009).

2.5 Conclusion

Variability in the fishery yield for *T. lepturus* in south-east Australia has been confirmed in this study and potentially arises from the sporadic aggregation, migration or recruitment of *T. lepturus* in south-east Australia. The variation in fishery yield through time and between commercial fishing gears, habitats, latitudinal zones and sectors, makes it unlikely that catch rate data will be representative of relative population abundance. A stock assessment for *T. lepturus* in NSW would lack accuracy if it was primarily based on commercial catch data, this could leave populations vulnerable to increases in anthropogenic pressure such as fishing pressure, habitat degradation and climate change. To successfully monitor *T. lepturus* populations in NSW a dedicated recreational fishing survey designs is recommended and a consistent annual design for sampling the commercial fishery. It is also recommended that ongoing sampling of the commercial and recreation catch includes biological data, to allow for population modelling such as length at maturity and growth curves to inform on the population life history traits and productivity to a local population decline for *T. lepturus* in NSW.

Chapter 3: Life-History and Demographic Characteristics of Largehead Hairtail (*Trichiurus lepturus*) in South-Eastern Australia.

3.1 Abstract

In this study, life history traits of *T. lepturus* from south-eastern Australia are described for the first time, providing insights into the potential productivity of the population. Samples collected ranged in length from 12 - 78 cm pre-anal length (PL) and 44 cm to 193 cm estimated total length (TL). The sampled length distributions differed between sexes and the largest individuals in the collection, from 56 cm PL to 78 cm PL were all female. The lengths at which 50% of females and males reached sexual maturity (L_{50}) were 40.15 cm PL and 40.65 cm PL respectively. The mean gonado-somatic index for females (GSI) peaked in June at 1.9% and elevated GSIs suggested a spawning period from approximately June to September, during winter and into the start of spring. Male GSIs and macroscopic maturity staging displayed similar patterns, in support of the estimated spawning period. Females dominated the samples, with a F:M sex ratio of 1: 0.4. Females dominated sampling from estuarine and coastal habitats as well as during the spawning and non-spawning periods. There was significant variation in the sex ratios among catch events, with one event being dominated by males, while others were entirely female. The relationships between PL and body weight (BW) differed between males and females and differed between the collection of *T. lepturus* from south-eastern Australia and a collection from the Arabian sea off Oman. Otolith annual increments indicated an age range from 0 years to a maximum of 8 years. The age-length relationship was estimated with von Bertalanffy growth (VBG) parameters of L_∞ (76.3 PL/189.3 TL), k (0.13 yr-1) and t_0 (-0.99 yr) and the modelled growth rates did not differ between sexes. Individuals reached 7, 24, 30, 36, 41, 45, 49 and 52 cm PL by the end of year 1–8, respectively. The literature was reviewed for the life history traits of populations globally and the parameters were compared with the south-eastern Australian population regarding population productivity. The life history traits of *T. lepturus*, including a larger maximum body length, longer lifespan, and larger body length at maturity indicate a population with less fishing pressure than in other areas, while the slower individual growth rate and shorter spawning period suggest that the population of *T. lepturus* in south-eastern Australia may be

less productive and therefore, potentially more vulnerable to increasing anthropogenic pressure than populations in other global regions.

3.2 Introduction

T. lepturus has a global distribution and there are large differences in the fishery characteristics for *T. lepturus* across global regions. *T. lepturus* distribution extends from the temperate waters of approximately 60°N throughout equatorial waters to the southern temperate waters of South America, South Africa and southern Australia, approximately reaching 45°S (Al-Nahdi et al., 2009, Carvalho and Luque, 2011, CHIOU et al., 2006, FAO, 2018, Shih et al., 2011) (Figure 13). The species' occurrence in fisheries is most frequent within the Food and Agriculture Organization (FAO) fishing area 61 (15-65°N, 110°E-175°W), in the North-west Pacific, with China consistently taking the highest proportion of global landings, typically between 60-80%, followed by Korea. *T. lepturus* is reported to have a high abundance between 25°N and 35°S (Martins and Haimovici, 1997), however, within this latitudinal range, there is a large variation in the fishery yields across different countries (Table 3). While there are substantial yields from countries bordering the north-west Pacific Ocean, there are relatively low yields from the US and Mexico (Table 3). When the fishing effort is not reported with catch it is difficult to infer relative abundance of populations among countries; however, the substantial landings reported throughout the regions of the north-west Pacific Ocean do suggest high relative abundance in these regions. Reports of over-exploitation and localised depletions from China, India, Japan and Korea provide evidence that the fishing pressure in these countries is likely to have been excessive (Zhang et al., 2018, Ghosh et al., 2009, Watari et al., 2017, Kim et al., 2005).

Table 3. Comparison of reported mean yield of ribbonfish and *Trichiurus lepturus* from global fisheries.

~Latitude	Country/region	Annual catch weight	Citation
31-41°N	China/North east China (Yellow Sea and Bohai Sea)	**73,000 – 910,000 t** from 1995-2007	(Wang and Liu, 2013)
30-42°N	Korea/ all Korea	**96,311 t** from 1975-2004	(Kim et al., 2005)
24-44°N	US/ All US	**21 t** in 2004	(Bryan and Gill, 2007)
24-44°N	US/ All US	**13 t** from 1991-2014	(De la Cruz-Torres et al., 2014)
22-35°N	Taiwan/ South west Taiwan	**165 t** from 1998-2002	(CHIOU et al., 2006)
23-25°N	Pakistan/ All Pakistan	**31 623 t** in 1999 and **20 375 t** in 2009	(Muhammad et al., 2017)
20-24°N	India/ North west India (Gujarat)	**49,190 t** of ribbonfish from 2007-2010 (*T. lepturus* form major component of ribbonfish catch)	(Ghosh et al., 2014)
20-24°N	India/ North west	**33,000 t** from 1995-2000	(Khan, 2006)
13-22°N	India/ North-east India	**44,336 t** of ribbonfish from 2007-2010 (*T. lepturus* form major component of ribbonfish catch)	(Ghosh et al., 2014)
13-22°N	India/ North-east India	**31,944 t** by trawl, from 2007-2010	(Ghosh et al., 2014)
21°N	India/ Veraval	**18,813 t** by trawl, from 2003-2006	(Ghosh et al., 2009)
20-24°N	India/ North west India (Gujarat)	**42,649 t** by trawl, from 2007-2010	(Ghosh et al., 2014)
20-24°N	India/ Gujarat	**58,196 t** of ribbonfish, from 2002-2006 (*T. lepturus* form major component of ribbonfish catch)	(Ghosh et al., 2009)
18.5°N	Mexico/ State of Tabasco	**40 – 300 t** from 2000-2014	(De la Cruz-Torres et al., 2014)
17° N	India/ Kakinada	**1,047 t** ribbonfish by trawl, during 1990s (*T. lepturus* 92% of ribbonfish catch)	(Abdussamad et al., 2006)
8-22°N	India/ all India	**111,000 t** ribbonfish in 1992 (*T. lepturus* form major component of ribbonfish catch)	(Reuben et al., 1997)
16-19°N	Oman/ Al-Duqum and Salalah	**5815 t** from 1995–2005	(Al-Nahdi et al., 2009)
28-37°S	Australia/ NSW	**35 t** from 1986–2015	(Current study)

Excessive fishing pressure has resulted in the over-exploitation of *T. lepturus* stocks in some regions. *T. lepturus* was reported as overfished in the North-West Pacific Ocean (FAO 2016), which is a region that hosts the fisheries of multiple countries including China, Japan, Korea and Taiwan. There are also reports of over-exploitation and local depletion of *T. lepturus* from each of the countries of China (Zhang et al., 2018), India (Ghosh et al., 2009), Japan (Watari et al., 2017) and Korea (Kim et al., 2005). A stock assessment from the Bungo Channel of Japan carried out between 2006 and 2011 indicated that the biomass of populations was decreasing (Watari et al., 2017) and a report from India indicated there was over exploitation of *T. lepturus* in the waters off Veraval, India between 2003 and 2006 (Ghosh et al., 2009).

T. lepturus has a history of taxonomic uncertainty. For a time*, T. lepturus* was thought to be part of a species complex including *Trichiurus lepturus, Trichiurus nanhaiensis* and *Trichiurus japonicas* (Shih et al., 2011)*,* but the complex has recently been confirmed as three distinct species in the North-West Pacific ocean (Hsu et al., 2009, Wang et al., 2017). According to Froese and Pauly (2019), there are nine valid species under the genus *Trichiurus*, including *T. lepturus, T. australis and T. nanhaiensis.* However, FAO continues to report all the *Trichiurus* species under the name *Trichiurus lepturus* in global fisheries statistics reports (Wang et al., 2017). The samples collected for the present study were all confirmed as *T. lepturus* by observing dorsal fin colour (Wang et al., 2017), morphological measurements, and gill cusps (Chakraborty et al., 2005, Tzeng et al., 2007). Some of the global studies reviewed in this paper classify the species as *T. lepturus*, but do not specify their species identification procedure and therefore, it is possible their sample collections were made up of multiple *Trichiurus* species that might display different life history traits.

T. lepturus across all global regions have been reported as a relatively short-lived and fast-growing species, therefore increasing the relative productivity of their populations and consequently decreasing their vulnerability to local population decline (Del Toro, 2001). However, there is little agreement among global literature on the maximum body length, length at maturity, length-weight relationship, spawning period and spawning strategies of *T. lepturus* populations, all which influence productivity. Global *T. lepturus* populations have been reported to have flexible reproductive strategies, depending on latitude (Nahdi 2009). Females often spawn more than once in a reproductive season and Kwok and Ni (1999) have reported group-synchronous spawning behaviour. In addition to the varied reporting on life history traits and spawning strategies between countries, Shih et al. (2011) has reported significant differences in life history traits among three *Trichiurus* species from the same region in the East China Sea*, T. japonicus*, *T. nanhaiensis* and *T. lepturus.* The different species were found to have different growth rates, length at maturity, spawning periods, sex ratios and spawning strategies. Numerous studies have reported that sex ratios favour females, especially in larger length classes (Al-Nahdi et al., 2009, Ghosh et al., 2014, Kwok and Ni, 1999). In southern Brazil, females dominated sex ratios, dependent on the location and season. Martins and Haimovici (2000) used seasonal changes in sex ratios to make conclusions on a local population potentially dividing during some seasons to implement two different reproductive strategies, which could mediate potential risks posed by detrimental perturbation. Martins and Haimovici (2000) also suggested a further need for more published data on sex ratios at lower latitudes.

In contrast to other global regions, the fishery for *T. lepturus* in south-eastern Australia, which includes a commercial trawl and handline fishery as well as a recreational fishery, has a generally low yield; however, on occasion catches are substantial. For the commercial fishery between 1991 and 2015, the annual yield was relatively low at an annual mean of 35 t (Chapter 2). Annual yield has been variable, and during some years, the yield was very low, in particular, in five out of 29 years, less than 1 tonne was reported. In some years, the yield is relatively high, peaking at 161 t during the financial year of 1990/91, when the annual yield was 4.6 times higher than the average.

As the demand for commercially and recreationally fished species in south-eastern Australia increases, the exploitation of *T. lepturus* and thus the pressure on its population will presumably increase. The population declines of *T. lepturus* reported in the north-west Pacific indicate the potential vulnerability of *T. lepturus* to fishing pressure, highlighting the need to understand the vulnerability of populations in south-eastern Australia.

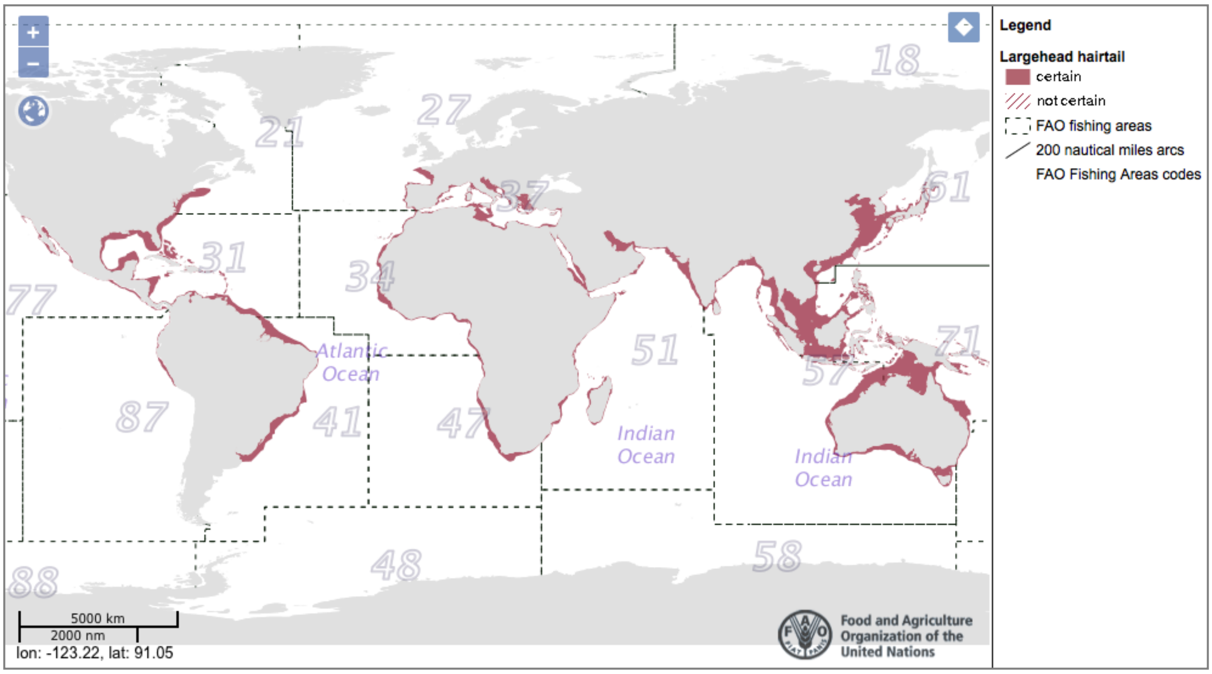


Figure 13. Geographic distribution of *Trichiurus lepturus* based on all reported fishery catch weight (FAO, 2018).

T. lepturus has a formal stock status of 'undefined' in NSW, due to a limited understanding of the population demographics in south-eastern Australia (Stewart et al., 2015). The demographics and life-history traits of the south-eastern Australian population may differ from populations in other global regions, given the taxonomic uncertainty, the relatively low yield of Australian fisheries compared with the yield of fisheries in the North-West Pacific, and the large-scale geographic separation of the south-east Australian population from the sampling regions where demographics have been reported to date. It is therefore pertinent to describe the demographics of the local population of *T. lepturus* in south-eastern Australia, and how these demographics can inform on the productivity and vulnerability of *T. lepturus*.

The main objectives for this study were to describe and investigate the maximum length, longevity, length at maturity, spawning period, sex ratios, length-weight relationship and growth, including whether otolith weight of *T. lepturus* in NSW grow linearly with somatic growth. The study also aimed to compare the length frequency distributions, growth curves and length-weight relationships of the population between sexes, compare the length-weight relationship of the south-eastern Australian population with a population from the Arabian Sea and investigate sex segregation inside and outside the reproductive season and between estuarine and coastal habitats.

3.3 Materials and Methods

3.3.1 Sample collection

Most of the commercial catch for *T. lepturus* in NSW is reported from three locations and sampling for the current study therefore focused on these locations. There were two estuarine sites and one ocean site: (1) the Hawkesbury Estuarine site at 33.55° S; 151.33° E; (2) the Hunter estuarine site at 32.93°S, 151.78° E’ and; (3) the Hunter coastal site 32° S – 33° S, 151.80° E – 152.65° E (Figure 14). The coastal fishery extends to 3 nautical miles offshore and the Hunter Coastal site overlaps the latitudinal range of the Hunter estuarine site. The Hawkesbury estuarine site is located approximately 83 km south of the Hunter estuarine site and the Hunter coastal site. The Hunter estuarine site is part of a river system with freshwater habitats at the upper reaches. The mouth of the Hunter River enters the Tasman Sea at the city of Newcastle and is a major industrial port and an important harbour for recreational activities including recreational fishing. The Hunter estuarine site is a bar-built estuary, with a sandbar built up by ocean waves at the mouth of the estuary, which restricts the tidal inlet from the coast (Roy et al., 2001). Compared with tidal-dominated estuaries, the estuary is shallow (average river depth: 3.3 m) and has a strong riverine influence. The Hawkesbury estuarine site is a part of the Hawkesbury River system, which also has a freshwater, riverine influence; however, in contrast to the Hunter River, it has a wide mouth and therefore the riverine influence is less prominent (Roy et al., 2001). The Hawkesbury estuary enters the Tasman sea at the city of Sydney, the most populated city of Australia and therefore, similar to the Hunter estuary, is also an important estuary for recreational fishing (Roy et al., 2001). The coastal waters of eastern Australia between 32°S – 33°S are affected by wind-driven coastal upwelling and the East Australian Current, a poleward-moving current that transports warm water from the Coral Sea off Queensland down the east coast of Australia into the Tasman Sea (Oke and Middleton, 2000). The speed and intensity of the current varies according to multiple factors and therefore causes considerable variability in the characteristics of the coastal habitats of south-eastern Australia (Hughes, 2012, Suthers et al., 2011).

T. lepturus samples from the commercial fishery were obtained from September 2015 to December 2016, approximately every one to two weeks and, depending on availability, a random sample of up to 10 fish were collected from each of the three sites. Supplementary samples were obtained from an annual recreational fishing competition for *T. lepturus*, held between the 1st and 3rd of July 2016. The engagement of anglers at the event resulted in 83 angler-donated *T. lepturus* individuals from the Hawkesbury estuarine site. A second contribution of recreational angler-caught samples was provided by the NSW Fisheries Department compliance officers, after being seized from recreational anglers exceeding the enforced bag limit of 10 fish per person per day. All compliance-seized samples were caught within approximately 75 days, between mid-February and mid-April 2016, at the Hunter estuarine site. Twelve additional individuals were donated from an offshore trawl observer program run by the NSW Fisheries Department. These individuals were caught as bycatch on a commercial trawl vessel and would not have been retained for market due to their small size.

3.3.2 Length composition and maximum lengths

The pre-anal length (PL) of each individual was recorded to the nearest cm, from the tip of the lower jaw to the anal vent. PL is a more reliable length measure than total length (TL) because the tail of *T. lepturus* is prone to breaking (Al-Nahdi et al., 2009, Khan, 2006). The relationship between PL and TL of 356 intact fish (TL = 2.254 x PL + 17.322), detailed in Appendix A, was used to estimate the TL of all individuals. TL was estimated to allow for direct comparisons with studies that have only reported on TL. The length frequency distributions of male and female *Trichiurus lepturus* from the biological collection were compared using a Kolmogorov-Smirnov test.

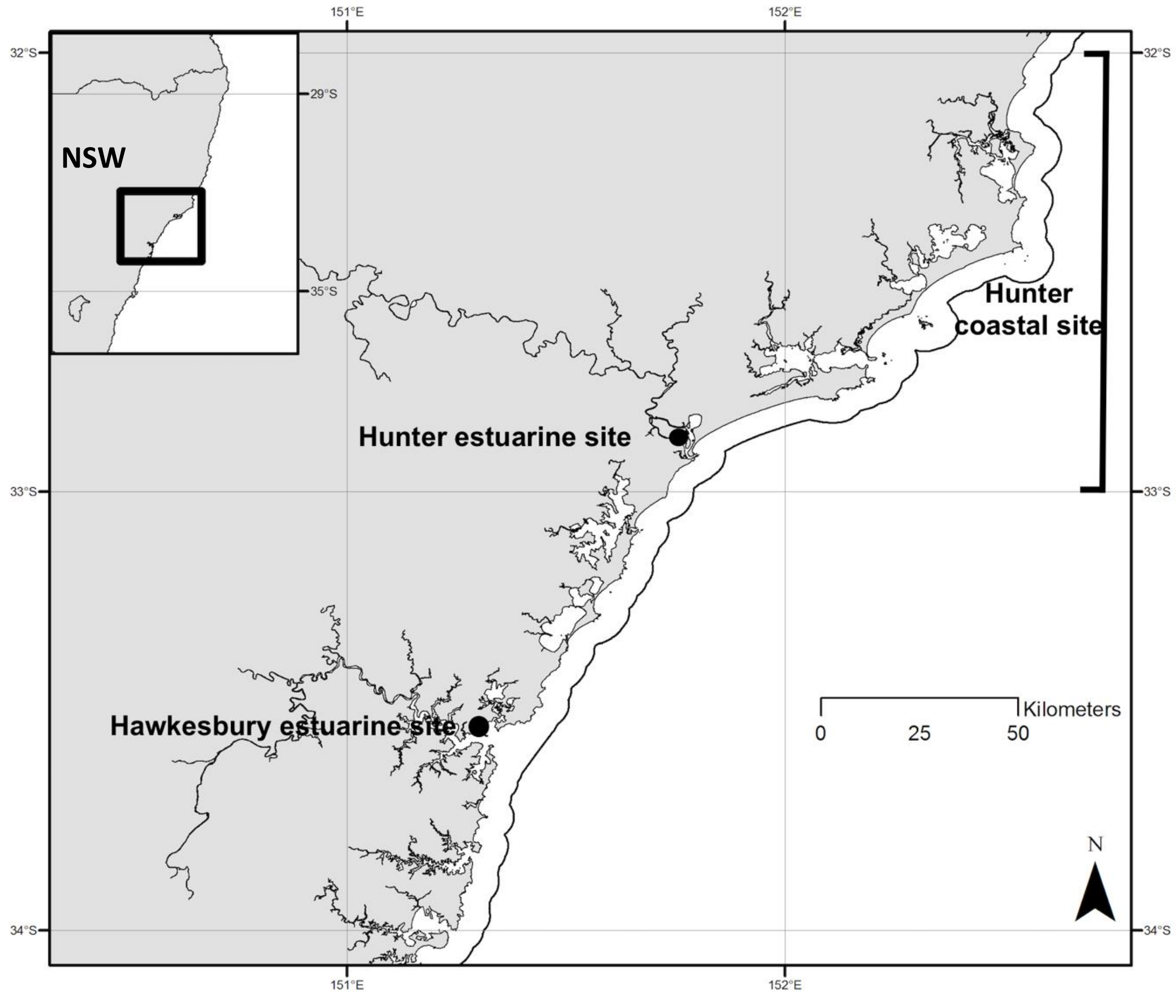


Figure 14. Map of the south-eastern Australian coast showing the three sites *Trichiurus lepturus* were collected between 2015 and 2017: Hunter estuarine and coastal sites and Hawkesbury estuarine site. The coastal site extends to 3 nautical miles from the coast, marked by a black line.

3.3.3 Age estimation using sagittal otoliths and longevity

The sagittal otoliths were excised, cleaned and stored dry. One otolith from each individual was weighed (to the nearest 0.00001g) using an electronic balance (Sartorius Australia PTY LTD) and embedded in a block of epoxy resin. The left otolith was selected for embedding wherever possible, but when necessary the right was used due to demonstrated consistency in the annual increments between otolith pairs of *T. lepturus* (Kwok and Ni, 2000, Shih et al., 2011). In order to expose a transverse section of the otoliths, a single diamond-embedded blade was used on a Gemmasta high-speed saw. The sections were then adhered to glass slides and ground down to a thickness of ~0.2 mm with 1200 grit polishing paper on a Struers model LaboPol-4 to reveal the otolith core (Campana, 2001). Kwok and Ni (2000) used the marginal increment method to validate the alternating opaque and hyaline growth increments on transverse sections of *T. lepturus* otoliths as annual increments. In the present study, the sections were viewed under reflected light on a black background. The methods of Kwok and Ni (2000) were followed to identify the first opaque increment and to count the opaque annual increments along the dorsal lobe to provide an age estimation in years. To avoid reading bias, each otolith was read without knowledge of the fish length or date and place of sampling. The relationships between pre-anal length and otolith weight and between estimated age and otolith weight were investigated to explore any potential use of otolith weight for the determination of age-structure of *T. lepturus.*

3.3.4 Maturity

The wet weight (W) of each individual was recorded, the gonads were weighed and assigned as either female or male and placed into categorical stages between 1 and 5 based on macroscopic characteristics. Stages 1 and 2 were categorised as immature and stages 3, 4 and 5 were categorised as mature (Table 4). Mature specimens were defined as females with visible oocytes through the ovarian wall and males with enlarged, opaque testes with a discernible tapered edge running the length of the teste.

Verification was carried out by identifying the microscopic characteristics of a subset of gonads from each sampling site, each sex, a range of lengths and from each of the 5 maturity stages. Gonads were kept in a fixative solution of 10% formaldehyde for histology and then transferred to ethanol. The fixed gonad tissue was embedded in paraffin wax and a 5 µm cross-section was taken through the middle of the gonad and stained with haematoxylin and eosin. Histological sections were examined under a compound microscope and a microscope mounted camera was used to collect images of each gonad stage. Microscopic staging criteria for each stage are detailed in Table 4.

Female mature specimens were defined with vitellogenic and migratory-nuclei and/or hydrated oocytes in their ovaries. Males were deemed mature only if spermatozoa were present in the seminiferous tubules.

The length at which 50% of all specimens were sexually mature (L_{50}) was estimated for males and females separately using a logistic function applying the maximum likelihood method (Haddon, 2010). Individuals from inside and outside the spawning season were used in the analysis. The logistic curves for maturity for each sex were compared using the bivariate form of Wald’s F-test (Kotz, et al., 1982 cited by Macbeth et al., 2005).

Table 4. Macroscopic and microscopic characteristics of male and female gonads of *Trichiurus lepturus,* following the methods of Kwok and Ni (1999) and Shih et al. (2011).

Female		
Stage	**Macroscopic characteristics**	**Microscopic characteristics**
1. Juvenile	Very small. Small thread-like strand. No eggs visible. Difficult to determine sex.	Tissue is made up of primary growth oocytes, chromatin nucleolar and perinucleolar oocytes, the nucleus takes up a large proportion of these small cells. At the later stage of primary growth, nucleoli form at the periphery of the nucleus.
2. Developing	Larger than stage 1, approximately 50-70% of the ventral cavity. Orange in colour and tube-like shape allows an obvious distinction of female ovaries from male testes. Oocytes are not visible through the ovarian wall.	Cortical alveolar oocytes have developed, the first small yolk granules are forming in the cytoplasm. primary growth oocytes are still present.
3. Mature/ripe	Larger than stage 2, usually extending the full length of the ventral cavity. Orange colour and tube-like shape. Oocytes are visible through the ovary wall. Oocytes are opaque.	Vitellogenic oocytes have developed, there are more yolk granules in the cytoplasm and they are increasing in size. In the later stages the yolk begins to fuse together and migrates from the centre of the cell to the edge.
4.Spawning (running ripe)	Larger than stage 3. Large, hydrated ova can be seen through the ovary wall. Oocytes are large and almost translucent. Oocytes are shed through the anal vent when gentle pressure is applied to abdomen.	Running ripe oocytes are large and appear uniform due to the cell being filled with the fused, hydrated yolk mass.
5. Spent	Smaller than stage 4. Flaccid and bloodshot.	Tissue is predominantly made up of post ovulatory follicles, difficult to distinguish from stage 2.
Male		
1. Juvenile	Very small. Small thread-like strand. Difficult to determine sex.	Spermatagonia is predominant in the tissue with the initial development of spermatocytes and spermatids
2. Developing	Larger than stage 1, approximately 50-70% of the ventral cavity. Pale pink colour. Mostly translucent. Obvious distinction of male testes from female ovaries.	Spermatogenic cysts have formed and contain spermatogonia. Spermatozoa has developed
3. Mature/ripe	Larger than stage 2, usually extending the full length of the ventral cavity. Pale pink or cream colour. Opaque and milky. There is an obvious tapered edge that runs along the length of the teste.	Tissue predominantly made up of spermatozoa. Late stage spermatids also present within spermatogenic cysts.

4.Spawning (running ripe)	Larger than stage 3. Pale pink or cream colour. Opaque and milky. There is a discernible tapered edge that runs along the length of the teste. Milt is shed through anal vent when gentle pressure is applied to the abdomen.	Larger proportion of tissue has developed into spermatozoa. Spermatids have developed further.
5. Spent	Smaller than stage 4. Pale violet in colour. Flaccid and bloodshot.	Reduced volume of residual spermatozoa.

3.3.5 Reproductive season

The gonadosomatic index (GSI) was calculated for each male and female where a gonad weight was taken, using the formula $[W_g (W_w - W_g)] \times 100$, where W_g is the gonad weight and W_w is the body weight. The monthly proportion of individuals at each maturity stage, assigned by macroscopic gonad staging, was calculated for all individuals where gonad staging was possible.

3.3.6 Sex segregation

To investigate sex segregation of *T. lepturus* in NSW, sex ratios were investigated for smaller and larger individuals, based on the length related difference in sex ratios observed by Al-Nahdi et al. (2009) in the Arabian sea off Oman. Sex ratios were also investigated between the reproductive season and the non-reproductive season and between estuarine and coastal habitats based on the spatial and temporal patterns of sex segregation reported by Martins and Haimovici (2000) from Southern Brazil. Chi-square tests were used to test whether sex ratios deviated from the expected ratio of 1:1 during the spawning season and during the non-spawning season. Further Chi square tests were used to test whether sex ratios deviated from the expected ratio of 1:1 in the Hunter coastal habitat and the Hunter estuarine habitat. $P < 0.05$ was considered significant for all tests. Sexual segregation was subsequently examined among catch events because it was the sampling unit with the best resolution in time and space. A catch event was defined as a catch by a commercial fisher on a single day and at a single location. A Chi square test of independence was used to quantify the difference between the observed and expected sex ratio of each catch event, based on the pooled sex ratio of the commercial collection of *T. lepturus* in NSW from 2015-2017.

3.3.7 Length-weight analyses

The length-weight relationship for *T. lepturus* in NSW was estimated with non-linear regression, using the equation $W = a L^b$, where W is the body weight (g), L is the body length (cm), 'a' is a coefficient related to body form and 'b' is the growth exponent (Chakravarty, Pavani, & Ganesh, 2012; Ghosh, Pillai, & Dhokia, 2009; Muhammad et al., 2017). Analysis of covariance (ANCOVA) was used to test whether the length-weight relationship differed between sexes. Weight was treated as the response variable, while sex with two levels was treated as an independent factor and length was treated as a covariate. An interaction term between sex and length was included to test for a difference in the rate of weight increase with length between the sexes and to verify the assumption of homogeneity of regression slopes. If the interaction term was not significant, the term was eliminated and the ANCOVA was rerun. The data were log transformed prior to analysis to meet the assumption of linearity. A similar approach was used to test whether the length-weight relationship differed between the regions of south-eastern Australia and the Arabian Sea off Oman. The raw total length and weight data were available from a recently published research paper on *T. lepturus* in the Arabian sea, off Oman (Nahdi et al., 2016) and were used to make a direct comparison with the length (calculated total length) and weight data from south-eastern Australia. The sex was not specified for the data published from Oman so the data for both sexes from south-eastern Australia were pooled for a regional comparison. In this analysis, region with two levels (Oman, NSW) was treated as the independent factor. The data were log transformed and truncated to approximately equal length ranges, between 66.69 cm TL and 134.28 cm TL, from each region. An ANCOVA was used to compare regressions between regions with the same design as the previous ANCOVA used for the comparison between sexes.

3.3.8 Growth

The von Bertalanffy growth function (VBGF) was fitted to the length-at-age data using the equation $L_t = L_\infty [1 - e^{-k(t-t_o)}]$, where L_t is length (cm) at age t, L_∞ is the asymptotic length (cm PL), k is the rate at which the curve approaches L_∞ (year^{-1}), t is age (years), and t_o is the theoretical age of the fish at zero length. The curve was used to model growth for pooled sexes and then for males and females. The growth curves for both sexes were compared using the analysis of residual sums of squares (ARSS) method (Chen et al., 1992).

3.4 Results

3.4.1 Sample collection

A total of 439 individuals were used for demographic investigation. Gonads were weighed and assigned macroscopic maturity stages for 431 individuals, otoliths were collected from 429 individuals and weighed, age estimations in years were made for 418 individuals.

3.4.2 Length composition and maximum lengths

Individuals ranged from 12 - 78 cm PL and 44 cm to 193 cm estimated TL and the majority (86.53%) were between 30 and 60 cm PL. Length-frequency distributions differed between males and females during the sampling period (Figure 15, Kolmogorov-Smirnov D= 0.16, P= 0.0021). Female fish had a larger length range with PL ranging 12 cm to 78 cm, while male fish PL ranged from 30-56.

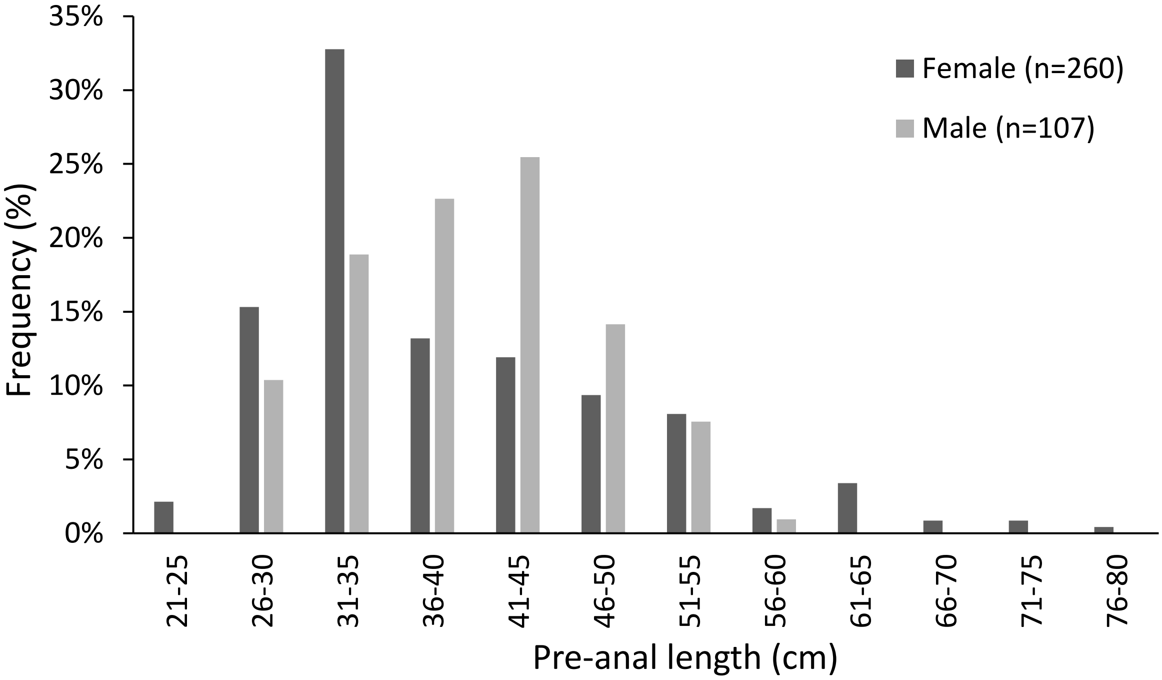


Figure 15. Length frequency distribution of male and female *Trichiurus lepturus* collected from the commercial fishery in south-eastern Australia between 2015 and 2017. Individuals are from all three collection sites and all fishing methods. Only individuals that could be macroscopically assigned to a sex were included.

3.4.3 Age estimation and longevity

Sectioned sagittal otoliths, viewed under reflected light, had an opaque core and subsequent broad hyaline increments (Figure 16). Otolith annual increments indicated an age range from 0 years to a maximum of 8 years and 4 years was the most numerous age group (27.51%). The relationship between otolith weight (OW) and PL was best described by the linear relationship OW= 0.0005 (PL) - 0.0056 (Figure 17 r^2= 0.92), indicating that otoliths of *T. lepturus* in NSW grow linearly with somatic growth. The relationship between OW and estimated age was described by the linear relationship OW= 0.0024 (age) + 0.0018 (Figure 18, r^2 = 0.48), also giving the indication that otoliths continuously increase in weight, throughout the life of the individual.

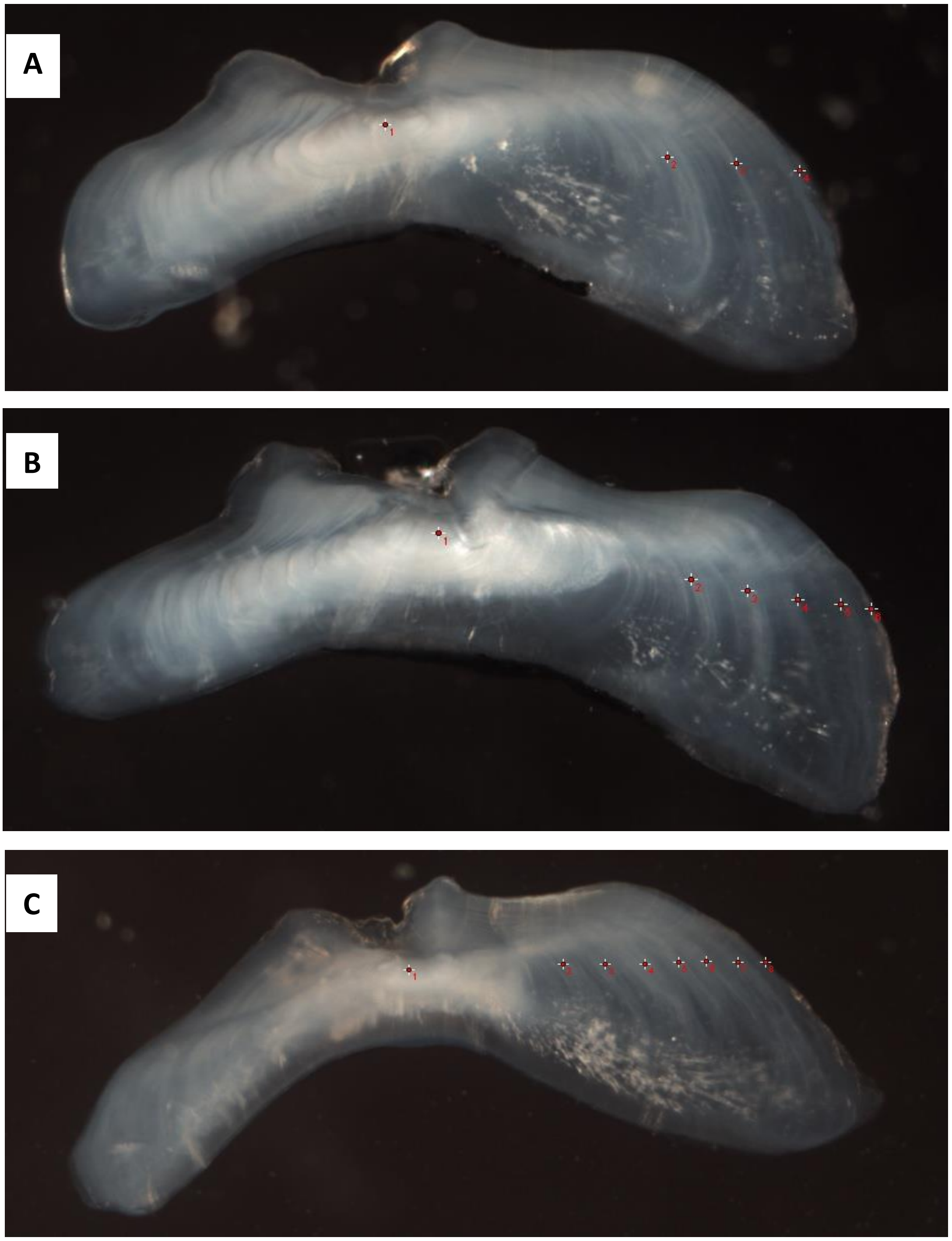


Figure 16. Sectioned otoliths of *Trichiurus lepturus* viewed using reflected light at x4 magnification. The red dot in the middle of the cross section marks the core, the last red dot on marks the edge and each of the other red dots in between mark fully formed annual increment. A) 29 cm PL Female age 2, B): 32 cm PL Female age 4, C): 36 cm PL female age 6.

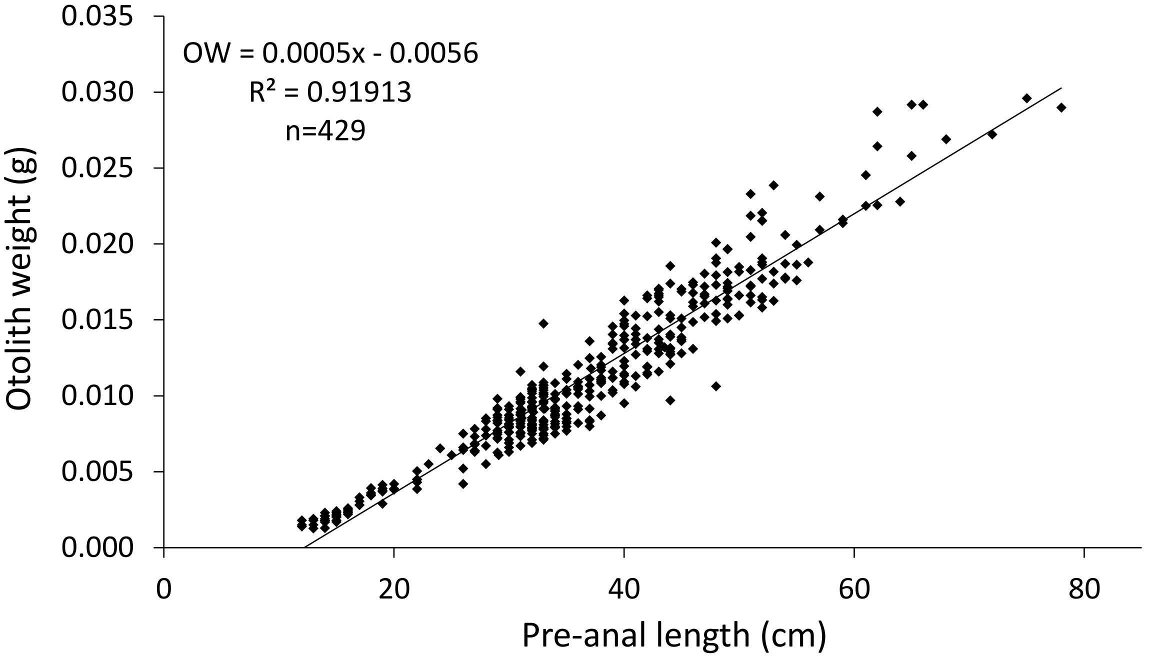


Figure 17. Relationship between pre-anal length and otolith weight for *Trichiurus lepturus* collected from NSW between 2015 and 2017.

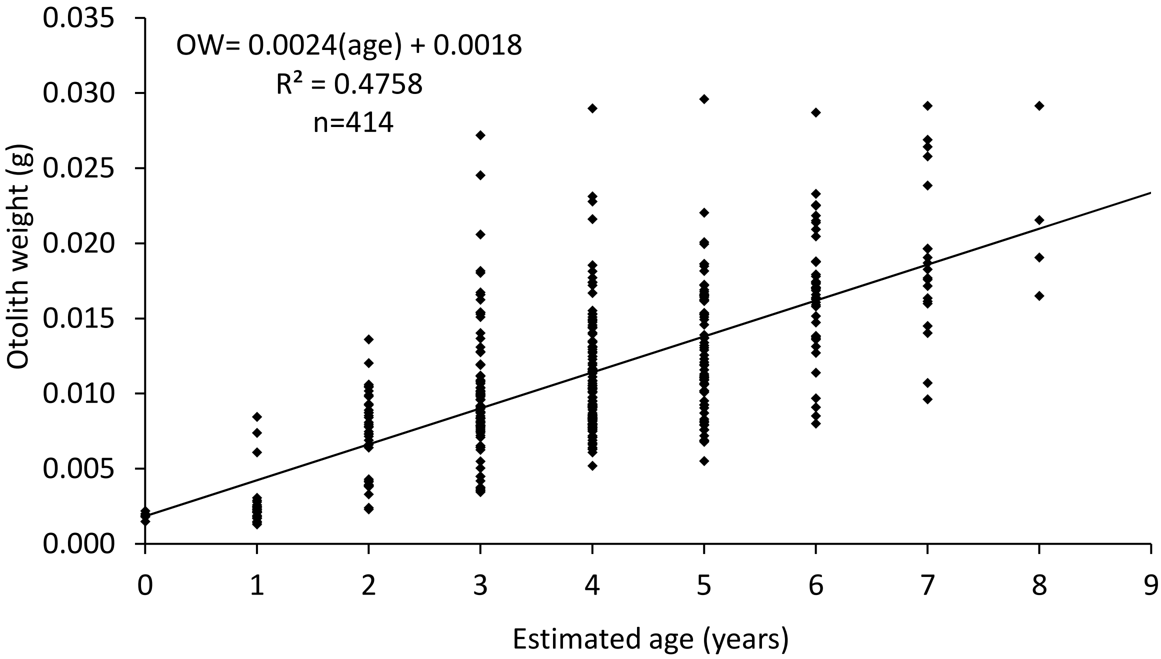


Figure 18. Relationship between estimated age and otolith weight for *Trichiurus lepturus* collected from NSW between 2015 and 2017.

3.4.4 Maturity

Histological slides verified the macroscopic staging done for *T. lepturus* gonads in south-eastern Australia. Female Stage 1 immature gonads were made up of primary growth oocytes in two stages, chromatin nucleolar and perinucleolar. Chromatin nucleolar are oocytes in their first stage of development, small cells with one uniform nucleus which takes up a large proportion of the cell (Kurobe et al., 2016). Perinucleolar are the second stage oocytes, they are larger than chromatin nucleolar and can be recognised by the nucleoli forming at the perimeter of the nucleus (Figure 19.a). Stage 2, developing gonads, were identified by the development of larger primary cortical alveolar oocytes. Cortical alveolar oocytes are identified by the formation of yolk granules outside the nucleus, in the cytoplasm. The tissue still had a large amount of primary growth oocytes present (Figure 19.b). Stage 3, mature/ripe gonads, could be verified by the presence of vitellogenic oocytes as well as fused yolk and migratory nucleolar oocytes, all of which indicate a stage of development just prior to running ripe/hydrated oocytes. Vitellogenic oocytes are larger, they have larger yolk bodies forming in the cytoplasm, and at the later vitellogenic stages, the yolk droplets fuse together and begin to migrate from the centre of the cell to the edge. (Figure 19.c). Stage 4, running ripe gonads, were defined by hydrated oocytes and all oocytes were either hydrated or vitellogenic oocytes, with a very small number of perinucleolar oocytes also occurring (Figure 19.d).

Male Stage 1 immature gonads were made up predominantly of spematagonia tissue with the initial development of spermatocytes and spermatids (Figure 20.a). Stage 2, developing gonads were identified by the development of spermatozoa. Spematogenic cysts, containing spermatogonia have also developed in the tissue (Figure 20.b). Stage 3, mature/ripe gonads, could be verified by the high proportion of spermatozoa in the tissue and spermatids at later stages, within spematogenic cysts (Figure 20.c). Male stage 4, running ripe gonads, and stage 5, spent gonads, were absent from the collection. Multiple oocyte development stages were observed in the same ovary and post ovulatory follicles (POFs) were observed alongside developing oocytes.

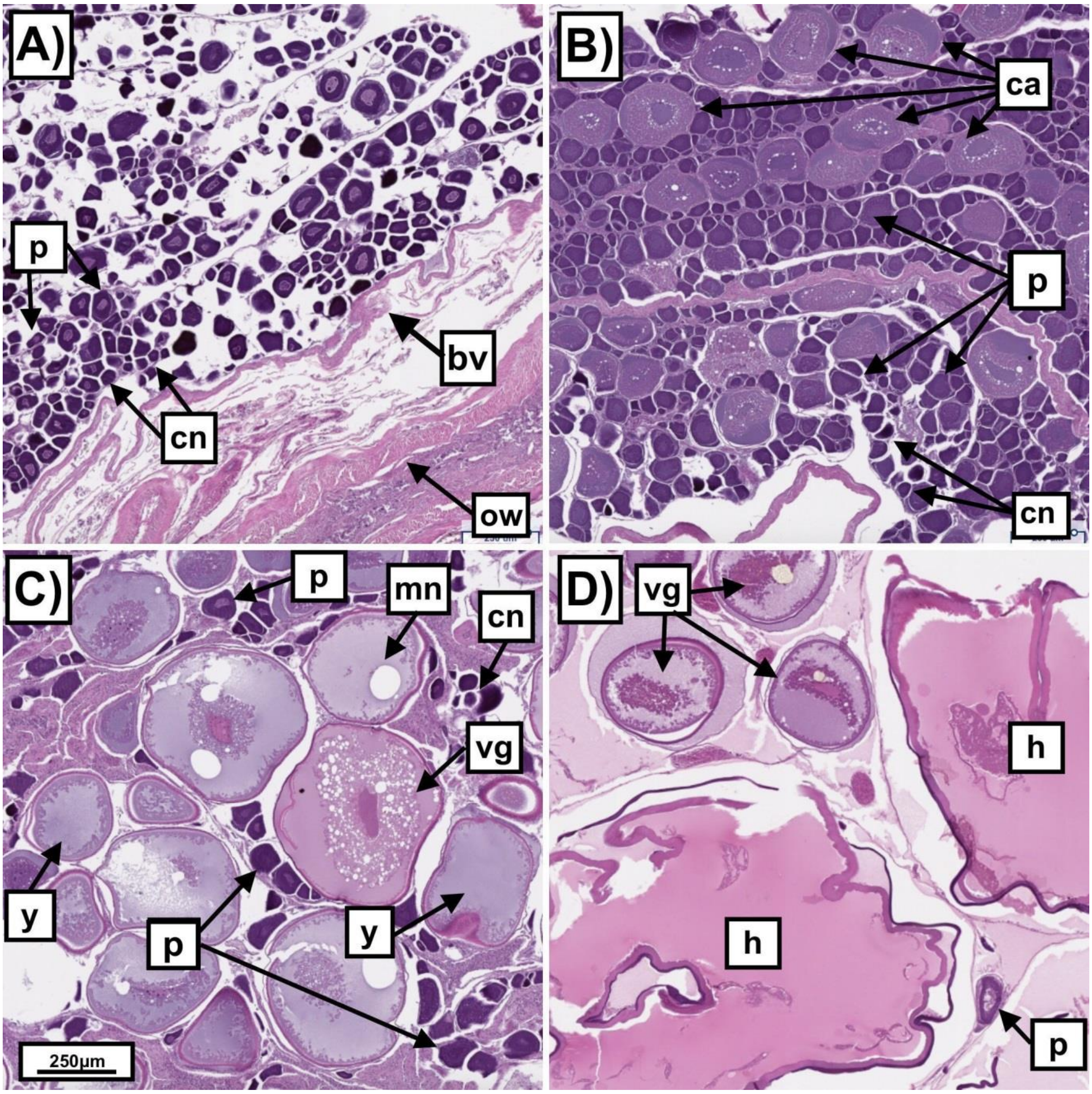


Figure 19. Histological sections of the development of *Trichiurus lepturus* ovarian tissue according to the criteria outlined In Table 2 A) Stage 1 (juvenile) ovary at 4x magnification, B) Stage 2 (developing/resting) ovary at 4x magnification, C) Stage 3 (ripe) ovary at 4x magnification, D) Stage 4 (Running ripe) ovary at 4x magnification Abbreviations: **cn,** chromatin nucleolar **p**, perinucleolar oocyte; **bv**, blood vessel, **ca,** cortical alveolar; **vg**, vitellogenic; **y**, fused yolk; **mn**, migratory nucleolar; **h**, hydrated.

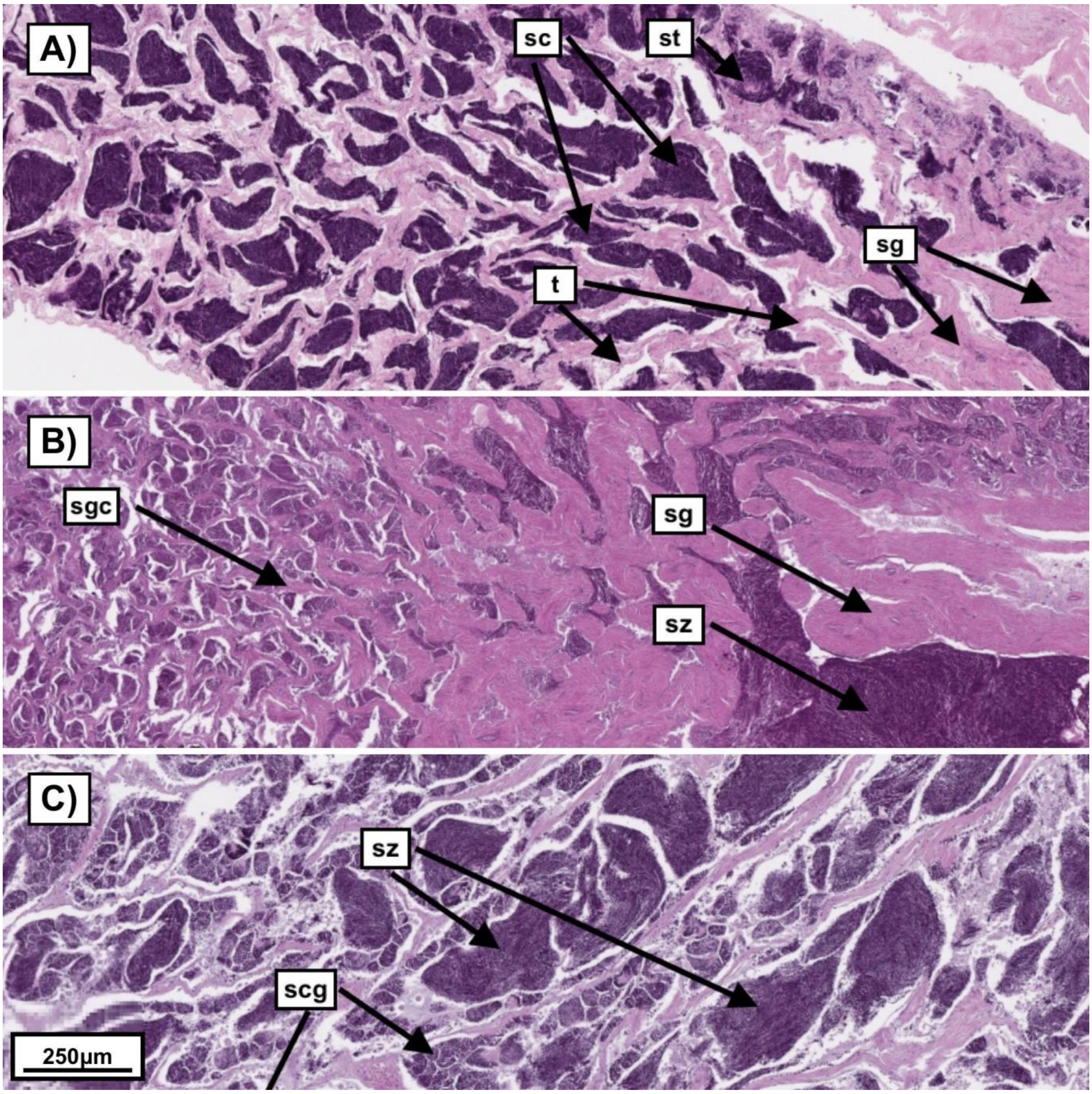
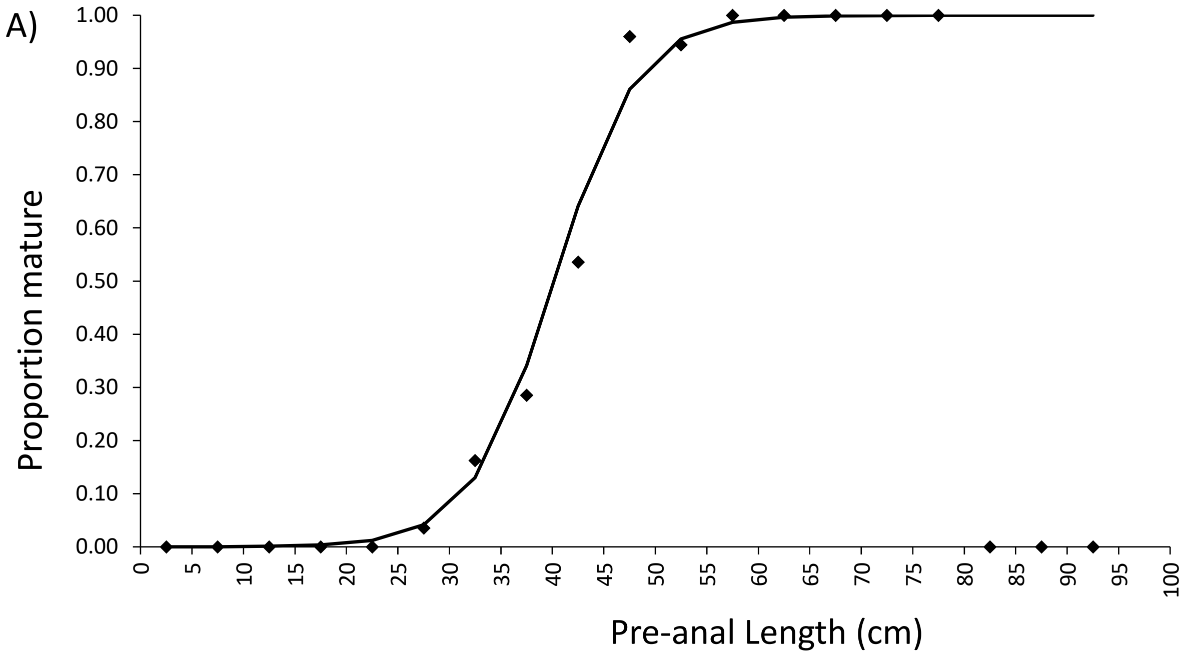


Figure 20. Histological sections of the development of *Trichiurus lepturus* testicular tissue according to the criteria outlined in Table 2 A) Stage 1 (Juvenile) ovary at 4x magnification, B) Stage 2 (developing/resting) ovary at 4x magnification, C) Stage 3 (ripe) ovary at 4x magnification. Abbreviations: **t**, seminiferous tubule: **sc**, spermatocytes; **st**, spermatids; **sg**, spermatogonia; **sz**, spermatozoa; **sgc**, spermatogenic cyst;

3.4.5 Length at maturity

The lengths at which 50% of females and males reached sexual maturity (L_{50}) were 40.15 cm PL and 40.65 cm PL, respectively (Figure 21). The shapes of the logistic curves describing maturity versus length for males and females were significantly different (Figure 21, Wald's test, $P < 0.05$). This difference was driven by the steeper slope of the female curve (selection range 8.90 cm) when compared to males (selection range 31.50 cm). However, The L_{50} values had overlapping S.E.s (Female 40.2 ± 0.8 cm; males 40.6 ± 2.8 cm) suggesting no significant difference in this parameter (Figure 21).



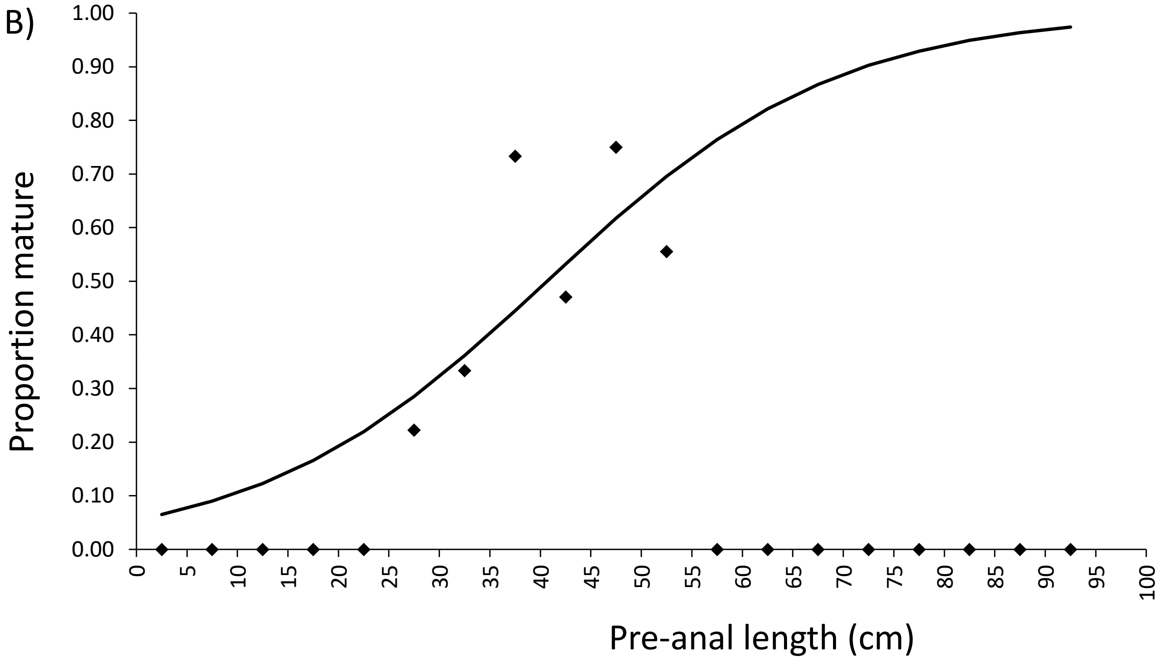


Figure 21. Length at maturity with fitted logistic curves for *Trichiurus lepturus* collected from NSW between 2015 and 2017 for A) females and B) males.

3.4.6 Reproductive season

Mean GSI calculated for female *T. lepturus* pooled over the sampling period and collection locations peaked in June at 1.9% and remained relatively high until September, suggesting the peak reproductive season for *T. lepturus* in NSW is from June to September (Figure 22.a).

Values were at their lowest point between January and April at 0.2% (Figure 22.a). The mean GSIs for male *T. lepturus* were much lower than for females but also suggested a peak reproductive season from June to September (Figure 22.b). There was insufficient data to calculate a GSI value for males in August, but for the other months, the highest values were from June to October (0.89%) and the lowest were from January to May (0.27%). Macroscopic maturity staging of male and female gonads indicate a similar pattern of reproductive activity to that of GSI (Figure 23); however, the macroscopic development suggested a longer reproductive season, lasting from April to September. During April to September, there was a higher proportion of ripe gonads, corresponding to high GSI values and during January and February, when GSI values were low, there was an absence of macroscopically staged ripe gonads.

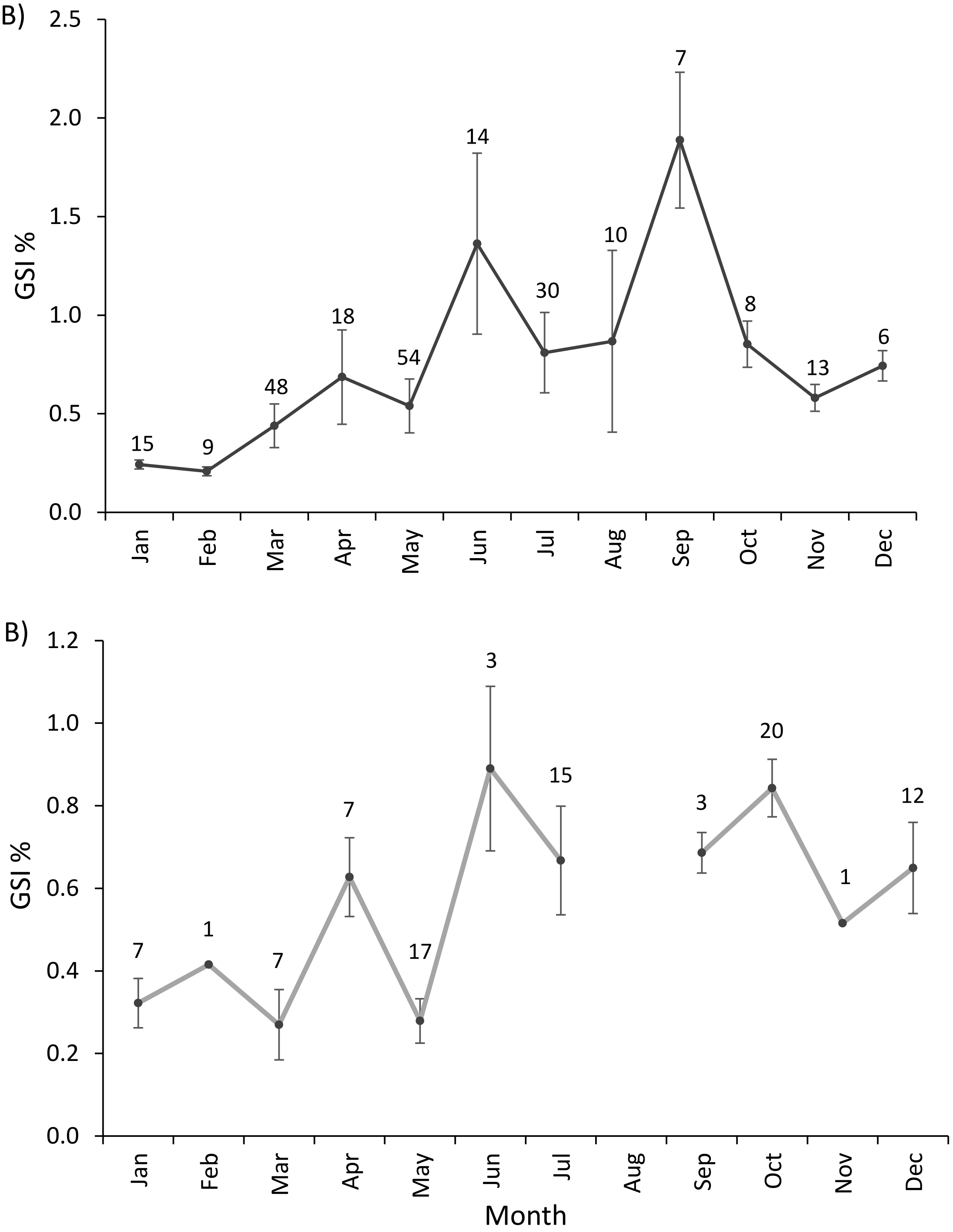


Figure 22. Monthly variation in gonadosomatic index (mean±SD) of (A) female and (B) male *Trichiurus lepturus* collected from NSW between 2015 and 2017. Numbers above error bars represent sample sizes.

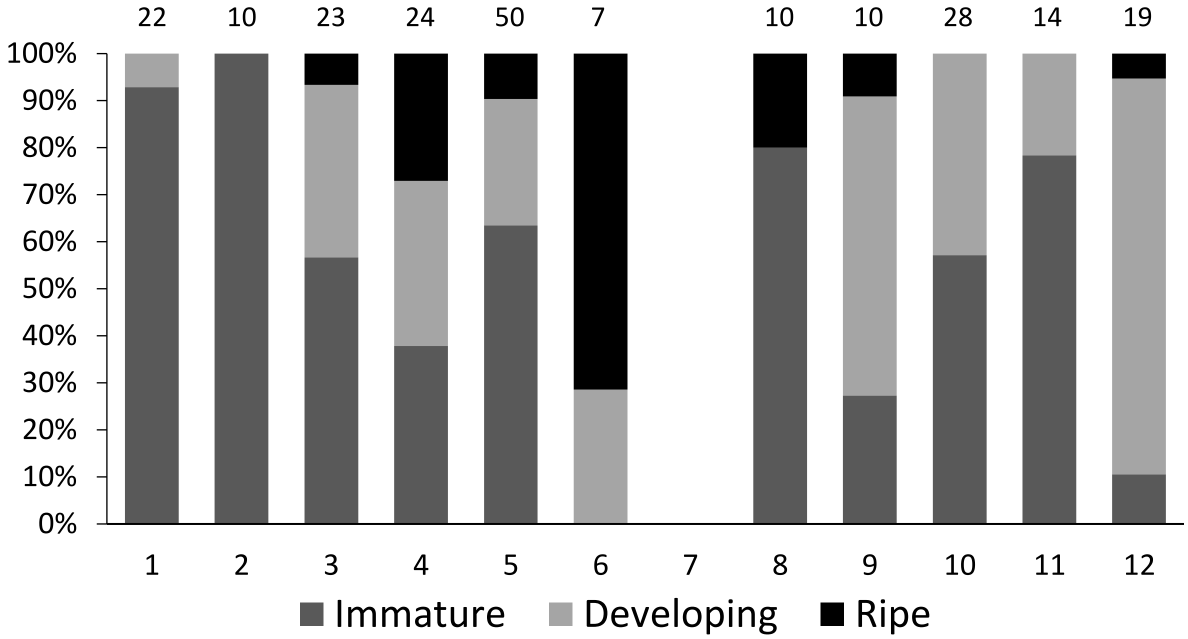


Figure 23. Monthly variation in macroscopic maturity stages for *Trichiurus lepturus* collected from the NSW commercial fishery between 2015 and 2017. No sample was available for July. Numbers above columns represent sample sizes.

3.4.7 Sex segregation

Most of the overall collection of *T. lepturus* from NSW was female, with a F:M sex ratio of 1: 0.4. For individuals > 40cm PL (equating approximately to > 110cm TL) the ratio was 1: 0.7 and < 40cm PL was 1: 0.29. All individuals over 60cm PL were female. Females dominated in both the reproductive and non-reproductive seasons (Chi square, df= 1, P< 0.001) and between the estuarine and the coastal habitats (all collected outside of the spawning period) (Chi square, df = 1, P< 0.001). There was significant variation in the sex ratios among catch events (Chi square, df = 4, P< 0.001). At least two catches which were entirely female, one which was during the reproductive season and one which was outside the season (Table 5). Twelve out of 17 catch events were predominantly female and four catch events were majority male, which were all taken outside the reproductive season.

Table 5. Sex ratios by season, separated by catch event, for *Trichiurus lepturus* collected from the commercial trawl and handline fishery in south-eastern Australia between 2015 and 2017. The shaded rows indicate the catch events during the reproductive season for *Trichiurus lepturus* in south-eastern Australia. Only the catch events with >6 individuals from the Hunter estuarine site and Hunter coastal site were included.

Month	Site	Method	Catch event ID	F:M	n
1	Hunter Estuarine	Handline	Catch ID #1	1: 0.5	9
	Hunter coastal	Handline	Catch ID #2	1: 0.44	13
2	Hunter coastal	Handline	Catch ID #3	1: 0.11	10
3	Hunter Estuarine	Handline	Catch ID #4	1: 0	19
4	Hawkesbury Estuarine	prawn trawl	Catch ID #5	0.63: 1	13
	Hunter Estuarine	Handline	Catch ID #6	1: 0.08	13
5	Hawkesbury Estuarine	prawn trawl	Catch ID #7	1: 0.27	19
	Hunter Estuarine	Handline	Catch ID #8	1: 0.63	31
	Hunter coastal	Ocean Trawl	Catch ID #9	1: 0.05	21
6	Hunter coastal	Handline	**Catch ID #10**	1: 0.17	7
8	Hunter coastal	Fish trawl	**Catch ID #11**	1: 0	10
9	Hunter coastal	Fish trawl	**Catch ID #12**	1: 0.43	10
10	Hunter Estuarine	Handline	Catch ID #13	0: 1	10
	Hunter Estuarine	Handline	Catch ID #14	0.8: 1	11
	Hunter coastal	Fish trawl	Catch ID #15	0.75: 1	7
	Hunter coastal	Handline	Catch ID #16	1: 0	11
12	Hunter coastal	Handline	Catch ID #17	0.5: 1	19
Total				1: 0.43	240

3.4.8 Length-weight relationships

The relationships between PL and Body weight (BW) for males and females were best described by the power relationships BW= 0.0449*PL$^{2.6296}$ and BW= 0.0172*PL$^{2.8909}$ respectively and for pooled individuals, W= 0.0149*PL$^{2.93}$. For the length-weight comparison between sexes, a significant interaction between sex and length was identified, indicating the weight of females increased faster with length relative to males (Figure 24. ANCOVA, $F_{1,290}$ = 6.884, P= 0.009). Due to the truncation of the data sets, conclusions on the effect of sex on length-weight relationships can only be drawn for individuals between 27.11 and 56.26 cm PL.

For the length-weight comparison between south-eastern Australia and the Arabian Sea, the rate of weight increase with length did not differ between regions, evidenced by a non-significant interaction between region and length (Figure 25, ANCOVA, $F_{1,1434}$ = 3.689, P= 0.055) so the model was refitted without the interaction term. The reduced model indicated that both length and region were significantly different between regions. A higher exponent in a length-weight equation generally indicates that a population is heavier at any given length, however the coefficient in the equation also affects the result. Despite the length-weight equation for NSW having a higher exponent than the equation for the Arabian Sea, the *T. lepturus* from the Arabian Sea were heavier at any given total length, across the length range examined, compared with *T. lepturus* from NSW (Figure 25, ANCOVA, $F_{1,1434}$ = 5474.790, P< 0.001). Due to the truncation of the data sets, conclusions on the effect of region on length-weight relationships can only be drawn for individuals between 66.69 cm and 134.28 cm TL.

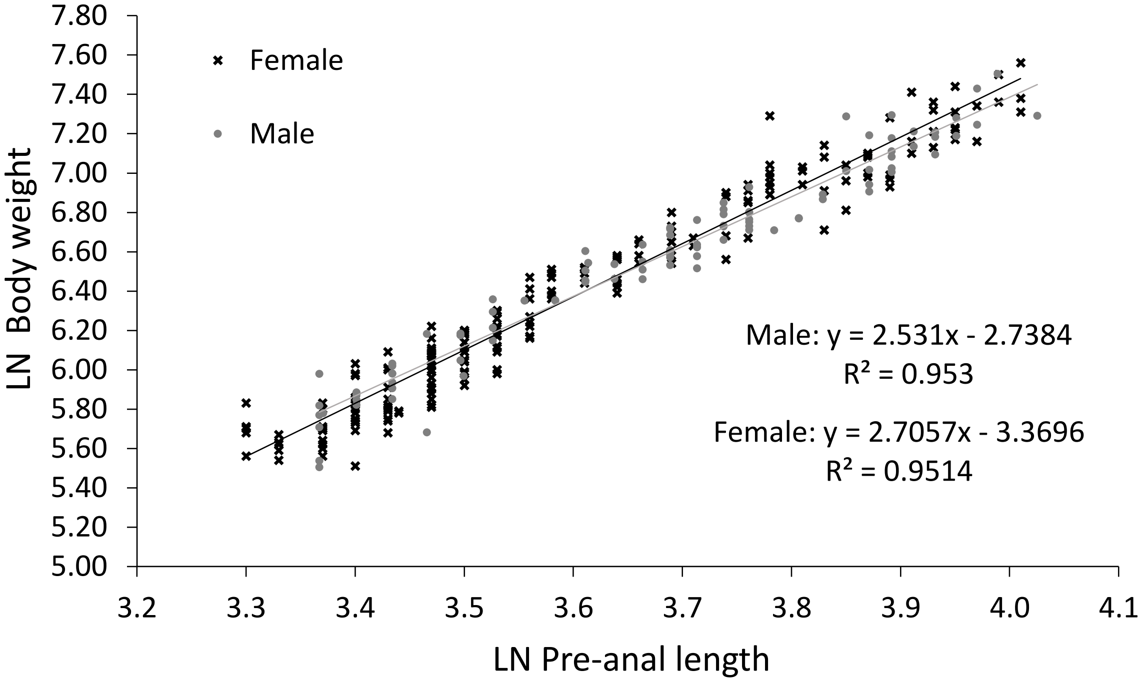


Figure 24. Comparison of the pre-anal length to weight between male and female *Trichiurus lepturus* collected from NSW between 2015 and 2017.

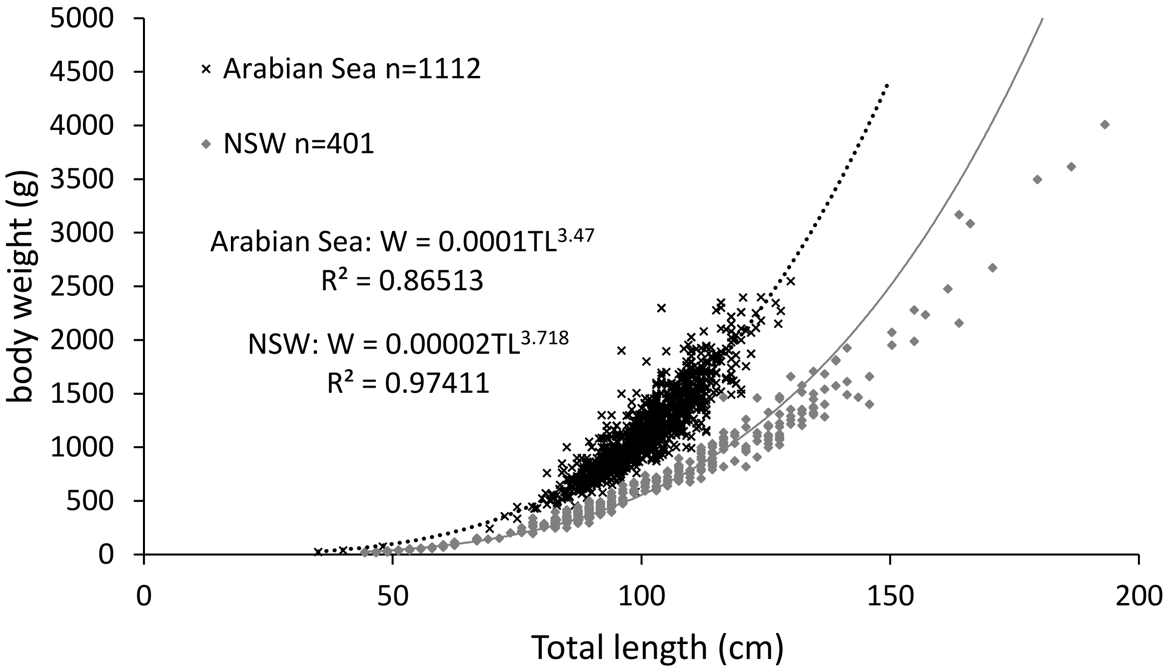


Figure 25. Comparison of the total length to weight relationships for *Trichiurus lepturus* from NSW and the Arabian Sea. Data for the Arabian Sea were taken from Nahdi et al. (2016).

3.4.9 Growth

Fitted VBGF growth parameters indicated the growth rates of male and female *T. lepturus* were not significantly different (Figure 26.a, ARSS, $F_{3,\ 324}$ =2.38, P= 0.0694), so males and females were combined in the same curve (Figure 26.b). The growth rate for *T. lepturus* in NSW was estimated by von Bertalanffy growth (VBG) parameters of $L_\propto$ (76.3 PL/189.3 TL), K (0.1277 yr-1) and t_0 (-0.9930 yr). Individuals reached on average 7, 24, 30, 36, 41, 45, 49 and 52 cm PL by the end of years 1, 2, 3, 4, 5, 6, 7, 8 respectively.

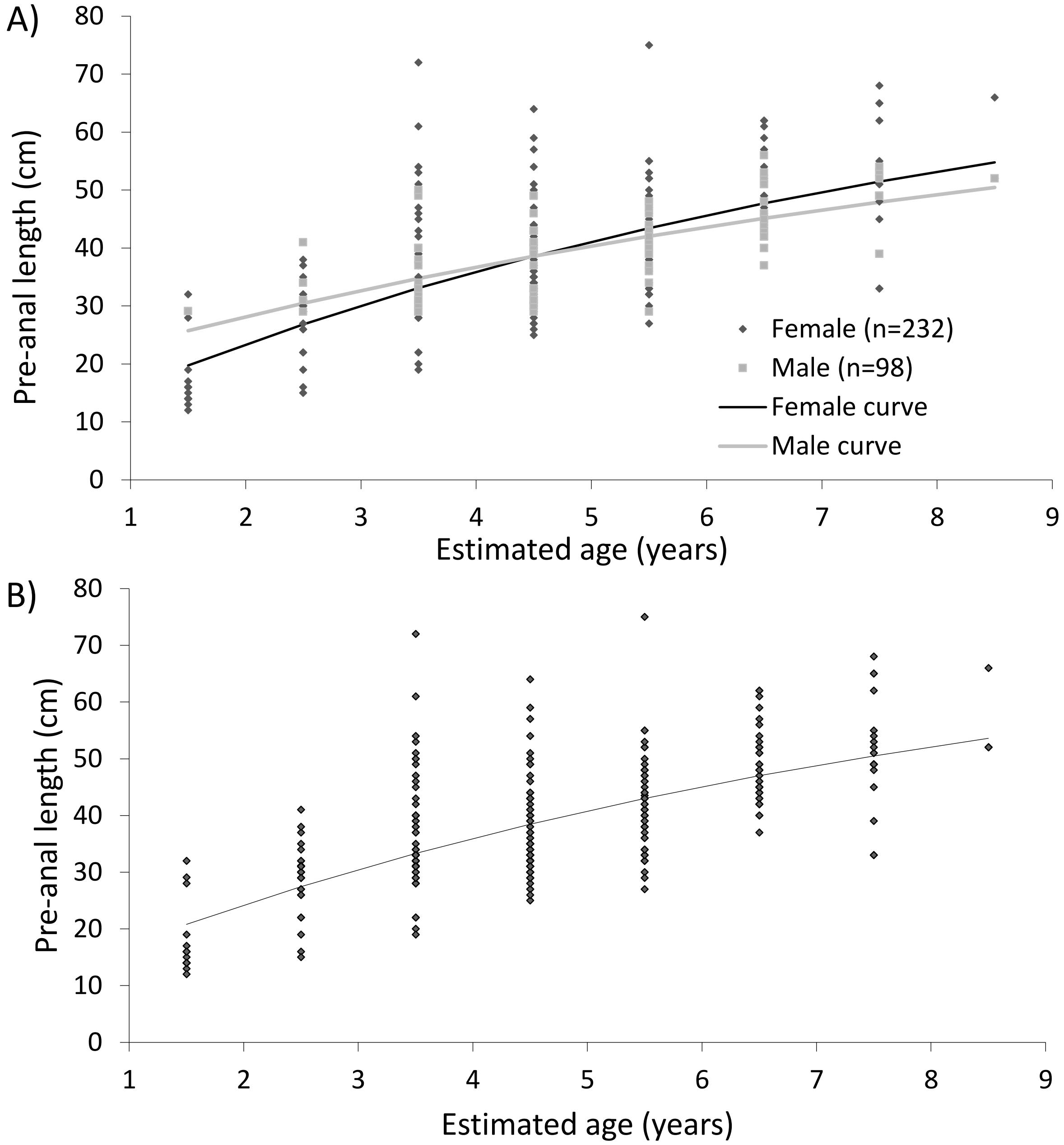


Figure 26. Von Bertalanffy growth function fitted to A) males and females and B) pooled length at age data for *Trichiurus lepturus* collected from NSW between 2015 and 2017.

3.5 Discussion

3.5.1 Maximum lengths and longevity

The maximum recorded lengths from the collections of *T. lepturus* in other global regions were substantially smaller than the maximum length from south-eastern Australia, except the maximum TL in the collection by Shih (2011) from Taiwan, which was similar at 79.3cm PL (Table 6). The sample collection for *T. lepturus* in south-eastern Australia ranged from 12 – 78 cm PL (44 – 193 cm estimated TL) and all fish that were larger than 56 cm were female, indicating that females may grow to larger lengths or it is possible large males were missed during sampling due to fishery dependent bias (Chapter 2). The smallest fish for both sexes in the collection were 12 cm PL, but the observed maximum length for males was 56 cm PL, whereas females reached 78 cm. The larger maximum length of *T. lepturus* in South-eastern Australia compared with other regions may indicate the population is less productive. In general, populations with individuals growing to larger lengths will have individuals with slower growth rates which is associated with lower productivity (Coulson et al., 2012, Wakefield et al., 2013). Alternatively, the population in south-eastern Australia may have more individuals that reach higher ages and therefore larger lengths due to lower fishing pressure. In some cases, larger maximum length can be an indication of higher productivity if the large females have a higher oocyte count and oocytes of a better quality (Randall et al., 2013).

It should be noted that small individuals could be under-represented in the current study because the gear used by recreational anglers and commercial handliners is selective of large length classes. In addition, small individuals landed by commercial trawl vessels are often not retained for market due to the fragility of small *T. lepturus*. Large males may have also been underrepresented if they were segregated from the rest of the population due to the timing and spatial extent of fishing effort. Spatio-temporal segregation of sexes has been suggested by Martins and Haimovici (2000) and is discussed below .

Table 6. Length summary of *Trichiurus lepturus* collected from NSW between 2015 and 2017 and length summary other global regions.

Region/ Sample	**Latitude**	**Mean L (±SE)**	**Min L**	**Max L**	**N**	**Citation**
NSW, Australia	32-34°S	36.16 (0.55)	12 PL/ 44 TL	78 PL/ 193 TL	438	Current study
Female		47.93 (1.31)	29 PL/ 83 TL	78 PL/ 193 TL	75	
Male		43.34 (1.09)	30 PL/ 85 TL	56 PL/ 139 TL	36	
Juvenile		29.83 (0.70)	12 PL/ 44 TL	56 PL/ 144 TL	205	
Recreational		37.45 (0.65)	27 PL/ 28 TL	62 PL/ 157 TL	121	

Southern Brazil	30-34°S	-	0.05 TL	160 TL	3232	Martins 2005
Female		-	-	-	-	
Male		-	-	-	-	
Kakinada, India	16.99°N	52.1 TL	8 TL	114 TL	-	Abdussamad 2006
Female		-	-	-	-	
Male		-	-	-	-	
Arabian Sea, Oman	17.50°N	-	16 TL	126 TL	-	Al Nahdi 2009
Female		-	-	-	-	
Male		-	-	-	-	
Visakhapatnam India	17.69° N	-	32 TL	81 TL	669	Reuben 1997
Female		-	29 TL	101 TL	406	
Male		-	32 TL	81 TL	363	
South-west Puerto Rico	18.00°N	-	13 PL	38 PL	184	Del Toro 2001
Female		-	13 PL	38 PL	133	
Male		-	13 PL	28 PL	51	
Veraval, India,	20.91°N	-	12 TL	126 TL	6409	Ghosh 2009
Male		-	-	-	276	
Female		-	-	-	256	
Taiwan	22-24°N	-	25.7 PL	79.3 PL	70	Shih 2011
Female		-	-	-	-	
Male		-	-	-	-	
Mumbai			46.5	124.4	1021	Khan 2006
Female		-	-	-	-	
Male		-	-	-	-	

3.5.2 Length at maturity

In south-eastern Australia, 50% of females and males reached sexual maturity (L_{50}) at 40.15 cm PL (107.82 cm TL) and 40.65 cm PL (108.95 cm TL), respectively, which are substantially larger than the length at maturity reported for populations of *T. lepturus* from all other regions (Table 7). Among global populations, there is a wide range of lengths at maturity reported and the exceptionally large length at maturity estimated from south-eastern Australia, 28% larger than the next most large from the Arabian Sea (Table 7), provides further evidence that *T. lepturus* are well adapted to a wide range of environments (Martins and Haimovici, 2000). There is evidence to suggest that *T. lepturus* populations from environments with marked temperature cycles or regions with lighter fishing pressure are more likely to reach maturity at larger lengths. Marked temperature cycles and lighter fishing pressure are reported from the regions of southern Brazil, the Bay of Bengal off India, the Gulf of Mexico and the Arabian sea off Oman, but not from Japan, south-eastern Brazil, India or the South China Sea (Al-Nahdi et al., 2009). The reported length at maturity for *T. lepturus* are larger from the regions of the first group relative to the second group (Table 7). The population in south-eastern Australia is exposed to temperature variability, caused by the EAC (Suthers et al., 2011), and relatively low fishing yield (see Chapter 2), implying relatively light fishing pressure, therefore these processes, along with taxonomic uncertainty among global regions are all possible explanations for the larger length at maturity. Environmental instability and other external pressure on populations including climate change, natural and anthropogenic habitat degradation and fishing pressure, all can cause populations to trend toward sexual maturity at smaller lengths (Del Toro, 2001). At present, the relatively low fishing yield for *T. lepturus* implies that fishing pressure has less of a detrimental impact on fish populations in south-eastern Australia compared with the north-west Pacific. If these external pressures increase, as anticipated in Australia, the length at maturity of fish populations may decrease. It is important to note the length at maturity in south-eastern Australia was calculated using individuals from inside and outside the spawning season which may have influenced the results. Further analysis will be done using individuals caught during the spawning season to improve accuracy.

Table 7. Length at maturity parameters of *Trichiurus lepturus* collected from south-eastern Australia between 2015 and 2017 and other global locations, presented in order of latitude from north to south. *Citation not seen, referenced from (Al-Nahdi et al., 2009). F = female. M = male. The regions with marked temperature cycles or regions with lighter fishing pressure are shaded in grey.

Latitude	**Region**	**L_{50} (cm)**	**Minimum length at maturity**	**Citation**
35.5-36°N	Japan	F = 59 TL	-	*(Munekiyo and Kuwahara, 1984)
30-35°N	South-west Atlantic, Southern Brazil	F = 69.3 TL M = 63.9 TL	-	(Martins and Haimovici, 2000)
16-19°N	Arabian Sea, Oman	F = 79 TL	-	(Al-Nahdi et al., 2009)
18-30°N	Gulf of Mexico	F = 35 TL	-	*(Sheridan et al., 1984)
20-24°N	Northern Arabian Sea, India	61.2 TL	45 TL	(Ghosh et al., 2014)
1.5-22.5°N	South China Sea	F = 25.5 PL	F = 18.5 PL	(Kwok and Ni, 1999)
13-22°N	Northern Bay of Bengal, India	52.9 TL	39 TL	(Ghosh et al., 2014)
8-19°N	Arabian Sea, India	F = 60 TL M = 58 TL	-	(Thiagarajan et al., 1992)
17.9-18.3°S	SW Puerto Rico	F = 18.7 PL 52.0 TL	-	(Del Toro, 2001)
7-34°S	SE Brazil	F = 39 TL	-	*(Bellini, 1980)
28-37°S	Australia/ NSW	F = 40.2 PL/ 107.8 TL M = 40.7 PL/ 108.9 TL		(Current study)

3.5.3 Spawning period

The estimated spawning period for *T. lepturus* in south-eastern Australia, from June to September, is relatively short in comparison to some reports from other global regions (Table 8). In the East China Sea, a protracted spawning period for *T. lepturus* was reported from March to October, which spans across the warmer season from mid spring to the start of autumn, when rainfall is high (Kwok and Ni, 1999, Ye and Rosenberg, 1991). Two spawning stocks were reported, both thought to spawn twice per season, meaning there were four main spawning events across the season (Kwok and Ni, 1999). The spawning period in the South China Sea was reported as similar to the East China Sea, but even longer, spanning February to October, encompassing colder months. From both areas, inter-annul variation was also reported (Kwok and Ni, 1999). From Southern Brazil, at a lower latitude (30°-35°S), with lower temperatures, a shorter spawning period was reported from November to February, lasting four months across late spring to early summer (Martins and Haimovici, 2000). Del Toro (2001) suggested the observed differences in spawning strategies among the regions was driven by the difference in latitude and temperature. South-eastern Australia is similar in latitude and environmental characteristics to southern Brazil, and spawning periods are similarly short, but they occur in opposite seasons. In addition, reports from the US at 26°N (Bryan and Gill, 2007) and the Arabian Sea, off Oman at 18°N (Al-Nahdi et al., 2009), which are both more closely aligned in latitude and temperature with the east and south China Seas, have both documented *T. lepturus* to have short spawning periods. This comparison also does not lend support to consistent differences in length at maturity driven by latitude and temperature. An alternative explanation for the process of varying spawning periods is the large-scale geographic separation between global populations and the cumulative effects of associated natural and anthropogenic factors that influence their habitats and consequently their populations. The conclusions from south-eastern Australia could be strengthened with a longer sampling period, across numerous years to make a more comprehensive assessment of the variability in the spawning time frames of the population.

Observations on the oocytes of *T. lepturus* indicated the spawning of the *T. lepturus* population in south-eastern Australia is likely to be asynchronous (Del Toro, 2001) and provides evidence that individuals will spawn more than once over a short time period, (Del Toro, 2001).

Table 8. Comparison of the reported reproductive season (shaded months) of *Trichiurus lepturus* at various global locations with the present study, presented in order of latitude from north to south. Spawning season was identified using various methods including: GSI[1, 3, 6, 10, 11]; gonad macroscopic maturity staging[5, 7, 8]; recruitment pattern[4, 7]; CPUE[9].

Citation	Country	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
[1]Bryan 2007	US (26°N)	▨ 24°	▨ 25°	▨ 26°	28°	30°*	31°*	32°*	32°*	32°*	30°*	27°	25°
[2]Ye and Rosenberg, 1991	China (26°N)	25°	28°	▨ 28°	▨ 28°*	▨ 28°*	▨ 25°*	▨ 21°*	▨ 17*	▨ 16°*	▨ 15°*	18°	22°
[3]Kwok and Ni 1999	China (26°N)	25°	28°	28°	▨ 28°*	▨ 28°*	▨ 25°*	▨ 21°*	▨ 17*	▨ 16°*	▨ 15°*	18°	22°
[3]Kwok and Ni 1999	China (22°N)	25°	▨ 28°	▨ 28°	▨ 28°*	▨ 28°*	▨ 25°*	▨ 21°*	▨ 17*	▨ 16°*	▨ 15°*	18°	22°
[4]Ghosh 2009	India (20°N)	28° Δ	28°Δ	30°Δ	32°Δ	▨ 30°Δ	▨ 31°+	29°+	28°+	▨ 29°	▨ 32°	▨ 32°Δ	▨ 29°Δ
[5]Khan 2006	India (18°N)	▨ 28°Δ	▨ 28°	▨ 28°	▨ 29°	▨ 30°	32°+	29º+	29º+	30º+	▨ 32°*	▨ 32°Δ	▨ 30°Δ
[6]Al-Nahdi 2009	Oman (17°N)	22°Δ	23°Δ	26°Δ	▨ 29°Δ#	▨ 31°Δ#	▨ 31°Δ	29°Δ	28°Δ	28°Δ	28°Δ	26°Δ	23°Δ
[7]Abdussamad 2006	India (16°N)	▨ 23°Δ	▨ 26°Δ	28°Δ	31°Δ	32°	32°+	29°+	29°+	29°+	28°+	25°	▨ 23°Δ
[8]Tampi 1968	India (13°N)	33°Δ	32°Δ	31°Δ	30°Δ	▨ 30°	▨ 28°	26°*	25°*	24°*	26°*	▨ 27°*	▨ 30°*
[9]Cheng 2001	Indonesia (7°S)	27°*	26°*	25°*	26°*	26°	27°	28°	28°	28°	28°	▨ 28°	▨ 27°*
[10]Martins 2000	Brazil (33°S)	▨ 25°*	▨ 24°*	22°*	19°*	16°*	15°*	15°*	17°*Δ	18°*Δ	20°*Δ	▨ 23°*Δ	▨ 22°*
[11]Current study	Australia (33°S)	22°*	22°*	21°*	18°*	14°*	▨ 12°*	▨ 11°	▨ 12°	▨ 15°	18°	19°*	21°

° Average air temperature Degrees Celsius
*Rainfall average over 100mm
Δ Rainfall average under 20mm
+ Monsoon
\# Indirectly effected by monsoon

3.5.4 Sex segregation

The observations of sex ratio patterns for *T. lepturus* in south-eastern Australia were like those found in populations from various regions around the globe. with a F:M sex ratio of 1: 0.4, females dominated the collection. This finding supports the global trend of female domination in *T. lepturus* populations, reported from south-west Puerto Rico (Del Toro, 2001), the Arabian sea off Oman (Al-Nahdi et al., 2009), Boca del Rio in Mexico (De la Cruz-Torres et al., 2014) and South Brazil (Martins and Haimovici, 2000) (Table 9). Al-Nahdi et al. (2009) divided their collection of *T. lepturus* from the Arabian Sea in Oman, into individuals above and below 110 cm TL. They found that 21 % of females to be >110 cm TL, compared to only 4 % of males, meaning that a heavy majority of the individuals >110 cm were female. In south-eastern Australia, individuals > 40cm PL (equating approximately to > 110cm TL) had a F:M sex ratio of 1: 0.7 and the individuals < 40cm PL had a sex ratio of 1: 0.3. Therefore, the pattern of sex ratios in south-eastern Australia does not directly reflect the findings of Al-Nahdi et al. (2009). However, all individuals over 60cm PL (~152.56 cm TL) were female in south-eastern Australia, which does support the typical global conclusion of females dominating *T. lepturus* populations, particularly in larger length classes (Kwok and Ni, 1999; Martins and Haimovici, 2000). While Martins (2000) used this observation to infer that females attain larger lengths than males of the same age, in the present study the length at age-based growth curves were not significantly different between males and females. The similarity of sex ratios between estuarine and coastal habitats, and between the spawning season and the non-spawning period in south-eastern Australia is in contrast to southern Brazil, where sex ratios varied between seasons and habitats (Martins and Haimovici, 2000). In Brazil, Martins and Haimovici (2000) observed female domination in populations of *T. lepturus* in autumn and winter in coastal shelf waters (outside the spawning period), whereas sex ratios in the same habitat on the coastal shelf were even during spring and summer (during the estimated spawning season). Martins (2000) used this observation to infer that males had either moved offshore or northward to participate in prolonged spawning seasons in other regions. In the current study, it should be noted that the individuals used from inside the reproductive season were all caught from the Hunter coastal site and those outside the reproductive season were from both estuarine and coastal sites, therefore the temporal results could be confounded by site selectivity. The hypothesis of sexual segregation occurring at a small scale, within sites was supported by a significant variation in the sex ratios among catch events from the commercial fishery. Two of the events were entirely female, one of which was taken outside the estimated reproductive season and one was taken during the season. This finding did not support a variation in sex ratios based on reproductive season. The segregation may be related to another temporal cause because four out of the five catch events that were predominantly male were within the last three months of the year, from October to December and all outside the reproductive season. As previously mentioned, it is possible that males, particularly large males, may have been underrepresented if they were segregated from the rest of the population due to the timing and spatial extent of fishing effort. The spatial and temporal variation in sex ratios found by (Martins and Haimovici, 2000) in Brazil lends support the possibility of fishery-dependent sampling bias.

Table 9. Sex ratios of *Trichiurus lepturus* collected from NSW between 2015 and 2017 and other global locations. Presented in order of latitude from north to south

Region	Latitude	F:M	Citation
Maharashtra, India	15-20°N	1:1	(Khan 2006)
Arabian Sea, Oman	16-25°N	1: 0.12	(Al-Nahdi et al., 2009)
Arabian Sea, Oman	16-25°N	1: 0.26 Individuals <100 cm TL	(Al-Nahdi et al., 2009)
East China Sea, China	21.5-22.5°N	>1: 1	(Kwok and Ni, 1999)
Boca del Rio, Mexico	19-19.1°N	Windy: 4:1 Dry: 1.86:1 Rainy: 1.5:1	(De la Cruz-Torres et al., 2014)
South-west Puerto Rico	17.9-18.3°S	2.59: 1	(Del Toro, 2001)
Mayagiiez, Puerto Rico	18.1-18.3°S	4.54: 1	(Del Toro, 2001)
Boqueron, Puerto Rico	17.9-18°S	1.79: 1	(Del Toro, 2001)
Current study	32-34°S	1: 0.4	Current study

3.5.5 Length-weight relationships and growth

The weight of females in south-eastern Australia increased faster with length relative to males, which is similar to the findings from the Mauritania shelf, where it was concluded that females are heavier than males at similar lengths after sexual maturity (Wojciechowski, 1972). For the length-weight comparison between sexes, the truncation of data sets to common lengths for both sexes considerably reduced the length ranges examinable, therefore the conclusions for the length-weight relationship comparison between sexes can only be made for *T. lepturus* in south-eastern Australia within the length range of 27.11 (78.43 cm TL) to 56.26 cm PL (144.13 cm TL). Potential implications of the restricted length range could include a missing convergence or divergence between the length-weight of male and females after the length of 56.26 cm PL. A divergence is arguably more likely to occur as individuals grow to larger lengths because there will have been more time for a convergence or divergence to present itself.

The growth rate of *T. lepturus* across all populations worldwide is relatively fast, however the growth rate (k) of *T. lepturus* in south-eastern Australia (0.1277yr$^{-1)}$ is amongst the slowest reported (Table 10). Growth of *T. lepturus* did not differ between sexes in the present study, but females were observed with a greater maximum length and all fish sampled > 60cm were female. The weight of females also increased faster with length relative to males. While the von Bertalanffy growth curves between males and females did not differ significantly, other evidence including, the length range, and the domination of females in the largest length class and how female weight increases with length, all suggest that females might grow larger than males, and at a faster rate.

The estimated L_∞ (76.3cm PL) for *T. lepturus* in south-eastern Australia was similar to the maximum length in the collection (78cm PL), supporting the generated model fit. *T. lepturus* from south-eastern Australia were lighter at any given total length, across the length range examined, compared with *T. lepturus* from the Arabian Sea off Oman. The difference in how each population increases in body weight could be due to unidentified taxonomic differences that influence growth rates or body proportions, or several environmental or anthropogenic processes caused by the large geographic separation. The slower growth rate estimated with von Bertalanffy growth parameters and the length-weight relationship is another indication that the population may be less productive than populations from other global regions, and therefore more vulnerable to local population decline, if the fishing pressure in south-eastern Australia continues to increase.

Table 10. Growth parameters of *Trichiurus lepturus* collected from NSW between 2015 and 2017 and other global locations, presented in order of latitude from north to south. *Citation not seen, referenced on Froese, R. and D. Pauly. Editors. 2018.FishBase. World Wide Web electronic publication. www.fishbase.org, (11/2018).

Latitude	Country	L_∞ (cm)	K (per yr)	t_0 (yr)	n	Citation
35.49° N	China	70.8 PL	0.110	-2.8200	-	*(Jingqi and Mingdi, 1981)
34.08° N	Japan	56.8 PL	0.2610	-0.6435	3739	*(Sakamoto, 1976)
34.87° N	Japan	43.4 PL	0.2826	-0.4130	505	*(Kosaka, 1967)
25°N-33°N	China	-	0.3103	-0.40	-	*(陳賽斌 and 渡邊精一, 1989)
30.40° N	China	76.6 PL	0.1390	-0.2660	869	*(Hamada, 1971)
22°N-25°N	Taiwan	86.9 PL	0.182	-2.161	-	(Shih et al., 2011)
20.91°N	India	134.1 TL	0.29	-0.275	6489	(Ghosh et al., 2009)
17.69° N	India	106.8 TL	0.6117	-0.1399	5976	(Reuben et al., 1997)
17.50°N	Oman	127.4 TL	0.399	-0.9815	10740	(Al-Nahdi et al., 2009)
16°N-17°N	India	145.4 TL	0.29	-0.20	-	(Narasimham, 1972)
16.99° N	India	128.2 TL	0.72	-0.003	-	(Abdussamad et al., 2006)
25.43° S	Brazil	245.0 TL	0.27	-	-	*(Rossi-Wongtschowski et al., 2006)
25°S- 33°S	South Africa	146.8 TL	0.292	-	-	(Torres Jr and Pauly, 1991)
32°S-34°S	Australia	76.3 PL 189.3 TL	0.1277	-0.9930	330	Current Study in NSW
34°S-41°S	Argentina	234.0 TL	0.550	-	-	*(Milessi) 2008

3.6 Conclusion

The demographics of the *T. lepturus* population in south-eastern Australia, including a larger maximum length, a longer lifespan, a larger length at maturity indicates a population that is lightly fished compared to populations in other areas. The shorter spawning period and slower individual growth rate suggests that the population may have lower productivity and could potentially be less resilient to perturbation, both natural and anthropogenic, than populations in other regions, and more vulnerable to increasing anthropogenic pressures on the population, including climate change, habitat degradation and increased fishing pressure.

Chapter 4: The Utility of Otolith Microchemistry to Inform on the Estuarine-Coastal Movements of Largehead Hairtail (*Trichiurus lepturus)* in Australia

Abstract

Largehead hairtail (*Trichiurus lepturus*) is an important species for both global and Australian fisheries, but knowledge is lacking about *T. lepturus* life history and movements in Australian waters. An evaluation was made of the utility of otolith microchemistry to investigate movements of *T. lepturus* between estuarine and coastal habitats. Otolith Sr, Ba, Mg, and Mn at the otolith edge (representing the most recently-deposited material and encompassing ~100-250 days prior to capture) were analysed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Sr and Ba displayed significant positive relationships with ontogeny (using body length as a proxy), while Mn showed a significant negative relationship, suggesting that elemental incorporation was influenced by ontogeny. Therefore, ontogeny was incorporated as a covariate into any further spatial or temporal analysis. Mg and Mn differed significantly among months, but no inter-annual variability was detected in any of the four elements. The findings indicate that the use of Mg and Mn for investigating individual movement patterns, unlike Sr and Ba, could be confounded by seasonal changes. The use of all four elemental concentrations are also unlikely to be confounded by annual variability. To determine if otolith chemistry differed between coastal and estuarine habitats, elemental concentrations on the otolith edge were compared between individuals collected during the same time of year. The method was unable to distinguish between Individuals caught in estuarine and coastal habitats, because no significant differences in elemental concentration were identified between the coastal site and either of the two different estuarine sites located ~83 km apart. An additional finding was the significant difference between the two estuarine sites, indicating that otolith elemental concentrations vary with geographic location, even among similar habitat types. Core-to-edge transects were utilised to investigate changes in otolith Sr and Ba profiles that might indicate movements over an individual's life. A pattern of substantial Ba elevation before the formation of the first annual increment was observed in 21 out of 30 individuals from all three capture sites. The current results have not allowed for *T. lepturus* movements between

coastal and estuary habitats to be determined in south-eastern Australia, however further analyses of otolith microchemistry from fish captured from a broader spatial scale may provide additional information on *T. lepturus* movements and stock structure in south-eastern Australia.

4.1 Introduction

Directly tracing the individual movements of fish is challenging. The most common method used is conventional, physical tagging (e.g. T-bar external tag) but this only yields a single start and end point, with individuals potentially making a number of unknown movements in between tagging and recapture. More comprehensive methods to track movements are acoustic tagging and monitoring systems, pop-up satellite archival tags (PSAT), and electronic data storage tags (DST). The drawbacks of these techniques include cost, battery life, low rates of return and high rates of mortality at the juvenile life stage (Sturrock et al., 2012). The challenges associated with these methods typically result in low replication, which is rarely sufficient to encapsulate the high degree of movement variability among individuals within a population.

Otoliths are calcium carbonate structures in the skull of teleost fishes, and the analysis of their chemical composition, has been identified as a cost-effective alternative to tagging for detecting geographic movements of fishes (Avigliano et al., 2017, Campana et al., 2000, Fowler et al., 2016, Yamane et al., 2012). Otoliths sit in endolymph fluid-filled cavities in the inner ear, and facilitate stability and orientation (Yamane et al., 2012). Otoliths grow as the endolymph fluid gradually accretes around the outer edge (Elsdon et al., 2008). Fish growth is faster during warm seasons and slower during cold season resulting in seasonal increments forming throughout the life of the individual (Fortunato et al., 2017). In most species, when a cross section is taken through the core and viewed under reflected light, the otolith shows alternating opaque and translucent (hyaline) increments which can be counted together as one annual increment. As the outer edge of the otolith accretes, minor and trace elements are incorporated in the otolith. The elemental incorporation in otoliths often scales with the elemental concentrations of the aquatic environment of residence, and because otoliths are metabolically inert (Elsdon and Gillanders, 2002), the accreted trace elements leave a permanent record of elemental incorporation (Campana and Neilson, 1985). The otolith has biochronological properties when it is analysed from the core (natal origin) to the edge (time immediately prior to capture) (Beer et al., 2011). The permanent record of chemical concentrations in otoliths have the potential to reflect the physical-chemical properties of the ambient water the individual resided in, at the time of accretion (Beer et al., 2011).

The quantification of otolith chemistry using laser ablation inductively-coupled plasma mass-spectrometry (LA-ICP-MS) is an increasingly popular research technique for the investigation of individual fish movements (Avigliano et al., 2017, Fortunato et al., 2017). LA-ICP-MS is a cost-effective method (Elsdon et al., 2008), with the potential to: (1) distinguish between fish stocks (Longmore et al., 2010); (2) describe connectivity between coastal and estuarine populations (Gillanders, 2005); (3) discern natal origins of individuals (Barnett et al., 2016); and (4) discern the type of migration undertaken by individuals (Fowler et al., 2016) and their habitat preferences (Fowler et al., 2015). In addition, the innovation of laser ablation, compared to the previous used method of solution based ICP-MS has allowed the targeted analysis of discrete areas of otolith cross-sections. This technique allows the chemical analysis to target biochronological life stages (Beer et al., 2011, Chang et al., 2012). If the ablation transect is run from the core-to-edge of an otolith, the results have the potential for movement patterns of the individual to be reconstructed from their natal origin to their capture (Avigliano et al., 2017, Crook et al., 2017, Fairclough et al., 2011, Fowler et al., 2016, Gillanders, 2005, Mercier et al., 2012, Morales-Nin et al., 2005).

The use of otolith chemistry to reconstruct the movement patterns of individuals is based on the assumption that the incorporation of trace and minor elements into the otolith are primarily driven by physical and chemical properties of the aquatic environment; however, inferences made on spatial movement patterns can be confounded by endogenous factors and temporal instability. Otolith elemental incorporation is known to vary with ontogeny for a number of species (Chittaro et al., 2006, Fowler et al., 2017, Morales-Nin et al., 2005, Moreira et al., 2018, Sturrock et al., 2012, Walther et al., 2010) but is not always the case (Elsdon and Gillanders, 2005). As fish develop, they undergo morphological and physiological changes that can affect otolith incorporation of trace elements (Elsdon and Gillanders, 2005). The effects of ontogeny could be attributed to a number factors including: age, growth rate, gonad development, stress, blood plasma, hormones or a combination of any number of these factors (Elsdon and Gillanders, 2005, Walther et al., 2010) and should be accounted for in spatial comparisons (Beer et al., 2011, Elsdon and Gillanders, 2005, Walther et al., 2010). Time, including yearly and seasonal cycles, might affect otolith elemental incorporation for a number of different reasons including: temporal variability of aquatic elemental concentrations or salinity in natural systems (Crook et al., 2016, Gillanders, 2002, Ruttenberg et al., 2008), or temperature effects, either directly on the uniformity of the crystallisation of the endolymph fluid or indirectly, by influencing temperature-dependent biological processes (Sturrock et al., 2015). Such factors can potentially confound spatial comparisons of otolith chemistry, leading to incorrect conclusions regarding residence within particular habitats or movement patterns between habitats. The incorporation of elements to the otolith and the factors affecting incorporation can also vary between species (Kraus and Secor, 2004, Mazloumi et al., 2017).

Strontium (Sr) and barium (Ba) have been the most reliable chemical elements for reconstructing movement patterns across numerous species, because: (1) they are readily incorporated into the calcium carbonate matrix of the otolith, (2) vary among aquatic habitats with key environmental variables like temperature and salinity, (3) their concentration in the otolith typically scales with concentrations in ambient water and (4) they are influenced less by dietary uptake and other physiological factors compared with other elements (Elsdon and Gillanders, 2003, Elsdon and Gillanders, 2005, Mazloumi et al., 2017, Milton and Chenery, 2001). Magnesium (Mg) and Manganese (Mn) are also readily incorporated into the calcium carbonate matrix of otoliths and have been used with some success for spatial discrimination among groups of fishes (Martin and Thorrold, 2005, Schuchert et al., 2010); however, results are less consistent than those for Sr and Ba.

Higher concentrations of Sr in coastal and ocean habitats are expected, due to higher concentrations of Sr salt in these habitats (Avigliano et al., 2017, Brass and Turekian, 1974). The aquatic Sr concentrations of coastal and ocean habitats remain at a relatively consistent level throughout the globe, more than an order of magnitude higher than the average freshwater habitat (Thorrold et al., 1997). Ba is expected in high concentrations in fresh water habitats, primarily due to the Ba presence in freshwater sediment (Avigliano et al., 2017, Coffey et al., 1997, Elsdon and Gillanders, 2005, Thorrold et al., 1997). There are also higher proportions of dissolved (bioavailable state for otolith incorporation) Ba compared with marine habitats (Elsdon and Gillanders, 2005). Dissolved Ba is variable among and within freshwater rivers, and in estuaries with connections to fluvial upwelling and run-off. The variability is relative to changes in the amount of terrestrial run-off and the flow rate, which directly relates to the increased movement of fluvial sediment, and the subsequent release of dissolved Ba into the aquatic environment (Elsdon and Gillanders, 2005, Li and Chan, 1979, Thorrold et al., 1997). Ba is also associated with deep sea habitats and at the surface of coastal upwelling systems. Dissolved Ba has nutrient type profiles in marine habitats, which means it is available in high concentrations in deep, nutrient-rich marine water and is generally less available near the surface, until it is brought up by wind-driven coastal upwelling. Despite the variability of Ba in natural systems, levels differ enough for water testing to distinguish between freshwater and marine habitats (Elsdon and Gillanders, 2005).

Otolith trace elements are typically expressed as a ratio to Calcium (Ca) for standardisation purposes, based on the broadly accepted assumption that the primary mechanism for otolith elemental incorporation of the relevant trace and minor elements, occurs through the substitution of calcium (Miller, 2009). Calcium substitution is the likely mechanism for otolith incorporation of Sr, Ba and Mg which are all alkali earth metals, divalent cations, whereas Mn is a transitional metal with a mechanism for otolith incorporation that is less clear (Miller, 2009).

The correlation of Sr:Ca and Ba:Ca ratios between otoliths and ambient water chemistry have been tested extensively in validating studies. Studies were carried out using captive reared fish, in aquaria with controlled, water chemical gradients and results were consistent between studies (Macdonald and Crook, 2010, Walsh and Gillanders, 2018, Wells et al., 2003). In contrast, the results from salinity tests vary between studies. Controlled aquaria studies with steep salinity gradient tests, typically show Sr:Ca and Ba:Ca to have an inverse relationship along a salinity gradient. Normally otolith Sr will display a positive correlation with salinity and otolith Ba will display a negative correlation (Mazloumi et al., 2017, Stanley et al., 2015, Sturrock et al., 2012). The typical relationships between otolith Sr and Ba and salinity are complimentary to the relationship between otolith Sr and Ba and ambient water concentrations. This is useful for tracing movement patterns between freshwater and marine habitats, in natural systems. However, some studies have reported a negative effect of salinity on otolith Sr (Campana, 1999, Elsdon and Gillanders, 2002), and a positive effect on otolith Ba (Dorval et al., 2007, Panfili et al., 2012), while others did not find any relationship with otolith Sr (Elsdon and Gillanders, 2005, Gillanders and Munro, 2012). Temperature has also been shown to vary among studies in a similar way to salinity. The varying results between studies is likely due to variation in otolith incorporation among species (Kraus and Secor, 2004, Mazloumi et al., 2017). This variability highlights the importance of determining the species-specific capabilities of otolith chemistry techniques, before the technique is used for spatial comparisons in natural systems or reconstructing movement patterns.

Mg:Ca and Mn:Ca ratios in otoliths are often cited as useful indicators for spatial discrimination among groups of fishes (Ferguson et al., 2011, Miller, 2009, Schuchert et al., 2010), whereas the use of otolith Mg and Mn concentrations for the reconstruction of migration histories is still ambiguous. Otolith Mg and Mn are often used for multivariate analysis studies to determine spatial discrimination between stocks however neither element has been used in standalone core-to-edge transects to reconstruct migration histories. Otolith Mg and Mn concentrations do not often scale with salinity (Elsdon and Gillanders, 2002, Gillanders and Munro, 2012, Martin and Wuenschel, 2006) and otolith Mn concentrations do not often scale with water concentrations (Dorval et al., 2007). The elements are useful for spatial discrimination due to their variation with other factors. Otolith Mg varies with factors associated with water temperature and Mg water concentrations (Barnes and Gillanders, 2013, Martin and Thorrold, 2005, Martin and Wuenschel, 2006, Miller, 2011), while otolith Mn varies with factors such as dietary uptake and temperature (Barnes and Gillanders, 2013, Miller, 2009). The high degree of variability, between species for the incorporation of Mg and Mn indicates the incorporation might also be under a high level of physiological regulation (Mazloumi et al., 2017).

Otolith Sr and Ba concentrations have been used successfully for investigating movement patterns between marine and freshwater habitats, in natural systems for numerous species (Avigliano et al., 2017, Fortunato et al., 2017, Fowler et al., 2016). However, when migration is across a narrow salinity gradient, for example between coastal and estuarine habitats, the chemical markers are often not as well defined (Hamer et al., 2006). There is evidence, however, that otolith Ba might be a particularly useful marker between estuarine-coastal habitats (Hamer et al., 2006). Ba in aquatic systems, has a nutrient type profile, meaning it is associated with cold, nutrient-rich, productive waters (Woodson et al., 2013). This means that dissolved Ba is often available in the surface water of coastal habitats with active wind-driven upwelling and inshore/coastal habitats (Woodson et al., 2013). Thorrold (1997) demonstrated that core-to-edge profiles of Ba varied more between oceanic and inshore/estuarine habitats compared with Sr profiles.

T. lepturus is a cosmopolitan, benthopelagic fish species, also known as cutlassfish, ribbonfish (Martins and Haimovici, 1997) and largehead *T. lepturus* (Tzeng et al., 2007). The species is known to occur in shallow estuarine and coastal waters, as well as deep water, up to 350 m (Al-Nahdi et al., 2009, Bakhoum, 2007). Studies from India suggest that *T. lepturus* is a migratory species, which makes large movements along coastlines and moves between inshore and offshore habitats. In the waters off Kakinada, India, marine-estuarine dependency has been observed during annual migrations between offshore spawning grounds and inshore, estuarine waters (Narasimham, 1972). More recently, Khan (2006) inferred that *T. lepturus* demonstrate opportunistic migration, due to the relative abundance of prey, between coastal habitats of the west India coastline, separated by a distance greater than 500 km. Observations from Brazil suggest *T. lepturus* to be a marine-opportunist, where *T. lepturus* demonstrates flexible spawning patterns and spawning has been reported in all seasons, both offshore and inshore (Martins and Haimovici, 2000). The aforementioned research has provided insights to inform the fishery and ecosystem management of *T. lepturus* in these regions (Khan, 2006, Martins and Haimovici, 2000, Narasimham, 1972), yet also raised questions regarding potential variability in migratory behaviour among regions.

T. lepturus is an important commercial species on a global scale and based on catch weight, it is consistently placed by the United Nations (UN) Food and Agriculture Organization (FAO) statistics in the top ten marine species landed worldwide. In south-eastern Australia, the overall catch weight is comparatively small, with generally low yield and episodically large catches in the commercial and recreational fisheries of NSW (Stewart et al., 2015). The *T. lepturus* fisheries in south-eastern Australia are not well understood which has resulted in a stock status of ‘undefined’ and there are no specific management regulations for the commercial harvest of *T. lepturus*. Due to the unpredictable nature of *T. lepturus* in south-eastern Australia, it was hypothesised that analyses of otolith chemistry would be of value for elucidating estuarine-coastal movements of the species in south-eastern Australia. The understanding of individual movement patterns would help increase commercial and recreational stakeholder engagement, provide insights on the life history traits and key habitats of the population (Crook et al., 2008, Milton et al., 2008), allow for evidence-based management decisions, and ensure the population remains resilient in this region (Elliott et al., 2007).

The overall goal of this study was to gain insights into the estuarine-coastal movements made by *T. lepturus* individuals from south-eastern Australia by exploring whether otolith microchemistry analyses could be useful for discerning these movements. Analysing the variation in otolith chemistry along a gradient from the coast through to upper estuarine habitats would have been ideal but was impractical for this study. As an alternative, the concentrations in the outermost growth edge of the otolith were analysed for specific elements ones that are most likely to differ between coastal and estuarine habitats, and it was hoped that this method would allow for the discernment of movement patterns between these habitats.

However, given the variability involved in the incorporation into otoliths of elements such as Sr and Ba that are useful for delineating estuarine versus coastal habitats, the specific capabilities of otolith chemistry techniques and the factors that affect elemental incorporation must first be determined. The ideal approach would be to test variability using aquaria with controlled environmental variables; however, this was impractical for *T. lepturus* because the species occurrence in fisheries is patchy and it is a challenging species to obtain alive. Additionally, there is limited information available for captive rearing and husbandry of the species. For an alternative approach to the investigation, an opportunistic and cost-effective sampling design was implemented. A collection of otolith samples was selected from a pre-existing field collection of *T. lepturus*. Subsets were selected for stratification by time and location, and to isolate variables that might be cofactors in the presence of elements of interest, considering the limited scope of the pre-existing collection.

The specific objectives of this chapter were therefore to: (1) establish the relationship between *T. lepturus* otolith chemistry and body length and age, to identify which variable is the best proxy to control for any potential effects of ontogeny in elemental incorporation; (2) explore the temporal stability of elemental incorporation into *T. lepturus* otoliths by establishing the relationship between otolith chemistry and month and year; (3) compare the individual elemental concentrations (Sr, Ba, Mg, Mn) of the outermost growth edge of *T. lepturus* otoliths, from individuals caught between two habitat types to determine the capability of the technique to distinguish between estuarine and coastal sites; 4) compare elements between two estuarine sites to determine if differences in marker elements are similar for a specific habitat type, and; (5) investigate core-to-edge transects from fish caught in different habitats to look for patterns of coastal – estuarine movements over the full lifespan of individuals.

4.2 Materials and Methods

4.2.1 Sampling design

There were limited resources available for the current study, therefore, *T. lepturus* samples were selected from a pre-existing biological collection, gathered by the New South Wales (NSW) Department of Primary Industries Fisheries from commercial seafood cooperatives and supplemented with samples from recreational anglers.

The pre-existing collection was obtained from the commercial fishery for biological assessment of the species in NSW; however, the collection was limited in its spatial and temporal stratification due to the dependence of sampling on the local commercial fishery which is sporadic in time and space. The limitations subsequently limited the sampling capabilities for the current study. The seafood cooperative coop samples, of the pre-existing collection, were obtained every one to two weeks and, depending on availability, a random sample of up to 10 fish were collected from each of three sites.

Most of the commercial catch for *T. lepturus* in NSW is reported from three locations and sampling for the current study therefore focused on these locations. There were two estuarine sites and one ocean site: (1) the Hawkesbury estuarine site at 33.55° S; 151.33° E; (2) the Hunter estuarine site at 32.93°S, 151.78° E' and; (3) the Hunter coastal site 32° S – 33° S, 151.80° E – 152.65° E (Figure 27). The coastal fishery extends to 3 nautical miles offshore and the Hunter Coastal site overlaps the latitudinal range of the Hunter estuarine site. The Hawkesbury estuarine site is located approximately 83 km south from the Hunter estuarine site and the Hunter coastal site. The Hunter estuarine site is part of a river system with freshwater habitats at the upper reaches. The mouth of the Hunter River enters the Tasman Sea at the city of Newcastle and is a major industrial port and an important recreational harbour. The Hunter estuarine site is a bar-built estuary, with a sandbar built up by ocean waves at the mouth of the estuary, which restricts the tidal inlet from the coast (Roy et al., 2001). Compared with tidal-dominated estuaries, the estuary is shallow (average river depth: 3.3m) and has a strong riverine influence. The Hawkesbury estuarine site is a part of the Hawkesbury River system, which also connects with freshwater habitats at the upper reaches and, in contrast to the Hunter River, has a wide mouth and enters the Tasman Sea at the city of Sydney. The Hawkesbury River is a drowned river valley; it is a tidal river with a large width to depth ratio (average river depth: 13.8m) (Roy et al., 2001). The coastal waters of eastern Australia, between 32°S – 33°S are affected by wind-driven coastal upwelling and the East Australian Current, a poleward-moving current that transports warm water from the Coral Sea in Queensland down the east coast of Australia into the Tasman Sea (Oke and Middleton, 2000). The speed and intensity of the current varies according to multiple factors and therefore causes considerable variability in the abiotic characteristics of the coastal habitats of south-eastern Australia (Hughes, 2012, Suthers et al., 2011).

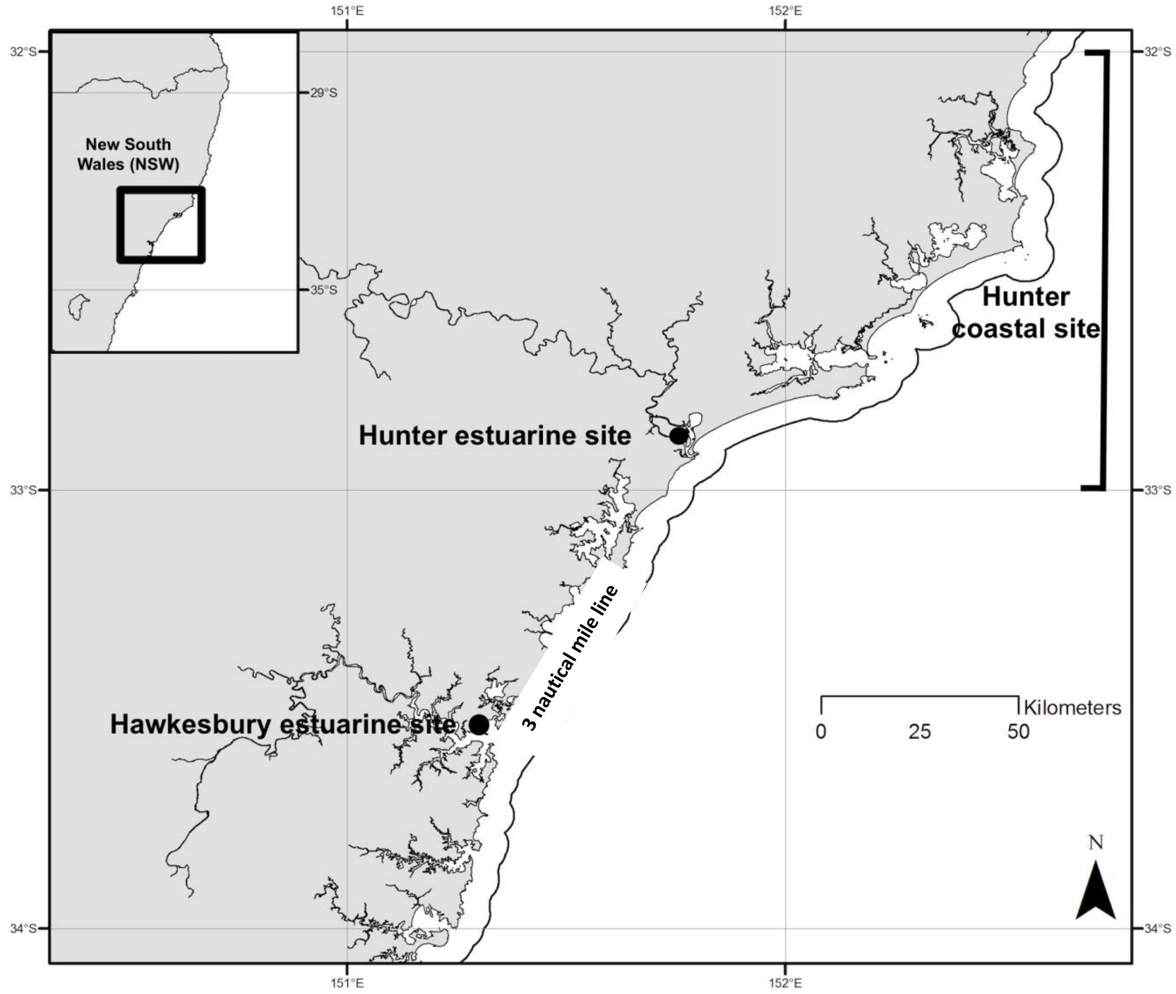


Figure 27. Map of the south-eastern Australian coast showing the three sites where *Trichiurus lepturus* were collected between September 2015 and December 2016: Hunter

estuarine and coastal sites, and Hawkesbury estuarine site are indicated. The coastal site extends to 3 nautical miles from the coast, marked by a black line.

Most supplementary, recreational fishery-caught samples were obtained through the attendance of an annual recreational fishing competition for *T. lepturus,* held at the Hawkesbury estuarine site, between the 1st and 3rd of July 2016. The engagement of anglers at the event resulted in angler-donated samples. A second contribution to recreational angler-caught samples were provided by the NSW Fisheries Department compliance officers from the Hunter estuarine site, after being seized from recreational anglers exceeding the enforced bag limit of 10 fish per person per day. All compliance-seized samples were caught within approximately 75 days, between mid-February and mid-April 2016.

The total sample, including commercial and recreational samples, of 140 individuals that were selected for the current study were collected from September 2015 to December 2016. All body lengths were recorded to the nearest cm and each pair of sagittal otoliths were excised, cleaned and stored dry.

Within the limitations of sample collection, individuals were selected to best align with the aims of the current study (Table 11). Selected individuals were partitioned into subsets to allow the examination of specific variables of interest, through time and space. The uneven availability of individuals across spatial and temporal factors of interest limited some investigations to a single site or year and did not allow for larger multi-factorial designs.

Before the reconstruction of elemental profiles from natal origin to capture, the elemental concentrations (Sr, Ba, Mg, Mn) were analysed from otolith material at the outer growth edge, so that analysis directly related to the site where an individual was caught. First, otolith edge concentrations from individuals caught at the same site, season and year, were compared with length and age. The comparisons were made to establish the relationship between *T. lepturus* otolith chemistry and both length and age, to identify which variable is the best proxy to control for any potential effects of ontogeny in subsequent comparisons among sites. Age would typically be the most suitable proxy for ontogeny, given the potential for body length to vary among individuals at similar ontogenetic stages. However, body length was also considered because it has been used as a suitable proxy for ontogeny in previous studies (Fowler et al., 2015, Longmore et al., 2010) and because of the significant relationship demonstrated between body length and age for *T. lepturus* in the region (n= 330), where age accounted for 94% of the explained variability in length (Chapter 3, Linear regression, r=0.94, p=<0.001). Second, otolith edge concentrations from individuals caught at the same site, with a constant age and length-class range, were compared among months and years, to explore the temporal stability of *T. lepturus* otoliths. The last comparison was among the three spatially segregated collection sites (estuarine and coastal habitats), using the otolith edge material of individuals caught in the same season and year, with an age and length-class range which was kept constant among sites. The comparison between the Hunter coastal and Hunter estuarine site was made to determine the capability of the technique to establish elemental markers for estuarine-coastal movements and the comparison between the estuarine sites was made to determine whether any subsequent investigations into movements of *T. lepturus* between estuarine and coastal habitats would need to be system specific.

Core-to-edge transects were utilised to investigate Sr:Ca and Ba:Ca profiles from natal origin to capture. The expected estuarine-coastal concentrations for the four trace-elements investigated, were established by observing pooled otolith edge values, then, any chemical deviations from the expected concentrations were examined, to look for patterns of coastal – estuarine movements over the full lifespan of individuals. Only individuals from the higher age classes (6-8 years) were selected, so that chemical profiles could be examined across a longer time span.

Individuals used for transect analysis were selected across three different age classes and were all caught in 2016, therefore, transect analysis encompassed individuals that were spawned in three different calendar years (2008, 2009, and 2010). Both sexes and a range of collection months/seasons were also dispersed between the three study sites to account for potential bias in any chemical profile deviations through time. The individuals that were selected for transect analysis were selected in equal numbers among each of the three collection sites (n=10 for each site), to investigate whether any observed patterns were likely to occur between the habitats or systems investigated.

Table 11. Summary of individuals selected to address each aim of the study. Individuals were partitioned in to subsets to allow the examination of specific variables of interest.

Aim	Influence on otolith chemistry	Sample size	Pre-anal Length range (cm)	Age range (yrs)	Site	Year	Season
1) Establish the relationship between otolith chemistry and both length and age.	Ontogeny	23	22-52	2-6	Hawkesbury estuarine site	2016	Autumn
2) Establish the relationship between otolith chemistry and month.	Year	28	29-78 (range constant) between years)	3-7 (range constant between years)	Hunter coastal site	2015 – 2016	Spring
2) Establish the relationship between otolith chemistry and year.	Season	56	22-55 (range constant between seasons)	2-8 (range constant between seasons)	Hawkesbury estuarine site	2016	All seasons
3) and 4) Determine the capability of the technique to distinguish within and between habitats types	coast/ estuary	59	22-52 (range constant between sites)	2-8 (range constant between sites)	Two estuaries and one coastal habitat	2016	Spring
5) Investigate movements between habitats over the fish's lifespan among systems and habitats.	-	30	33-62 (range constant between sites)	6-8 (range constant between sites)	Two estuaries and one coastal habitat	2016	All seasons

4.2.2 Otolith sample preparation and aging

The right otolith was used for chemical analysis wherever possible, but if necessary, the left was used, due to demonstrated consistency in the trace elemental concentrations between otolith pairs in other species (Arslan and Secor, 2008, Campana et al., 2000). Otoliths were weighed (to the nearest 1 x 10^{-5} g) using an electronic balance (Sartorius, Göttingen, Germany) and then embedded in blocks of epoxy resin. To expose a transverse section of the otoliths, a singular diamond-encrusted blade was used on a Gemmasta high-speed saw. The strips were then adhered to glass slides with thermoplastic glue (Crystalbond 509, Clarkstown, USA). To reveal the otolith core, sections were ground down to a thickness of ~0.2 mm with 1200 grit polishing paper on a Struers model LaboPol-4 polishing equipment. Each section was then polished using 9 and 3 micron lapping film. To observe the growth increments and determine the fish age, sections were viewed under reflected light at 4x magnification. Annual deposition of increments for *T. lepturus* populations have previously been validated using marginal increment analyses (Kwok and Ni, 2000). The annual increments are made up of opaque and hyaline increments. To age each individual, the opaque increments were counted starting from the core to the distal edge of the ventral lobe, following the methods of Kwok and Ni (2000). The estimated age of each individual was verified by a second reader. If an age was disputed the otolith was removed from analysis.

The slides were then sonicated for three minutes to remove any surface contamination, following the methods of Fowler et al. (2015) for ICPMS analysis

4.2.3 LA-ICP-MS

The otolith sample composition was analysed using an Agilent Technologies 7500ce inductively coupled plasma mass spectrometer (ICP-MS) located at the University of Technology Sydney, coupled with a New Wave UP-213 laser ablation unit with a Large Format Cell (Kenelec Scientific). The isotopes measured were Sr^{88}, Ba^{138} Mg^{24}, Mn^{55} and Ca^{43} which were chosen because they were expected to differ between estuarine and coastal habitats, based on the literature. The otolith sections were positioned in a random order on the slide to ensure that any instrument drift could not bias comparisons among sub-groups of individuals. Before each sampling ablation was made, a pre-ablation transect was made to remove any surface contaminants remaining after sonication (width: 100 μm). The sampling ablation transect was then made within the area of the pre-ablation, from the centre of the otolith core to the edge, along the primary ageing zone of the section. The positioning of transects on the otolith section were standardised among samples to account for spatial variation in the elemental composition of otolith material (Hamer and Jenkins, 2007) and the ablation diameter was selected as a compromise between detection power and temporal resolution of elemental concentrations (Figure 28). For the sampling transects, ablation diameter of 55 μm, a scan speed of 3 μm/s, a pulse rate of 10 Hz, and an energy output of 75% were used. Calibration of the signal intensity to the elemental concentration was performed using a NIST-612 glass standard (Pearce et al., 1997) for high sensitivity and low background intensity. One transect was made on the NIST glass after every five transects made on the samples to compensate for any instrument drift occurring throughout the sample run. Sample transects were also done in a random order to avoid systematic biases of instrument drift.

Count-per-second data for each isotope were converted to elemental concentrations by subtracting background counts, standardising to the external standard (NIST-612) and normalizing to the internal standard (Ca^{43}) for ablation yield following Elsdon and Gillanders (2005). All the recorded data for Sr^{88}, Ba^{138} and Mg^{24} were 100.0% above detection limits and Mn^{55} data were 58% - 97% above detection limits. Precision (% relative standard deviation) calculated from repeated ablations of the NIST standard were 2.02% for Sr^{88}, 2.75% for Ba^{138}, 5.42% for Mg^{24}, 3.06% for Mn^{55} and 0.00% for Ca^{43}. Concentrations of target elements were expressed as ratios to Ca.

4.2.4 Otolith edge material

Otolith material at the outer growth edge was analysed to obtain elemental concentrations as close as possible to the time of capture. The last two data points from the ablation transect, those closest to the otolith edge, were taken and averaged to ensure obtained values were not unduly influenced by outlying data values.

Edge analysis encompassed 59 μm of material from the edge of the otolith, calculated based on the ablation diameter and scan speed. The time encompassed in the analysis was determined using linear back-calculation based on the width of the previously completed annulus and the width of material encompassed in analysis and equated to approximately 100-250 days before capture.

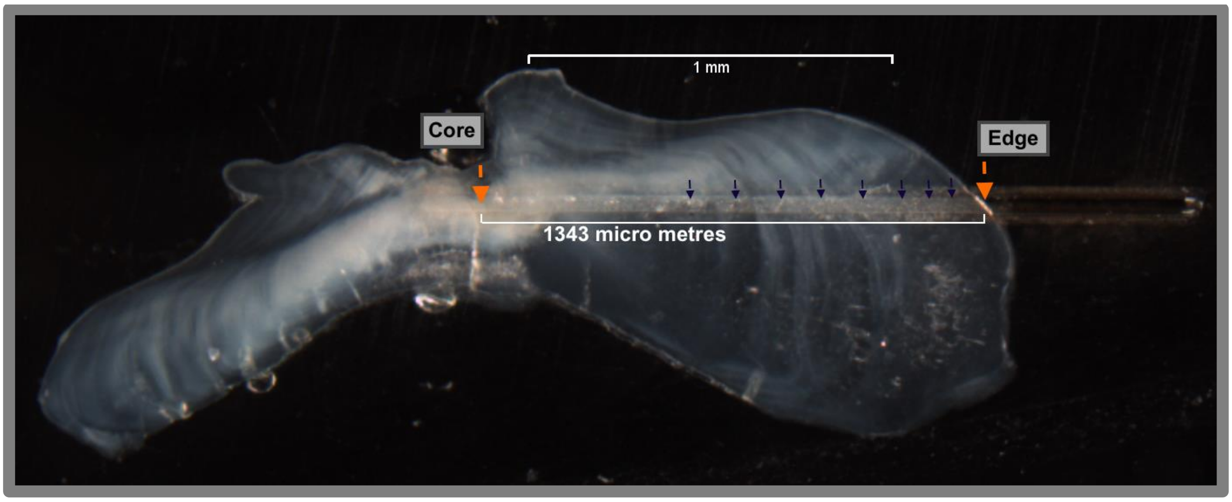


Figure 28. Image of otolith section from an 8-year-old *Trichiurus lepturus* showing the core-to-edge laser ablation transect. Unlabeled arrows represent annual increments (years 1-8).

4.2.5 Variation of trace elemental concentrations

To establish the relationships between trace elemental concentrations (Sr, Ba, Mg, Mn) in the otoliths of *T. lepturus* with factors of interest (Table 11), a series of independent, univariate tests were used to quantify the relationship between each of the elemental concentrations at the outer growth edge of otoliths and the five key factors of (1) age at capture, (2) length at capture, (3) month of capture, (4) year of capture, and (5) habitat/system of capture.

4.2.6 Data analysis

4.2.6.1 Ontogenetic factors

To determine the most suitable factor to control for the effect of ontogeny on elemental concentrations, linear regression was used to test if the relationship between Sr, Ba, Mg and Mn concentrations from the otolith edge and length or age were significant (α = 0.05) (Fowler et al., 2015). Individuals were selected (n=23) with a broad spread of lengths (22-51cm) and ages (2-6 years). The capture site (Hawkesbury River), year of capture (2016) and season of capture (autumn) were kept constant to isolate the effects of length and age on otolith chemistry (Table 11).

4.2.6.2 Temporal factors

To explore the temporal stability of *T. lepturus* otolith chemistry, a series of univariate ANCOVA tests were used to make comparisons between otolith chemistry and both month and year. To test for variability among months, individuals were available from four collection months: March (n=16), May (n=15), July (n=10) and November (n=15), while the collection year (2016) and the collection site (Hawkesbury River) were kept constant to isolate the effects of the collection month on otolith chemistry. To test for inter-annual variability, individuals were available between the years of, 2015 (n=11) and 2016 (n=17), the season of capture (spring) and the collection site (Hunter coastal site) were kept constant to isolate the effects of year on otolith chemistry.

Body length was used as a covariate in all temporal comparisons based on the significant relationship between *T. lepturus* otolith chemistry and body length, described below in results section. An initial ANCOVA including an interaction term between the covariate (length) and independent variable (month or year) was used to test the assumption of homogeneity of regression slopes. Sr, Ba and Mg all returned no significant interactions for either month or year and therefore met assumptions for ANCOVA. Subsequent ANCOVAs for these elements were conducted without the interaction term in the model. For the comparison of otolith Mn concentrations among months, a significant interaction between length and month was found (ANCOVA: $p = 0.011$). To meet assumptions, the data set was truncated to a subset of samples from a small length class (n= 38), because it had the highest sample size of useable data.

4.2.6.3 Comparison within and among habitat types

To determine the capability of the technique to indicate estuarine-coastal movements of *T. lepturus* using individual elemental concentration profiles, ANCOVA was used to make comparisons of otolith Sr, Ba, Mg and Mn concentrations between and within habitat types, including the Hunter coastal site and the Hunter estuarine site, to examine two habitat types at the same latitudinal range and the Hawkesbury estuarine site to examine a secondary estuarine system (Figure 27). The year of capture (2016) and the season of capture (Spring) was kept constant to isolate the effects of habitat on otolith chemistry. Length was used as a covariate to control for the effect of ontogeny on the main comparison. An initial ANCOVA with interaction term between the covariate (length) and independent variable (site) was run to test for homogeneity of regression slopes. Concentrations of Ba, Mg and Mn all returned no significant interactions and therefore met assumptions for the main ANCOVA. For Sr among sites, the initial data set returned a significant interaction between length and site, which was likely to be driven by varying length ranges among sites. Because the interaction violated the assumptions of an ANCOVA, the data set was truncated into 2 data subsets of smaller (30-40 cm PL) and larger (>40 cm PL) individuals and analysed separately. The subset of individuals from the smaller length class had useable data to make comparisons between all three collection sites and the subset of individuals from the larger length class had useable data to make a comparison between the Hunter estuarine and Hunter coastal site.

4.2.7 Core-to-edge transect analysis

To look for movements throughout the individual's lifespan, core-to-edge transects from each of 10 randomly selected individuals in each of the 3 collection locations were analysed for deviations in their otolith Sr and Ba concentration profiles. These deviations would indicate that the individual was exposed to conditions that differed from those experienced around the time of capture as determined by edge signatures. First, threshold boundary windows were constructed for otolith Sr and Ba concentrations, by establishing upper and lower threshold boundaries. Threshold boundaries were established using the 5th and 95th percentile of the pooled edge values of all otolith chemistry samples (n=140). Using the pooled samples was deliberate in order to increase the variability encompassed within the boundaries, so that only substantial deviations from the edge values would be detected, increasing the chance that the deviations were meaningful. Any pattern of specific deviation outside the boundaries may represent movements outside of the coastal – estuary systems studied. Edge values were used so they related to the time directly before capture (~100-250 days), when location was known. The threshold windows were, therefore, representative of the time frame of sample collection ranging between September 2015 to December 2016, the range of ontogenetic stages at capture, and the environment factors of the study region, including the: salinity; temperature and water chemistry. Next, the historical record of otolith Sr and Ba concentrations for each individual were examined along a growth axis, to look for deviations outside the defined threshold bounds.

Individuals were placed into one of four categories: individuals that either did or did not exhibit a deviation outside the defined threshold bounds in either Sr or Ba concentrations. Individuals were only placed in these categories if the deviation lasted 5 or more consecutive data points, in order to avoid potential data spikes caused by machine error.

To test for differences in the observed patterns in core-to edge elemental profiles among habitats and systems, an evaluation was made as to whether individuals were more or less likely to exhibit a deviation among the three collection sites (Fisher's exact test). The Fisher’s exact test was considered more suitable than a Chi Square test of goodness of fit, because some of the expected numbers in the comparison were less than 5.

4.3 Results

4.3.1 Variation of trace elemental concentrations

4.3.1.1 Ontogenetic factors

Body length was significantly related to the otolith elemental concentrations for Sr, Ba and Mn (Regression: p values < 0.05), but not Mg (Table 29, Figure 29), for *T. lepturus* caught from the Hawkesbury estuarine site in 2016. As length increased, both Sr and Ba increased (Table 29, Figure 29), with length accounting for 22% and 20% of the variability in concentrations, respectively. As length increased, Mn decreased, with length accounting for 53% of the variability in elemental concentration.

Table 12. Results of the linear regression tests on relationships for trace element-to-calcium concentration ratios with length (n=23) (a = 0.05).

Element	**Equation**	**r^2**	**df**	**p**
Sr	$y = 0.0426x + 1.0343$	0.22	22	0.02
Ba	$y = 0.025x + 0.2417$	0.20	22	0.03
Mg	$y = 0.0007x + 0.148$	0.02	22	0.50
Mn	$y = 0.0003x + 0.0199$	0.52	22	<0.001

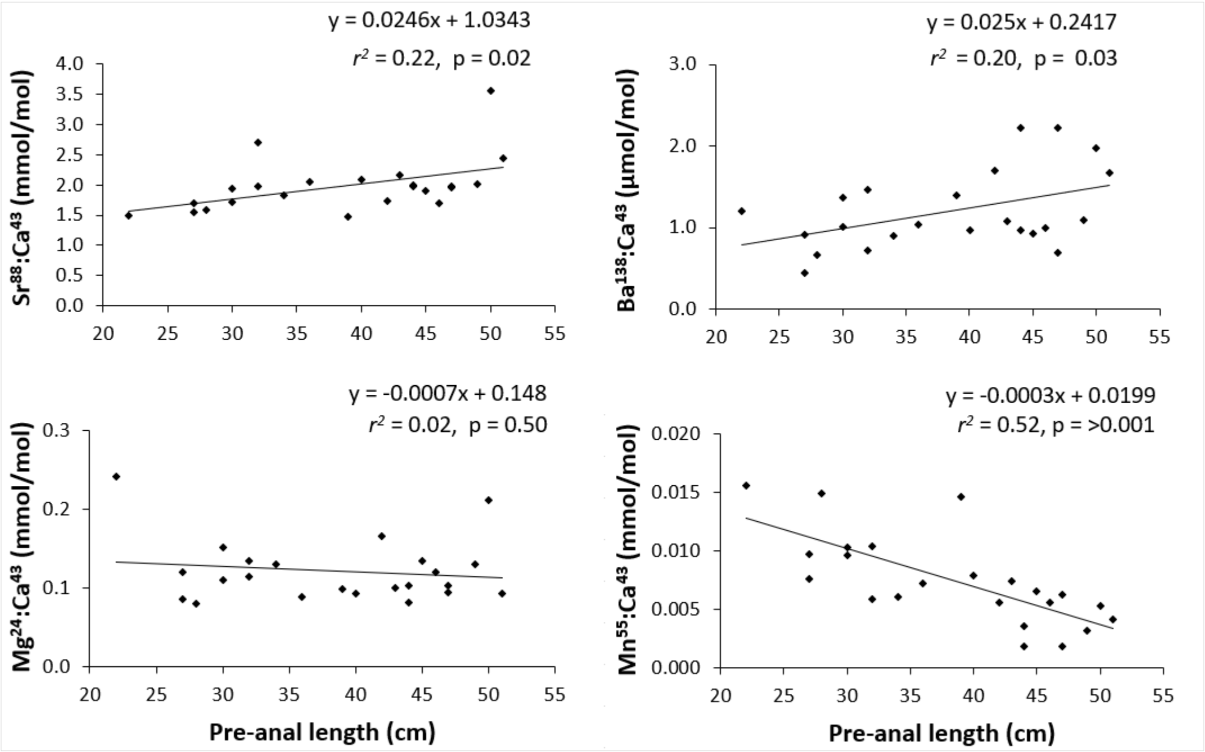


Figure 29. Linear relationship between ontogeny (using body length as a proxy) and the trace element concentrations on the edge of otoliths (n=23) for *Trichiurus lepturus* from the Hawkesbury River in 2016. Individuals ranged from 22-55 cm pre-anal length.

Age was significantly related to the otolith elemental concentrations of Mn (Regression: p values < 0.05) with a weak, negative relationship, where age accounted for 27% of the variability (Table 13, Figure 30). Age was not related to Sr, Ba and Mg (Table 13, Figure 30).

Table 13. Result of the linear regression tests on relationships for otolith trace element to calcium concentrations with age (n=23) (a = 0.05)

Element	**Equation**	**r^2**	**df**	**p**
Sr	y = 0.0044x + 1.9621	<0.001	22	0.95
Ba	y = 0.0839x + 0.8814	0.06	22	0.27
Mg	y = 0.0109x + 0.1632	0.14	22	0.08
Mn	y = 0.0014x + 0.013	0.27	22	0.01

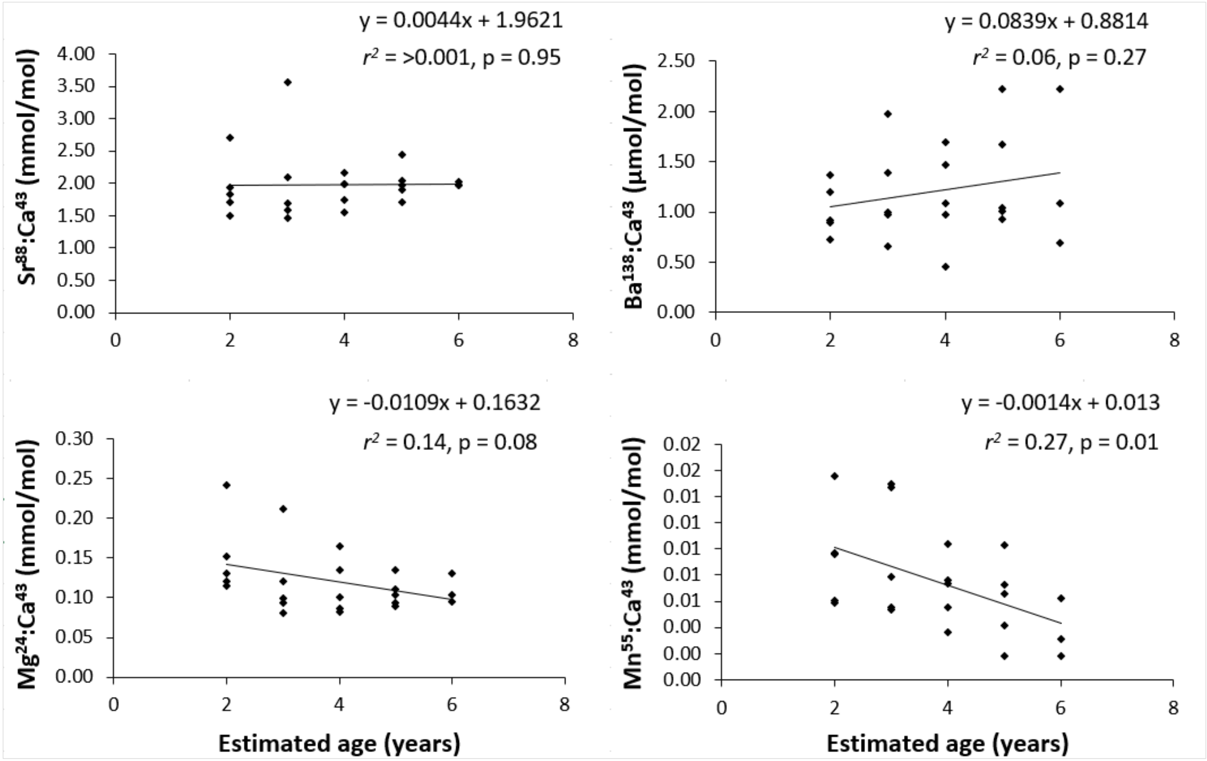


Figure 30. Linear relationship between ontogeny (using age as a proxy) and the trace element to calcium concentrations on the edge of the otolith (n=23) for *Trichiurus lepturus* collected from the Hawkesbury River in 2016. Individuals ranged in age from 2-6 years.

4.3.1.2 Time factors

Otolith Mg concentrations differed significantly among months (ANCOVA: Table 5, Figure 31), concentrations were higher in otoliths collected in March than those collected in May and July (Tukey's test: p = 0.007, 0.001, Figure 31). Mn concentrations also differed significantly among months (ANCOVA: Table 14, Figure 31); concentrations were lower in the otoliths collected in November than the otoliths collected in March and May (Tukey's test: p = 0.035, >0.001, Figure 31) There were no significant differences among collection months for otolith Sr or Ba concentrations (ANCOVA: Table 14).

Table 14. Result table for the main effect in ANCOVA comparisons between trace element to calcium concentrations on the edge of otoliths (n=56) among months (*a* = 0.05). Degrees of freedom (df) are indicated for the numerator and denominator of the F-ratio.

Element	**F**	**df**	**p**
Sr	2.130	3,51	0.108
Ba	2.114	3.51	0.110
Mg	0.5976	3,51	0.001
Mn	10.688	3,30	<0.001

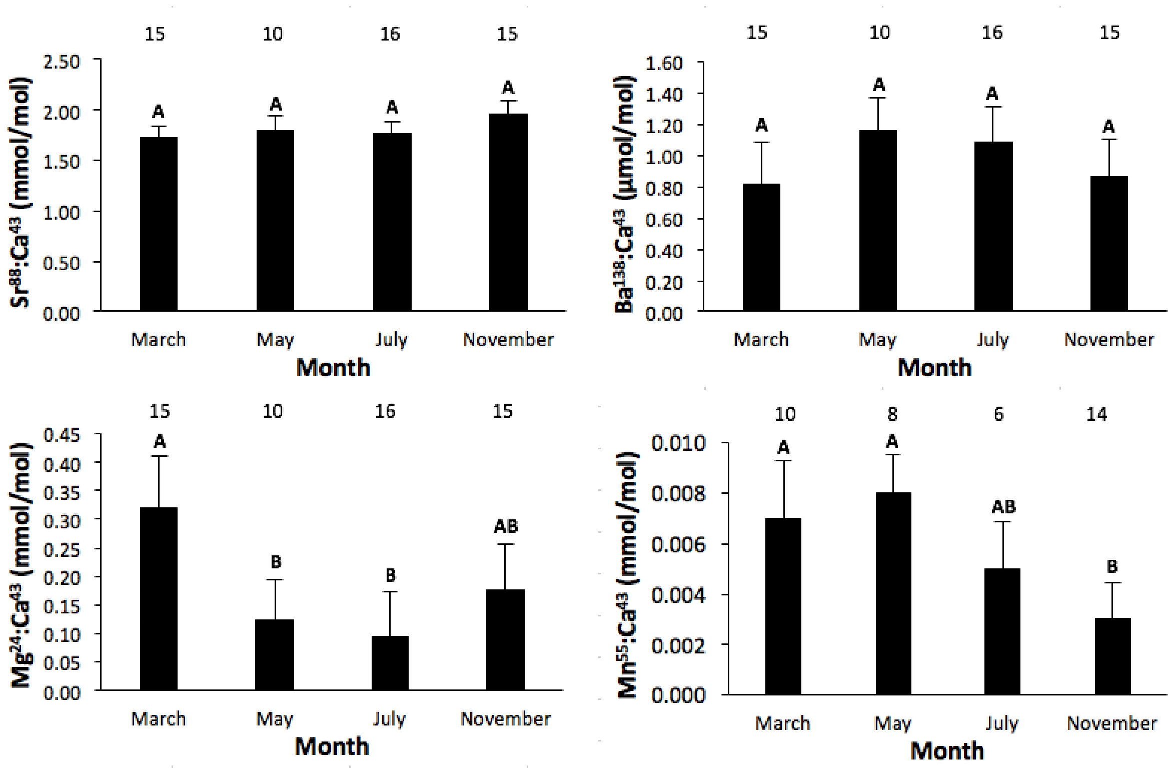


Figure 31. Mean (±SE) trace element concentrations on the edge of otoliths (n = 56) among collection months for *Trichiurus lepturus* from the Hawkesbury River in 2016. The body length range of individuals (22-55 cm pre-anal length) was kept constant among months. Months that share a capital letter were not significantly different from each other ($p \geqslant 0.05$) according to Tukey's tests. The sample size for each month is displayed above each column.

Otolith Sr, Ba, Mg and Mn concentrations did not differ significantly between the collection years of 2015 and 2016 (ANCOVA: Table 6, Figure 32).

Table 15. Result table for ANCOVA comparisons of trace element to calcium concentrations on the edge of otoliths (n=28), between 2015 and 2016 (*a* = 0.05).

Element	**F**	**df**	**p**
Sr	0.820	1,25	0.374
Ba	2.148	1,25	0.155
Mg	0.032	1,25	0.859
Mn	3.144	1,25	0.088

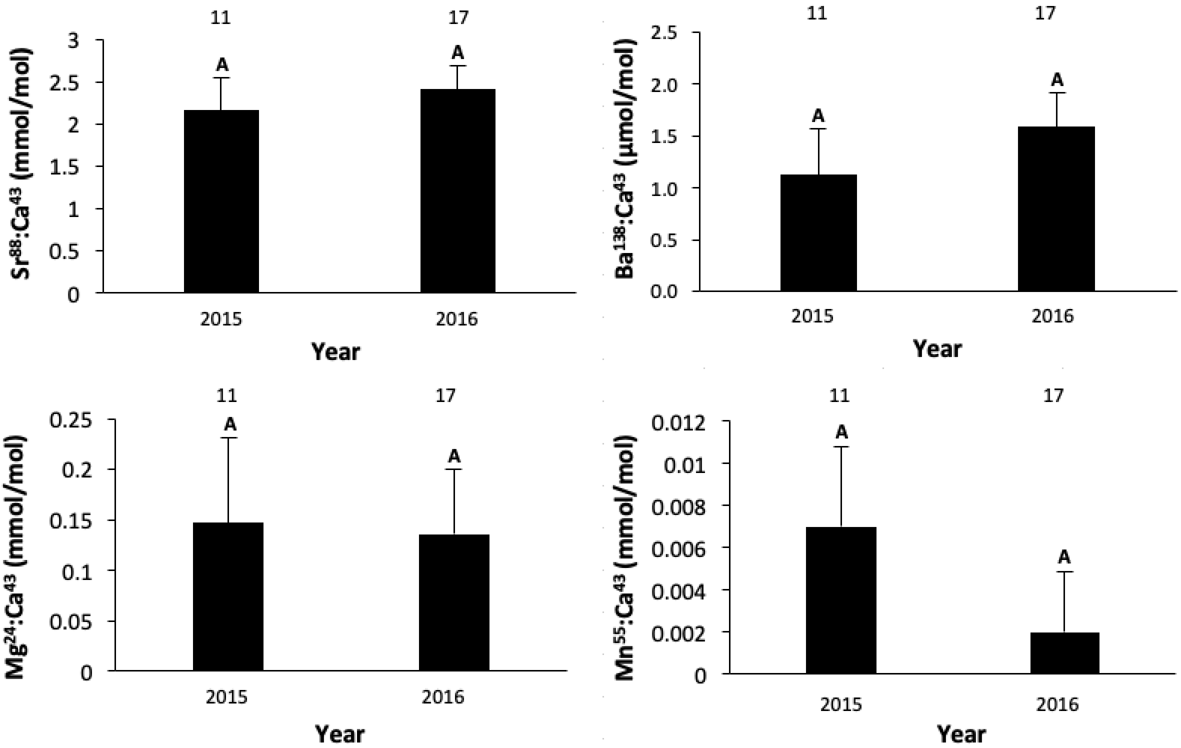


Figure 32. Mean (±SE) trace element concentrations on the edge of the otolith between collection years for *Trichiurus lepturus* (n=28) from the Hawkesbury River in 2016. The body length range of individuals (22-55 cm pre-anal length) was kept constant between years. Years that share a capital letter were not significantly different from each other ($p \geqslant 0.05$) according to ANCOVA analysis. The sample size for each year is displayed above each column.

4.3.2 Comparison within and between habitat types

Otolith Sr, Ba and Mn concentrations differed significantly within and between habitat types (ANCOVA: Table 7, Figure 33). The concentrations of Sr, in the otoliths of both the smaller individuals (30-40 cm PL) and the larger individuals (>40 cm PL) did not differ significantly between the Hunter estuarine site and the Hunter coastal site (Tukey's test: p= 0.065, ANCOVA: 0.276, Figure 33).

Otolith Sr concentrations were significantly higher from the Hunter estuarine site compared with the Hawkesbury estuarine site, for the subset of smaller individuals (Tukey's: p = 0.009, Figure 33). The comparison was not possible for the subset of larger fish due to the lack of larger fish in the sample collection for the Hawkesbury estuarine site. Otolith Ba concentrations were significantly lower from the Hawkesbury estuarine site than both the Hunter estuarine site (Tukey's test: 0.007, Figure 33) and the Hunter coastal site (Tukey's test: <0.001, Figure 33). Otolith Mn concentrations were significantly higher from the Hawkesbury estuarine site than the Hunter estuarine site (Tukey's test: 0.001, Figure 33) and the Hunter coastal site (Tukey's test: $p = 0.027$, Figure 33), whereas otolith Mg concentrations did not differ significantly among sites (ANCOVA: Table 7, Figure 33).

Table 16. Results table for ANCOVA comparisons of trace element to calcium concentrations on the edge of otoliths (n=59 for Ba, Mg and Mn and n=26 for Sr), among collection sites ($\alpha = 0.05$).

Figure	Element	F	df	p
7.A	Sr	5.898	2,22	0.009
7.B	Ba	10.175	2,55	>0.001
7.C	Mg	1.237	3,51	0.001
7.D	Mn	7.938	2,55	0.298

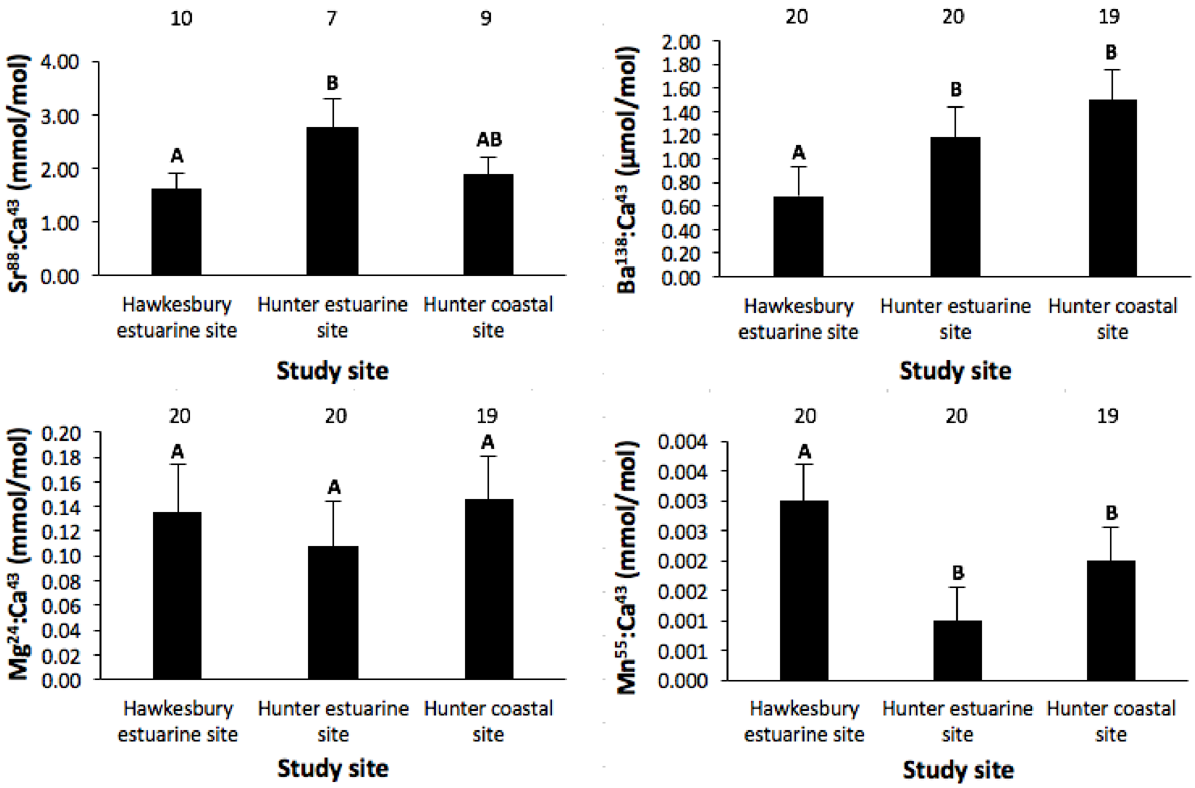


Figure 33. Mean (±SE) trace element concentrations on the edge of otoliths (n=59 for Ba, Mg and Mn and n=26 for Sr) among study sites for *Trichiurus lepturus* in Spring 2016. The length range of individuals (22-52 cm pre-anal length for Ba, Mg and Mn and 30-40 cm for Sr) was kept constant among sites. Sites that share a capital letter were not significantly different from each other ($p \geqslant 0.05$) according to ANCOVA analysis.

4.3.3 Core-to-edge transect analysis

The upper and lower threshold boundaries of otolith Sr and Ba concentration, based on the pooled edge values of all otolith chemistry samples, which are representative of the typical characteristics of all three estuarine and coastal collection sites, were calculated at: 1.50 (lower Sr), 3.16 (upper Sr), 0.51 (lower Ba) and 2.22 (upper Ba) mmol/mol $^{-1}$.

Otolith Sr values along the transects ranged between 1.19 and 5.50 mmol/mol, and Ba values between 0.31 and 8.34 mmol/mol. Both Sr and Ba profiles therefore deviated outside the defined threshold windows. Some Ba profiles deviated above the upper defined threshold bounds by more than double the upper boundary (Table 17, Figures 34, 35 and 36).

Ba deviations occurred in 25 out of the 30 *T. lepturus* core-to-edge transects, both above and below the defined threshold windows for Ba. For 21 out of the 25 deviations, the deviation was a spike that elevated above the upper Ba threshold and occurred before the formation of the first annual increment (Figure 34, 35 and 36). The 21 individuals were evenly dispersed among study sites, cohorts and sexes. The proportions of individuals that exhibited a Ba spike before the formation of their first annual increment was not significantly different among the habitats or systems investigated in this study (Fisher's test, p = 0.30).

There were four individuals with Sr profiles that exhibited a deviation outside the defined threshold bounds, however, most of the Sr profiles remained within the bounds. Of the 30 individuals observed in this study, Sr profiles rarely exhibited a decrease that corresponded with a Ba elevation.

Table 17. Sampling details of *Trichiurus lepturus* caught in south-eastern Australia, with reported changes in otolith chemistry profiles between birth and capture. Individuals that exhibited a Ba deviation before the formation of their first annual increment are highlighted in grey.

Fish #	**Site**	**Age**	**Season**	**PL (cm)**	**Low Sr**	**High Sr**	**Low Ba**	**High Ba**
323	Hawkesbury River estuarine	6	Autumn	49	✗	✓	✗	✓
329	Hawkesbury River estuarine	6	Autumn	45	✗	✗	✗	✓
330	Hawkesbury River estuarine	6	Autumn	52	✗	✗	✗	✗
296	Hawkesbury River estuarine	6	Winter	49	✗	✗	✗	✓
283	Hawkesbury River estuarine	6	Winter	42	✗	✗	✗	✓
262	Hawkesbury River estuarine	6	Winter	36	✗	✗	✗	✓
334	Hawkesbury River estuarine	7	Autumn	44	✓	✗	✓	✗
279	Hawkesbury River estuarine	7	Winter	53	✗	✗	✗	✗
328	Hawkesbury River estuarine	8	Autumn	52	✗	✗	✗	✓
319	Hawkesbury River estuarine	8	Winter	55	✗	✗	✗	✓
420	Hunter coastal	6	Spring	48	✗	✗	✓	✓
438	Hunter coastal	6	Spring	46	✗	✗	✓	✓
362	Hunter coastal	6	Summer	43	✗	✗	✓	✗
364	Hunter coastal	6	Summer	48	✓	✗	✓	✓
428	Hunter coastal	7	Spring	48	✗	✗	✗	✓
429	Hunter coastal	7	Spring	49	✗	✗	✗	✓
430	Hunter coastal	7	Spring	51	✗	✗	✓	✓
353	Hunter coastal	7	Summer	49	✗	✗	✗	✓
349	Hunter coastal	7	Summer	49	✗	✗	✗	✓
361	Hunter coastal	8	Summer	49	✗	✗	✗	✓
78	Hunter River Estuarine	6	Autumn	62	✗	✗	✗	✗
80	Hunter River Estuarine	6	Autumn	59	✗	✗	✗	✓
81	Hunter River Estuarine	6	Autumn	61	✗	✓	✗	✓
129	Hunter River Estuarine	6	Autumn	33	✗	✗	✗	✓
77	Hunter River Estuarine	7	Autumn	62	✗	✗	✗	✗
135	Hunter River Estuarine	7	Autumn	33	✗	✗	✗	✗
412	Hunter River Estuarine	7	Spring	45	✗	✗	✓	✓
413	Hunter River Estuarine	7	Spring	47	✗	✗	✓	✓
419	Hunter River Estuarine	8	Spring	52	✗	✗	✓	✓
410	Hunter River Estuarine	8	Spring	52	✗	✗	✓	✗

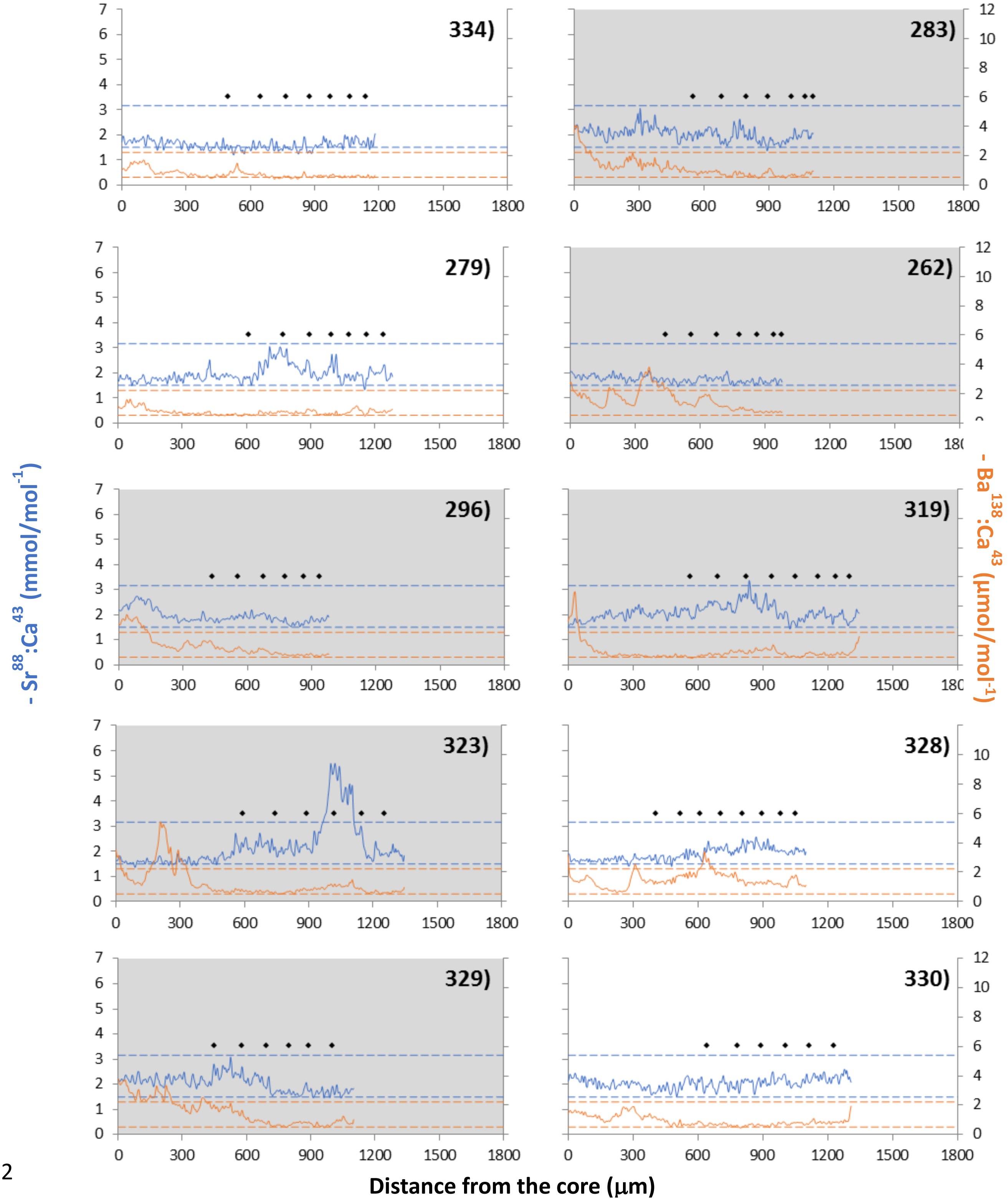


Figure 34. Otolith chemical profiles of *Trichiurus lepturus* from birth to capture for individuals caught from the Hawkesbury estuarine site in 2016. Black markers indicate otolith annuli age increments; ages were 6-8 years. Blue dashed lines indicate the upper and lower threshold bounds for Sr (1.50 - 3.16 mmol/mol $^{-1}$) and orange dashed lines indicate the upper and lower threshold bounds for Ba (0.51 - 2.22 mmol/mol $^{-1}$). Grey panels indicate an individual profile that exhibits the pattern of a Ba spike, occurring before the formation of the first annual increment.

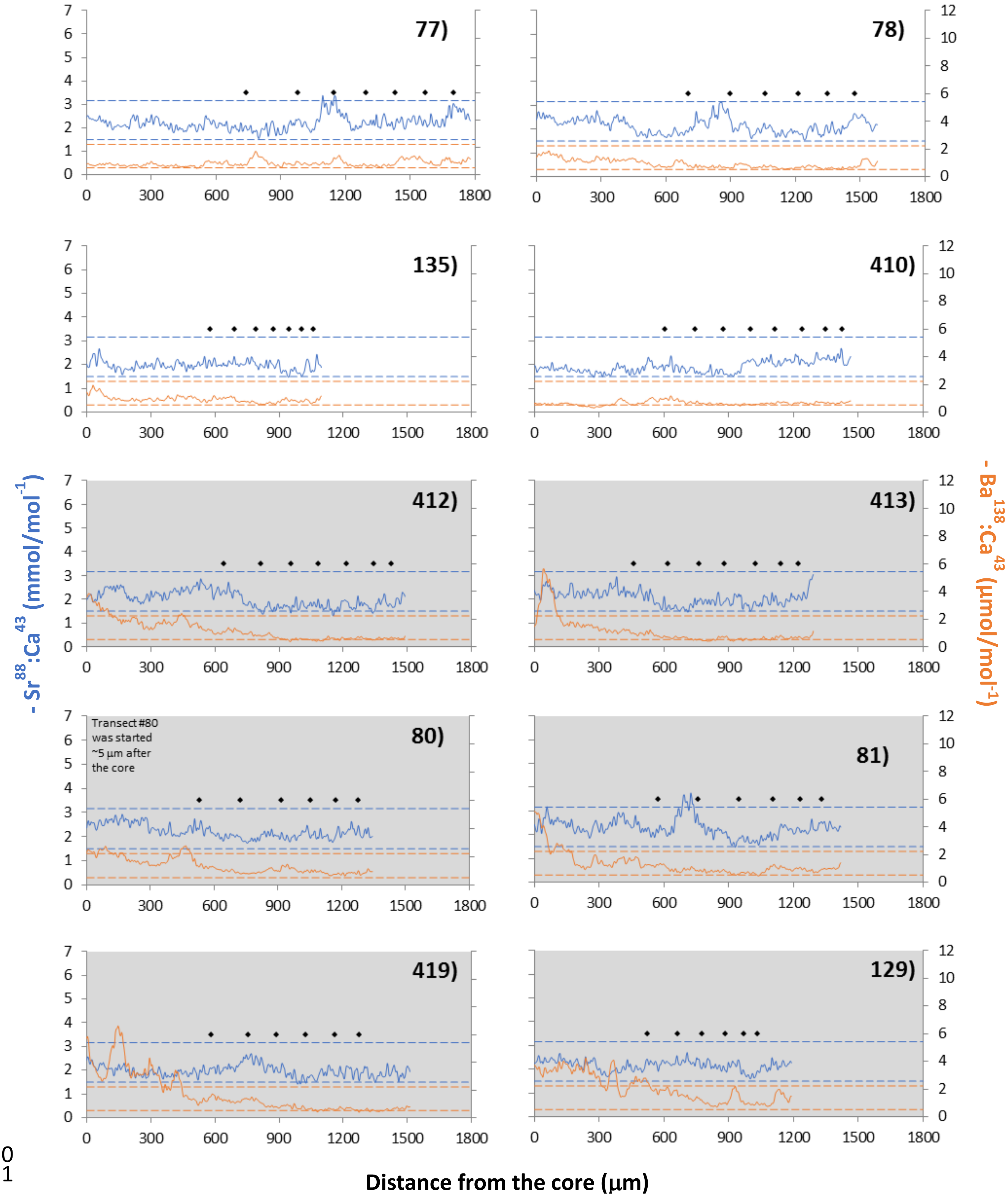


Figure 35. Otolith chemical profiles of *Trichiurus lepturus* from birth to capture for individuals caught from the Hunter coastal site in 2016. Black markers indicate otolith annuli age increments; ages were 6-8 years. Blue dashed lines indicate the upper and lower threshold bounds for Sr (1.50 - 3.16 mmol/mol -1) and orange dashed lines indicate the upper and lower threshold bounds for Ba (0.51 - 2.22 mmol/mol -1). Grey panels indicate an individual profile that exhibits the pattern of a Ba spike, occurring before the formation of the first annual increment.

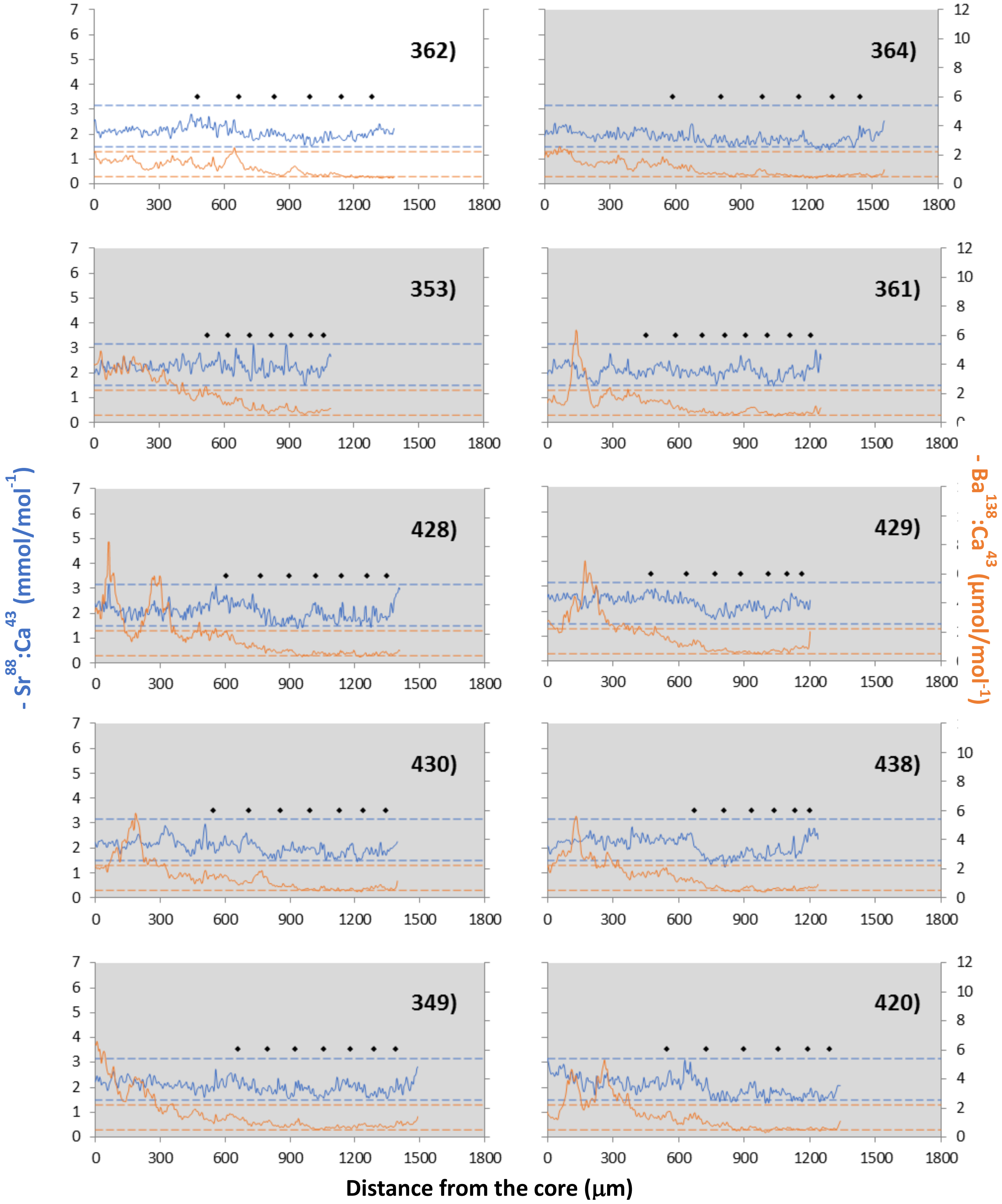


Figure 36. Otolith chemical profiles of *Trichiurus lepturus* from birth to capture for individuals caught from the Hunter estuarine site in 2016. Black markers indicate otolith annuli age increments; ages were 6-8 years. Blue dashed lines indicate the upper and lower threshold bounds for Sr (1.50 - 3.16 mmol/mol -1) and orange dashed lines indicate the upper and lower threshold bounds for Ba (0.51 - 2.22 mmol/mol -1). Grey panels indicate an individual profile that exhibits the pattern of a Ba spike, occurring before the formation of the first annual increment.

4.4 Discussion

In this pilot study, the broad aim was to evaluate the utility of otolith chemistry for investigating estuarine-coastal movements of *T. lepturus* on the south-eastern coast of Australia. However, the otolith chemistry methodology implemented in this pilot study showed no significant differences in any of the four elements that are typically used to distinguish estuarine from coastal habitats: Sr, Ba, Mg, and Mn. It was therefore not possible to determine whether estuarine-coastal movements of *T. lepturus* took place on the south-eastern coast of Australia. The core-to-edge transects of Ba and Sr profiles from natal origin to capture, therefore, were not conclusive of estuarine-coastal movements, however there was a pattern observed in a majority of the individual profiles, where Ba spiked above pooled average values, before the formation of the first annual increment.

T. lepturus are an irregularly caught species in south-eastern Australia with a generally low yield and episodically high catches. The patchy occurrence of the *T. lepturus* in NSW fisheries mean that sampling of the species for insights into their life history is challenging. It was anticipated that otolith chemistry analysis could provide insights on the estuarine-coastal movement patterns of *T. lepturus*. Due to the challenges of obtaining *T. lepturus* samples, the sample collection had a limited sample size and an uneven availability of samples across spatial and temporal factors of interest, which is why it was necessary to explore the effects of ontogeny and temporal factors on elemental concentrations. The investigation revealed that otolith chemistry displayed significant positive (Sr and Ba) and negative (Mn) relationships with ontogeny (body length as a proxy) but no relationship for Mg. Otolith Mg, and Mn concentrations differed significantly across months, but Sr and Ba did not. There was no inter-annual variability detected in otolith Sr, Ba, Mg or Mn concentrations. For core-to-edge transects, the technique revealed a pattern of otolith Ba profile spikes in the early development stage in most of the individuals analysed.

Body length was found to be a suitable proxy to account for ontogenetic effects in spatial and temporal comparisons, regardless of the specific mechanisms contributing to ontogenetic variation in otolith chemistry. Age would typically be the most suitable proxy for ontogeny, given the potential for body length to vary among individuals at similar ontogenetic stages. However, body length was found to be most suitable because of the demonstrated significant positive (Sr and Ba) and negative (Mn) relationships with otolith elemental concentrations. The relationship between elemental concentrations and age were expected to mirror the relationships with length, due to the strong linear relationship between body length and age for *T. lepturus* across the age/length range investigated. However, this was only true for the negative relationship between Mn and length and age. Mg was not significantly related to length or age and there was no significant relationship between age and Sr or Ba. The observed misalignment between length and age could be due to the age variable being restricted to categorical analysis whereas length is a continuous variable and therefore more suitable to detect the ontogenetic effects on otolith chemistry. Due to significant correlations, body length was incorporated as a covariate into any subsequent analysis that was used to compare otolith Sr, Ba and Mn concentrations through time, and among and within habitats. The nonsignificant relationship between Mg and length and age suggest it would not be necessary to integrate body length as a covariate for analysis on *T. lepturus* otoliths that only use otolith Mg.

In this pilot study, *T. lepturus* otolith chemistry varied significantly across months (Mg and Mn). The concentrations of Mg in March were more than double that in July and May. This was similar for Mn, with concentrations of Mn in March and May, more than double that of November. The demonstrated temporal instability of otolith Mn and Mg concentrations can affect their utility for reconstructing estuarine-coastal movement patterns, therefore, the detection of movements between habitats can be obscured, depending on when they occur relative to the changing seasons or years (Crook et al., 2016). Any future analysis using otolith Mg and Mn concentrations to make inferences on estuarine-coastal movements of *T. lepturus* will need to control for inter-monthly variability in the experimental design. In contrast, there was no inter-monthly or inter-annual variability in otolith Sr or Ba concentrations of *T. lepturus* collected in south-eastern Australia between 2015 and 2016 and no inter-annual variability in the uptake of any of Mg or Mn concentrations of *T. lepturus* otoliths. These results indicate that using otolith Sr and Ba to investigate individual movement patterns are unlikely to be confounded by seasonal changes. However, in this pilot study, inter-monthly stability was only tested on *T. lepturus* otoliths collected in the Hawkesbury estuarine site in 2016 and sampling on a broader spatial and temporal scale would be necessary for a more robust conclusion.

Results from this pilot study also indicated inter-annual stability in the otolith chemistry of *T. lepturus* (Sr, Ba, Mg and Mn). Otolith elemental concentrations did not vary between years, therefore, the signatures contained in previous growth zones may be useful for reconstructing estuarine-coastal movement histories. This finding could also assist the efficiency of future studies, because samples could be either pooled across years, or previous growth zones within the same individual could be useful for a temporal range across years in the study, rather than needing to collect samples across years. However, the conclusions from this pilot study are made tentatively, because the limitations on sampling resulted in the inter-annual stability only being tested on a limited number of *T. lepturus* otoliths and the annual comparison was limited to a comparison between 2015 and 2016. A broader study across more years would allow for more robust conclusions on the inter-annual variability.

Otolith Sr, Ba, Mg and Mn did not differ sufficiently between *T. lepturus* collected from the Hunter coastal and Hunter estuarine sites to allow distinction of these habitats and therefore has not allowed for the determination of specific estuarine-coastal movements of *T. lepturus* in south-eastern Australia. While there are broadly documented associations between otolith Sr and Ba and ambient water concentrations and the use of these relationships for reconstructing freshwater-coastal movement histories (Mazloumi et al., 2017, Stanley et al., 2015, Sturrock et al., 2012), the utility for otolith chemistry in the investigation of estuarine-coastal movement histories is not as well defined. The lack of distinction found in this study could be explained by: (1) the characteristics of the estuarine/coastal habitats varying too much over the time-frame encompassed in the study to allow a detectable difference in otolith chemistry with the sample size used; (2) the physical and chemical characteristics between the two habitats did not differ enough to reflect a difference in *T. lepturus* otolith chemistry; (3) *T. lepturus* moving between the habitats within the time-frame encompassed in the analysis (~100-250 days), therefore, the window of time analysed in the current study might have been too broad to capture the difference in the water chemistry or; (4) the incorporation of environmentally available elements being regulated by the physiology of *T. lepturus.* Physiological influences on otolith incorporation are difficult to isolate and test independently and therefore there is limited understanding on this mechanism (Sturrock et al., 2014). Sturrock et al. (2014) and Sturrock et al. (2015) have shown that physiological influences do have the potential to confound the relationship demonstrated between otolith concentrations and ambient water concentrations. It is unlikely, however to be the driving mechanism, because a majority of studies carried out on a number of species have shown that the effects of physiological influences are typically not large enough to confound environmental signals. In addition, the otolith incorporation of Sr and Ba is known to be unaffected by dietary uptake, across multiple species (Elsdon and Gillanders, 2005, Milton and Chenery, 2001, Walther and Thorrold, 2006). In addition, there was a significant difference in the otolith Sr, Ba and Mn between the two estuarine sites, which suggests that the estuarine-coastal effects on elemental incorporation found in this study are specific to region and not just habitat type.

These observations confirm that the utility of otolith chemistry needs further evaluation before any specific estuarine-coastal movements patterns for *T. lepturus* can be discerned. This pilot study was confined to one coastal site and two estuarine sites due to sampling constraints. A broader investigation which includes more estuarine and coastal sites, with a better geographic resolution on the capture locations and how far the locations are positioned between the estuary mouth and the estuarine upper reaches may still allow for a distinction of *T. lepturus* between coastal and estuarine habitats and subsequently allow for reconstruction of estuarine-coastal movement patterns.

The results of this pilot study have provided additional insights into the stock structure of *T. lepturus* in south-eastern Australia. There were significant differences in otolith Sr, Ba and Mn concentrations identified between regions on the NSW coast, separated by ~83km. The difference in elemental concentrations from individuals caught during the study period from the Hawkesbury estuarine site differed from both sites located ~83km north, the Hunter coastal site and the Hunter estuarine site. To address the aims of the study, otolith concentrations were compared between two spatially segregated estuarine sites to identify whether the reconstruction of estuarine-coastal movements, using core-to-edge transects, would need to be system specific. The distinction in otolith chemistry between these two regions suggests that, over the amount of the time encompassed in the analysis (~100-250 days), the *T. lepturus* sampled were likely not moving between the two regions, which indicates a spatial discrimination of *T. lepturus* stocks between the two regions during this time. Comparisons of elemental concentrations may prove useful for distinguishing individuals from the two estuaries based on their otolith chemistry (Hamer et al., 2003, Ruttenberg et al., 2008, Thorrold et al., 1997). The insight also highlights the potential to document a library of otolith chemistry characteristics for a broader range of *T. lepturus* otoliths across more estuaries along the east coast of Australia and a broader temporal scale to gain further insights into the species stock structure in this region (Thorrold et al., 1997).

The core-to-edge transects of *T. lepturus* were utilised to investigate changes in otolith Sr and Ba profiles between the natal origin and capture of individuals. The methodology from the current study did not allow for the determination of specific estuarine-coastal movements of *T. lepturus* in south-eastern Australia. As an alternative, patterns in the otolith Sr and Ba profiles between birth and capture of individuals were utilised to provide evidence of the displacement of individuals from within the estuarine/coastal environmental conditions documented by this study, potentially representing substantial movements made by individuals outside the boundaries of the estuarine and coastal region of this study. The displacement of individuals from within the boundaries of the study were defined by individual profiles of Sr and Ba making deviations above and below the constructed threshold windows, which were generated from the three pooled collection sites. Otolith Ba deviations both above and below the defined thresholds were observed in the chemical profiles of 25 out of 30 *T. lepturus* individuals. For 21 out of the 25 deviations, there was an observed spike above the upper threshold for Ba which always occurred before the formation of the first annual increment. The observed pattern of Ba spikes may have been caused by a number of natural processes, including: (1) individuals moving into different habitats from the defined collection sites with different water physical and chemical properties during their early development; (2) individuals remaining in their habitat and the spikes being caused by an environmental change, resulting in an increase bioavailable Ba; (3) temporal variation in natural systems outside of the scope defined by this study (2015 and 2016) and; (4) physiological changes in the early development of individuals that result in the elevated incorporation of dissolved Ba to the otolith.

The observed pattern of Ba spikes could be caused by individuals moving up river into freshwater habitats, but this is unlikely because none of the individuals with observed Ba spikes also exhibited corresponding decrease in Sr. Otolith Ba in high concentrations and Sr in low concentrations is typically associated with freshwater residence, due to the substantial difference in ambient water concentrations between freshwater and marine environments

(Mazloumi et al., 2017, Stanley et al., 2015, Sturrock et al., 2012). The absence of this inverse relationship in the chemical profiles of *T. lepturus,* does not support a hypothesis for individuals making freshwater migrations. An alternative explanation for the spiking Ba patterns that may account for the absence of Sr deviations, is the movement of *T. lepturus* individuals from lower to upper estuarine habitats. Individuals that occupied the upper reaches of the estuary, closer to the freshwater component of the river systems, relative to the study collection area, would be more likely to have exposure to fluvial run-off, which has been directly associated with dissolved Ba (bioavailable state), released from riverine sediment (Elsdon and Gillanders, 2005).

Another possible scenario for explaining the pattern of Ba spikes in *T. lepturus* profiles, is the movement of *T. lepturus* into deep marine habitats (Woodson et al., 2013). There are suggestions that dissolved Ba in marine environments increases with depth/distance from the shore (Henkel et al., 2012, Longmore et al., 2010), however there is still limited understanding of the dissolved and solid state cycle of Ba and its vertical distribution in marine habitats, and how that would relate to otolith incorporation (Woodson et al., 2013).

The observed pattern of Ba spikes in *T. lepturus* chemical profiles could have been influenced by natural weather events. There are weather events with the potential to increase dissolved Ba and subsequently Ba incorporation to otoliths, in both estuarine and coastal habitats. In estuaries, high rainfall and freshwater upwelling cause terrestrial run-off and higher fluvial flow rates, which are both directly associated with the release of dissolved aquatic Ba from sediment (Elsdon and Gillanders, 2005, Ferguson et al., 2011). In coastal habitats, dissolved Ba is not generally available near the surface but wind-driven upwelling brings cold, nutrient rich water to the surface and with it, dissolved Ba (Henkel et al., 2012, Woodson et al., 2013). The scenario of a natural weather event to explain the Ba spikes was thought to be unlikely, however, because the pattern was observed across 21 individuals, all before the formation of the first annual increment. All of the individuals selected for transect analysis were all caught in 2016 and were selected from three different age classes, therefore the 21 deviations occurred in three separate calendar years. If the pattern was caused by whether events, it means the events had to occur across three calendar years and only expose individuals in their early development stage to increased Ba.

The pattern of Ba spikes consistently occurs before the formation of the first annual increment and therefore, the phenomenon is likely to be associated with the early development stage of the individuals. The phenomenon is more likely to be associated with the physical and chemical characteristics of the habitat of residence during early development, rather than the physiological factors occurring during early development. Although a physiological effect cannot be ruled out, it is unlikely for two reasons: (1) As part of this pilot study, a positive relationship between otolith Ba concentrations and ontogeny (body length as a proxy) was demonstrated (linear regression). While the juvenile stage was not tested directly, the relationship between body length and Ba concentrations was a continuous, linear relationship based on a broad length range for the species and; (2) while there are many individuals that do exhibit a Ba deviation before the formation of the first annual increment, the pattern is not consistent across all individuals.

The proportion of individuals that exhibited a Ba spike before the formation of their first annual increment, was not significantly different between estuarine and coastal habitats (Fisher's test, $p = 0.30$). The comparison between habitats was implemented to explore whether individuals with observed otolith Ba spikes were more likely to be caught in estuarine habitats or coastal habitats. If individuals exhibiting Ba spikes were more likely to be caught in an estuarine habitat, it would provide evidence to support that Ba spikes are caused by upper estuarine movements. If individuals exhibiting Ba spikes were more likely to be caught in a coastal environment, it would provide evidence to support that Ba spikes are caused by exposure to coastal upwelling. The evaluation however was not conclusive and therefore, does not provide evidence to support either explanation. The non-conclusive result also does not provide evidence that the estuarine-coastal migration strategies of *T. lepturus* in south-eastern Australia are similar to *A. japonicas* in the Pacific Ocean, where individuals exhibit unique migration strategies depending on whether they are estuarine or coastal residents (Tsukamoto et al., 2002).

4.5 Conclusion

It has been established that the otolith chemistry methodology implemented in this pilot study, did not allow for the determination of estuarine-coastal movements of *T. lepturus* on the east coast of Australia. In the process of addressing the overall aim, body length was identified as the most suitable proxy for ontogeny by demonstrating significant positive (Sr and Ba) and negative (Mn) relationships with otolith elemental concentrations. It was possible to establish these relationships with body length because it was a continuous variable as opposed to age which was categorical. *T. lepturus* otolith chemistry showed signs of temporal stability, with no inter-annual variability detected in otolith Sr, Ba, Mg or Mn. Otolith Mg and Mn differed significantly among months, but Sr and Ba did not. Otolith chemistry was also useful for distinguishing elemental concentrations in the otoliths of individuals caught between regions separated at a scale of 10s of km, highlighting a potential to investigate the stock structure of *T. lepturus* in south-eastern Australia. The core-to-edge transect analysis revealed that a large proportion of individuals exhibited a substantial elevation in otolith Ba before the formation of the first annual increment. However, it is challenging to disentangle the potential processes driving this pattern. While there where some promising results for *T. lepturus* otolith chemistry regarding temporal stability and potential for stock discrimination, the results do not suggest it would be worthwhile to contribute resources to a comprehensive investigation on *T. lepturus* otolith chemistry in south-eastern Australia. Other methods such as tagging of live fish may provide more conclusive results regarding movements.

Chapter 5: General Discussion

The south-eastern Australian population of *T. lepturus* is, like all marine species, is exposed to the detrimental impacts of a dynamic range of anthropogenic pressure, including climate change, habitat degradation and fishing pressure (Ding et al., 2017, Molinos et al., 2016, Poloczanska et al., 2013, Vasconcelos et al., 2007). There is a general global increase in natural and anthropogenic pressures impacting on the productivity of marine populations (Enberg et al., 2009, Heino et al., 2015). Characterising the anthropogenic pressure on marine populations and understanding how a population will respond to those pressures is becoming increasingly important to ensure that adequate safeguards are employed to minimises the risk of local population decline (Castilla and Defeo, 2005, Hilborn and Walters, 1992, Randall et al., 2013, Wakefield et al., 2013). *T. lepturus* is a major contributor to the overall catch of global fisheries and is consistently placed in the top ten marine species landed worldwide by the United Nations (UN) Food and Agriculture Organization (FAO) statistics, based on catch weight (FAO 2018). The FAO has also reported that *T. lepturus* is overfished in the north-western Pacific Ocean (FAO 2018). In contrast, the fishery for *T. lepturus* in south-eastern Australia generally has a low yield and the yield is sporadic in time and space (Stewart et al., 2015). The fishery for *T. lepturus* in Australia is relatively small-scale and has limited data available for population assessment and management (Stewart et al., 2015). As the demand for commercially- and recreationally fished species in south-eastern Australia increases, the exploitation of *T. lepturus* and thus the detrimental pressure on its population will presumably increase. The population declines of *T. lepturus* reported in the north-west Pacific indicate the potential vulnerability of *T. lepturus* to fishing pressure, highlighting the need to understand the vulnerability of populations in south-eastern Australia.

Understanding the magnitude and type of anthropogenic and natural pressures on a population, as well as how a population will respond to these pressures is critical to the sustainable management of the local population. This understanding should be inclusive of the population productivity, its vulnerability to increasing fishing pressure and its resilience to perturbation. For a complete and comprehensive assessment of the population, it would be ideal to have a thorough understanding of the vital rates, including birth, growth and survival with would require a thorough understanding of population life history traits, the migration strategies, and habitat use of populations (Randall et al., 2013, Wakefield et al., 2013). It would then be pertinent to characterise the range of external processes influencing these traits (Hilborn and Walters, 1992). This thesis has directly contributed to addressing the limited understanding of *T. lepturus* population in Australia regarding their life history traits and the way individuals use habitat. Addressing these knowledge gaps has contributed to the sustainable management of the local population and has provided knowledge which allows for a broader understanding of the local ecosystem.

In Chapter One, the general introduction to the thesis, the concepts of increasing external pressure on marine populations were introduced, as well as the concept of population productivity and how populations with higher relative productivity are less vulnerable to increasing external pressure (Randall et al., 2013). The first chapter introduces the research presented in this thesis and its value.

In Chapter Two, the investigation in to the spatial and temporal dynamics of the fishery yield (landings by weight) and length composition of *T. lepturus* in south-east Australia revealed there was variability in yield, and that length distributions varied depending on fishing gear, habitat and geographic segregation. The findings suggested that monitoring the population according to fishery yield and length frequency data was unlikely to provide an accurate indication of population abundance or accurate quantifications of life history parameters (Vincent et al., 2007). These finding therefore indicated that further investigations were necessary in order to quantify life history traits of *T. lepturus* in south-east Australia.

In Chapter Three, biological samples were used to quantify numerous life history traits of *T. lepturus* in south-east Australia. The investigation of life history traits was based on biological population data collected from fishery-dependent data in NSW. The relative productivity of the south-east Australian population was then established by comparing the biological life history parameters of the local population with published parameters of populations from the global literature. The demographics of the *T. lepturus* population in south-eastern Australia, including a larger maximum length, a longer lifespan, a larger length at maturity were more similar to an unfished stock, while a shorter spawning period and slower individual growth rate indicated that the population is likely to have a lower productivity than populations in other global regions. Thus, the population could be vulnerable to natural and anthropogenic pressures (Coulson et al., 2012, Randall et al., 2013, Wakefield et al., 2013).

Thus far, the fishing yield in south-eastern Australia has been low relative to other regions. The yield for *T. lepturus* in south-eastern Australia is also low relative to the yield of other species in the region (Stewart et al., 2015). The lower fishing pressure relative to other global regions has meant there has been less influence of fishing on life history traits and population productivity in south-eastern Australia (Enberg et al., 2009, Heino et al., 2015). Considering the external pressure on this population is anticipated to increase, due to the trends of global increase in fishing pressure, the population should be monitored closely to avoid local population decline.

In order to implement a comprehensive monitoring strategy for an exploited species, it is essential to understand the ecological stock structure. Investigating the migration strategies of the individuals within a population contributes to an understanding of ecological stock structure. It is also essential to gain an understanding of the way individuals use habitats to assess how critical specific habitats are for the particular life history strategies of a population (Crossin et al., 2017). In Chapter Four, the utility of otolith chemistry for investigating the movements of *T. lepturus* between estuarine and coastal habitats was explored. The method, which has been applied to other species (Avigliano et al., 2017, Fowler et al., 2015), was in this case unable to distinguish individuals between estuarine and coastal habitats in a way that allowed for determination of movements between the estuarine and coastal habitats investigated. The otolith elemental core-to-edge transect analysis was useful and revealed that a large proportion of individuals exhibited a substantial elevation in otolith Ba:Ca before the formation of the first annual increment. However, it is challenging to disentangle the potential processes driving this pattern. Therefore, further investigation on the migration strategies and habitat use of *T. lepturus* is recommended to provide insights into movement and stock structure that can inform monitoring, assessment and management *T. lepturus* population in south-eastern Australia.

Otolith chemistry also revealed additional insights for investigating the stock structure of *T. lepturus* in south-eastern Australia. There were significant differences in otolith Sr:Ca, Ba:Ca and Mn:Ca concentrations identified between two regions on the NSW coast, separated by ~83km. The distinction in otolith chemistry between these two regions suggests that, over the amount of the time encompassed in the analysis (~100-250 days), the *T. lepturus* sampled were likely not moving between the two regions, which suggests a potential spatial discrimination of *T. lepturus* stocks between the two regions during this time. Comparisons of elemental concentrations may prove useful for identifying stock structure at small spatial scales within the geographic range of the NSW commercial fishery and therefore could indicate the necessary spatial scale of management for *T. lepturus* in south-eastern Australia (Hamer et al., 2003, Ruttenberg et al., 2008, Thorrold et al., 1997). The insight also highlights the potential to document a library of otolith chemistry characteristics for a broader range of *T. lepturus* otoliths across more estuaries along the east coast of Australia and the need for a broader temporal scale in sampling to gain further insights into the species stock structure in this region (Thorrold et al., 1997).

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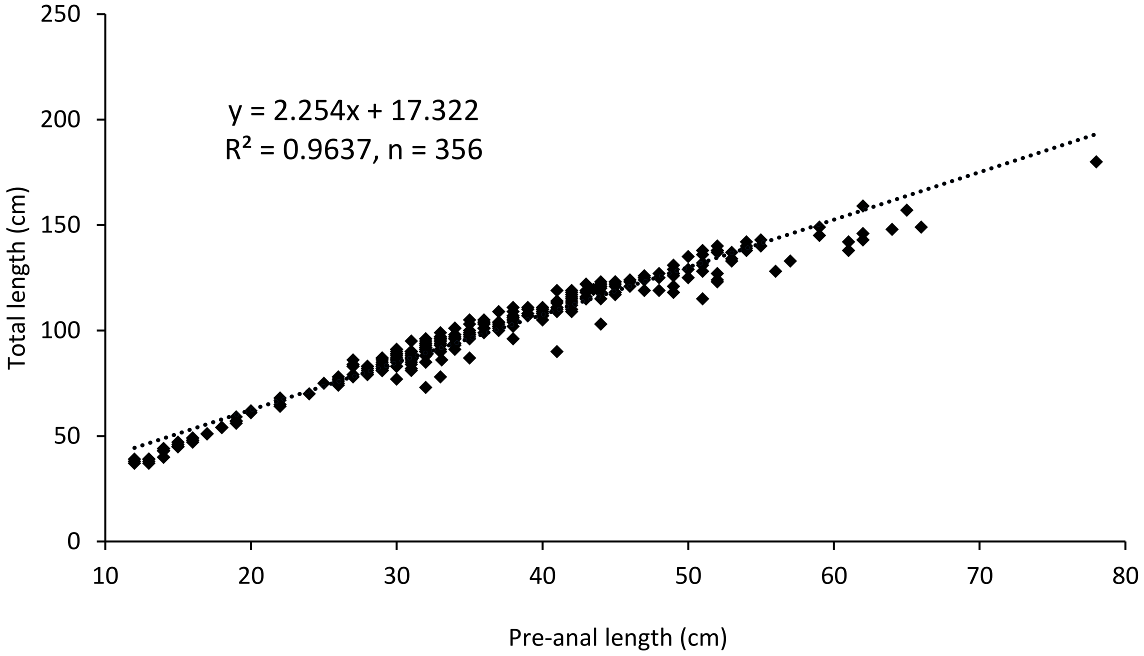
Appendices



Appendix A - The relationship between pre-anal length and total-length.