AGE AND GROWTH ESTIMATES FOR THE PORT JACKSON SHARK, *HETERODONTUS PORTUSJACKSONI*, (MAYER, 1793) FROM NEW SOUTH WALES, AUSTRALIA.

By

ROBERT ØSTLIE RAMOS

Cand. mag.

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School of Environmental and Life Sciences

The University of Newcastle

Australia

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THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Declaration of Authorship

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

ROBERT ØSTLIE RAMOS

31 May 2007

Cover image: The Port Jackson shark, *Heterodontus portusjacksoni*, (photo by the author).

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Table of Contents

Declaration of Authorship 2

Acknowledgments 3

Table of Contents 5

Abstract 13

Chapter 1: General Introduction 18

1.1 Problem Statement 18

1.2 Introduction to the Literature Review 21

1.3 Status and Threats 22

1.4 Ageing Techniques 26

1.5 Validation 29

1.6 Growth Models 34

1.7 Application of Techniques 37

1.8 Port Jackson Shark 38

1.9 Conclusion 40

Chapter 2: Anatomy of the Vertebrae and Dorsal Spine of the Port Jackson Shark (*Heterodontus portusjacksoni*). 43

2.1 Introduction 43

2.2 Materials and Methods 46

2.2.1 Sampling 46

2.2.2 Preparation of Vertebral Centra 47

2.2.3 Preparation of Dorsal Spines 49

2.3 Results 49

2.3.1 Vertebral Centra 50

2.3.1.1 Number of Vertebrae 50

2.3.1.2 Gross Anatomy 51

2.3.1.3 Relationship to Size 60

2.3.2 Dorsal Spines 62

2.3.2.1 Length and Width 62

2.3.2.2 Gross Anatomy 63

2.3.2.3 Relationship to Size 68

2.4 Discussion 73

2.4.1 Vertebral Centra 73

2.4.2 Dorsal Spines 75

2.5 Conclusion 76

Chapter 3: The Use of Vertebrae for Age Estimating the Port Jackson Shark (*Heterodontus portusjacksoni*). 78

3.1 Introduction 78

3.2 Materials and Methods 81

3.2.1 Sampling 81

3.2.2 Preparation of Vertebrae 83

3.2.3 Validation 85

3.2.3.1 Sampling 85

3.2.3.2 Maintenance 85

3.2.3.3 Chemical Markers 87

3.2.3.4 Marginal Increment Analysis and Centrum Edge Analysis 89

3.2.4 Ageing 91

3.2.4.1 Techniques and Methods 92

3.2.4.2 Whole Vertebrae 93

3.2.4.3 Sectioned Vertebrae 93

3.2.5 Analyses 94

3.2.5.1 Influence of Vertebra Number on Age Estimation 94

3.2.5.2 Comparison of Whole and Sectioned Vertebral Centra 94

3.3 Results 96

3.3.1 Relationship Between Length and Vertebral Diameter 96

3.3.2 Validation 96

3.3.3 Whole Vertebrae 100

3.3.3.1 Female 101

3.3.3.2 Male 103

3.3.4 Sectioned Vertebrae 104

3.3.4.1 Female 106

3.3.4.2 Male 107

3.3.5 Comparison Between Methods 109

3.3.5.1 Female 109

3.3.5.2 Male 111

3.4 Discussion 112

3.4.1 Position on the Vertebral Column 112

3.4.2 Validation 113

3.4.3 Age Estimation 114

3.4.4 Comparison of Methods 116

3.5 Conclusion 117

Chapter 4: The Use of Dorsal Spines for Age Estimating the Port Jackson Shark (*Heterodontus portusjacksoni*). 120

4.1 Introduction 120

4.2 Materials and Methods 123

4.2.1 Sampling 123

4.2.2 Dorsal Spine Preparation 123

4.2.3 Validation 124

4.2.4 Ageing 125

4.2.5 Analyses 126

4.2.5.1 Comparison of Methods and Structures 126

4.3 Results 127

4.3.1 Validation 127

4.3.2 Whole Dorsal Spines 131

4.3.3 Sectioned Dorsal Spines 134

4.3.4 Comparison Between Methods 136

4.3.5 Comparison Between Vertebral Centra and Dorsal Spines 138

4.4 Discussion 139

4.4.1 Validation 139

4.4.2 Age Estimation 141

4.4.3 Comparison of Methods 143

4.4.4 Comparison of Structures 144

4.5 Conclusion 145

Chapter 5: A Comparison of Age-Growth Models for the Port Jackson Shark (*Heterodontus portusjacksoni*). 147

5.1 Introduction 147

5.2 Materials and Methods 150

5.2.1 Sampling and Measurements 150

5.2.2 Growth Models 150

5.2.3 Statistical Analyses 152

5.2.4 Annual Growth 153

5.2.5 Longevity 153

5.2.6 Length-Frequency Distribution 154

5.3 Results 154

5.3.1 Vertebral Centra 156

5.3.1.1 Growth Curves 156

5.3.1.2 Annual Growth 160

5.3.1.3 Longevity 160

5.3.2 Dorsal Spines 160

5.3.2.1 Growth Curves 160

5.3.2.2 Annual Growth 165

5.3.2.3 Longevity 165

5.3.3 Length-Frequency Distributions 165

5.4 Discussion 166

5.4.1 Vertebral Centra 166

5.4.2 Dorsal Spines 168

5.4.3 Growth Models 169

5.5 Conclusion 172

Chapter 6: Validation of the Reading Techniques for Ageing the Port Jackson Shark (*Heterodontus portusjacksoni*). 174

6.1 Introduction 174

6.2 Methods 176

6.2.1 Sampling 176

6.2.2 Readers and Training 177

6.2.3 Experimental Design 177

6.2.4 Statistical Analysis 178

6.2.4.1 Bias 178

6.2.4.2 Precision – Within and Between Readers 179

6.3 Results 180

6.3.1 Within Readers 180

6.3.2 Between Readers 183

6.4 Discussion 186

6.4.1 Bias 186

6.4.2 Precision 188

6.4.3 Level of Experience 189

6.4.4 Experience in Ageing Other Structures 190

6.5 Conclusion 192

Chapter 7: General Discussion and Conclusion 194

7.1 Introduction 194

7.2 Anatomy of the Ageing Structures 194

7.3 Vertebral Centra and Dorsal Spines for Age Estimation 195

7.4 Growth Models and Parameters 196

7.5 Future Research 197

Appendix A 199

References 206

Abstract

World shark populations are declining rapidly from the increased demand for shark meat and fins and from their capture as by-catch. Techniques to accurately and reliably estimate the age of elasmobranchs are required to understand life history and develop management strategies. Although some biological information exists for the harvested species, few of the many shark species caught as by-catch have been investigated. In the waters of New South Wales, Australia, the Port Jackson shark (*Heterodontus portusjacksoni*) is a non-targeted species, however it is a major contributor to the elasmobranch by-catch in the bottom trawl and prawn fisheries. Although some biological and ecological information exists on Port Jackson sharks from New South Wales, there is no understanding of suitable techniques to age this species and of the age structure of the New South Wales' population. Hence, the main purpose for the present study was to determine a suitable technique to age Port Jackson sharks and thereby provide essential age and growth data for its future management, sustainable use and conservation.

This study consisted of research into: (1) the macroscopic anatomy of the vertebral centra and dorsal spines from 652 *H. portusjacksoni* to explore their suitability as ageing structures; (2) a comparison of whole and sectioned vertebral centra and of whole and sectioned dorsal spines for ageing; (3) validation of the annual formation of growth bands; (4) a comparison of the effect of prior experience on readers' ability to count annual growth bands in vertebral centra; and (5) the development of age-growth models for the New South Wales' population *of H. portusjacksoni*.

Two types of vertebral centra (trunk and caudal vertebrae) were found in the vertebral column. Both vertebrae consisted of a centrum, 2 ribs, 4 neural arches, basiopophyses and neural foramen and decreased in size from anterior to posterior. A strong linear relationship was identified between vertebral diameter (V_D) and fork length (F_L). Each dorsal spine was positioned anterior to the dorsal fin and consisted of a stem and the cap. A strong linear relationships were identified between spine base width (S_{BW}) and $F_{L.}$ The existence of alternating opaque and translucent band pairs in both structures further suggested they may be suitable for ageing purposes.

Validation (calcein and oxytetracycline injections, marginal increment analysis, and centrum edge analysis) confirmed that translucent band pairs were formed during the winter (September-November) and were annual in both vertebral centra and dorsal spines. Whole and sectioned vertebral centra and dorsal spines were individually examined to determine which method best estimated the age. Although both methods had individual low reading bias and high precision, there was a difference between the two methods. The average bias between the two methods using vertebral centra was -0.158 ±0.05 and the coefficient of variance (CV) was 92.32% for females and -0.157 ±0.03 and CV was 56.95% for males. This indicated that sectioned vertebral centra returned higher ages than whole vertebral centra. When comparing whole dorsal spine age estimates to sectioned dorsal spines, there was an average bias between methods of -0.22 ±0.026 and a low precision 73.09%. Again indicating that sectioned dorsal spines returned higher ages than whole dorsal spines. A comparison of ages estimated from sectioned vertebral centra and sectioned dorsal spines showed that vertebral centra aged older than dorsal spines. Although the two structures each had low reading bias and high reading precision, there were differences between the

two structures. The average bias between the two structures was -0.4 ±0.03 and the CV was low (74.7%), indicating that there was a significant difference in age derived from the two structures. Therefore, great caution should be taken when choosing which structure to use for age estimation. Ages of sharks estimated from the recommended sectioned vertebral centra ranged from 0.2 to 32.5 years for females and 0.2 to 23.8 years for males.

Four different growth models were compared using both sectioned vertebral centra and sectioned dorsal spines to estimate the best suitable growth curve. Out of the 4 growth models the Gompertz Growth Function (GGF) best described the growth of *H. portusjacksoni*. von Bertalanffy growth parameters estimated from ageing data using the GGF for females were $t_0 = 2.584$ yr, $k = 0.111$, $L_\infty = 1134.1$ mm F_L, and for males $t_0 = 1.629$ yr, $k = 0.131$, $L_\infty = 1012.9$ mm F_L. This indicates that *H. portusjacksoni* has a slow growth rate and a longer life span than most other elasmobranch species.

The effect of reader's experience on the bias and precision of age estimates of sectioned vertebral centra was determined in an experiment that compared the author (an experienced vertebral centra reader) with an experienced fish otolith reader, two readers recently trained in reading vertebral centra (but lacking experience), and two untrained and inexperienced readers. Bias and precision were determined for each reader from two ageing trials with the same structure three months apart. Between-trials bias for each reader (except the author) ranged from 0.033 to 0.13 and precision ranged from 8.2 to 19.5%, while the author had low bias 0.006 and high precision 1.1%. When comparing all the readers with the author, bias ranged from 0.033 to 0.13 with an 8.2 to 19.5% precision for trial one, and 0.017 to 0.5 with precision between 2.5 to 5.4% in trial two. The age-bias plots comparing the two trials all indicated that only the author was consistent. And the age-bias plots between the author

and the other readers all indicated that neither experience in ageing teleost otoliths or limited training in ageing enhanced the bias or precision compared to inexperienced elasmobranch agers.

Chapter 1

A female Port Jackson shark resting on the bottom besides egg capsules (photo by the author)

Chapter 1

General Introduction

1.1 Problem Statement

Sharks and rays are particularly vulnerably to overfishing because of their *k*-selected life history. Migratory patterns, oceanic and deep water habitat preferences of some species, place them outside the responsibility of both national and international authorities (Bonfil, 1994a). As fishing pressure on the world's shark population increases, the need to harvest these resources sustainably is becoming more and more important. Information on life history, longevity, growth and sexual maturity are crucial to maintaining a sustainable fishery. Sharks have no scales, and therefore cannot be aged by the use of any parts on the outer body. The only hard parts that are available for age estimation are the vertebrae and for some species the dorsal spines. The hardening of the cartilage imprints small circular growth bands, which can be used for this purpose. Although elasmobranchs have limited hard structures to be used for ageing, many different techniques have been used on different species. Some are species-specific and others are more common and can be applied to different species. Therefore, for each new shark that is aged it is important to validate different techniques and adapt these to the species in order to find which is most accurate. Further comparison to different subpopulations may give information on the structure of the species. Ultimately, validation of any technique must be undertaken so that crucial data on population age structure is not missed.

The aims of the present study are to investigate a range of techniques to age the Port Jackson shark, *Heterodontus portusjacksoni*, from the waters of the central coast of New

South Wales (NSW), Australia, and to use this information to develop age-growth models. Although *H. portusjacksoni* is not a commercially targeted species in NSW, it is being heavily caught as by-catch by demersal fish trawlers and in the prawn fisheries. In the Bass Strait of South Australia the species is the third most importantly caught in the shark gillnet fishery and the fourth in the longline fishery (Walker *et al.*, 2005) and accounts for over 3% of the total elasmobranch catch (220 tonnes year^{-1}) (Tovar-Avila, 2006).

Although, some information on the biology of *H. portusjacksoni* exists for NSW (McLaughlin, 1969; McLaughlin and O'Gower, 1971; Powter, 2007) there is no understanding of the age structure and growth of this species in the coastal waters of central NSW. Tagging studies (O'Gower and Nash, 1949) and population studies (Tovar-Avila, 2006) suggest the existence of a distinct population of *H. portusjacksoni* in these waters. Hence it is critical that knowledge of key biological parameters from the NSW population is estimated. Although this species is not commercially targeted, nor under any threat at the present time, some catches are retained for commercial use and the species is extremely sensitive due to its narrow geographical distribution to any influences that may change its normal lifecycle. Powter (2007) concluded from a modelling study using that the NSW population of *H. portusjacksoni* was vulnerable to minor increases in mortality of adults. A gap in Powter's (2006) estimates of mortality was that they were not based on age-at-length data or age-at-maturity data, which is a fundamental requirement of any fisheries-related assessment of a capture species. Life-history traits such as maturity, growth and fecundity might be the most important traits and must be clearly understood when considering the extreme pressure that commercial fishing will inflict on any population. Ideally the management of a population should determine life history traits before harvesting the resource (Musick, 1999)

Age and growth studies provide basic biological information needed to sustainably harvest and manage the use of a species. Although researchers have been estimating the age and growth of elasmobranch species since the early 1900s, no two species or populations are similar and each therefore requires individual estimates of population parameters. *H. portusjacksoni* possess two hard structures that are potentially useful for ageing: vertebrae and dorsal spines. The specific objectives of this research were:

1. To describe the macroscopic anatomy of the vertebral centra and dorsal pines of *H. portusjacksoni* and, using this, determine if each of these structures are potentially suitable for further investigation as ageing structures.
2. To validate the formation of visible band pairs in vertebral centra and compare the suitability of whole and sectioned vertebral centra as ageing structures.
3. To validate the formation of visible band pairs in dorsal spines and compare the suitability of whole and sectioned dorsal spines as ageing structures, and to compare the suitability of vertebral centra and dorsal spines as ageing structures.
4. To determine the most suitable model for vertebral centra and dorsal spines that describes the age-related growth of *H. portusjacksoni* from the central coast of NSW
5. To quantify the effects of readers' experience on estimates of the age of *H. portusjacksoni* and to use this information to recommend the level of prior experience and training needed to successfully age this species.

Chapter 2 will describe the macroscopic anatomy of the vertebral centra and dorsal spines of the Port Jackson sharks (*H. portusjacksoni*) in NSW. Chapters 3 and 4 will establish validated ageing techniques using both whole and sectioned vertebral centra and dorsal spines. A comparison between the different ageing methods and structures will determine the most suitable ageing procedure for this species. Based on the previous results, Chapter 5 will use the most suitable method and structure to estimate age and growth rates by comparing four different growth models and therefore establishing the growth parameters. Chapter 6 will test the effects of readers' prior experience and establish the importance of experience in ageing vertebral centra. Finally, Chapter 7 discusses the general conclusions of this thesis and Appendix A lists the species that have been successfully age estimated to date.

Each chapter of this thesis presents the original data (Chapters 2-6) and has been written in a style suitable for publication in a scientific journal. Tables and figures appear within the text and all references cited in this thesis are compiled and presented at the end of the thesis and not at the end of each chapter.

1.2 Introduction to the Literature Review

The increasing worldwide demand for shark fins and the public insight into shark finning and discarding of live sharks has generated international concern regarding the sustainability of the world's shark populations. Most shark species have a life history which is characterized by patterns of slow growth, late attainment of sexual maturity, low fecundity and long life spans, and a close relationship between the number of young produced and the size of the breeding biomass, making populations extremely vulnerable to

overexploitation (Branstetter, 1987a). To avoid overexploitation, the use of demographic analysis incorporating life history information will give insight into the population dynamics of shark species and assess their vulnerability to varying exploitation rates. Age and growth are two such important life history characteristics. In addition, the techniques and models used to establish them are important factors to understand before the life history can be determined. Nevertheless, the need to validate and compare these techniques and methods are not to be underestimated and are a crucial part of any age and growth study.

The following review of the literature will first summarise the current status and threats to the world shark populations. Then it will introduce the ageing and validation techniques for estimating the age and growth of sharks. It will also introduce the different models used to estimate growth and then the role of age and growth data in managing shark fisheries. Lastly, it will introduce the general biological characteristics of *H. portusjacksoni*.

1.3 Status and Threats

Elasmobranchs (sharks and rays) have been exploited for thousands of years. Since the 1870s *Squalus acanthias* have been exploited for their oil rich liver (McFarlane and Beamish, 1987b). Between 1947 and 1986, more than 20 million tonnes of *S. acanthias* were taken by target fisheries throughout the world (Last and Stevens, 1994). Up to 60,000 tonnes of stingrays are caught annually off the coast of India, and the dogfish fishery of the North Sea sustains the traditional "fish and chips" of the British Isles (Last and Stevens, 1994). The fishing of *Galeorhinus galeus* and *Mustelus antarcticus* is well established in the southern oceans of Australia, comprising 87% of the total shark catch from 1970 to 2002 (Walker *et al.*, 2003). The annual catch of *Carcharhinus obscurus* alone was 400–600

tonnes (live weight) during the 1980s. Reductions in abundance, restrictions on the level of effort and changing targeting practices, resulted in significant increases in catch during the 1990s, with current annual catches of between 200 and 250 tonnes (Simpfendorfer *et al.*, 2002a). Baum *et al.* (2003) estimated that all recorded shark species, with the exception of *Isurus* spp. have declined by more than 50% in the past 8 to 15 years in the northwest Atlantic Ocean. Hong Kong fish market, which is the world's most important market for shark fins, increased its shark fin imports by more than 214% from 1985 to 1998. After 15 years of industrial fisheries, large coastal predatory fish biomass is today only 10% of its pre-industrial value (Myers and Worm, 2003). This decrease is however a worldwide phenomenon. The United Nation Food and Agriculture Organization (FAO), which currently is the only global organisation that attempts to estimate world catches, estimates the global shark fishery to be between 0.39 to 0.60 million tonnes year^{-1}. However, new research using data from the shark fin markets in Asia, estimated world landing as high as 1.21 to 2.29 million tonnes year^{-1} (Clarke *et al.*, 2006) which is between 3 to 5 times higher than reported by FAO. The differences between the FAO and Clarke *et al.* (2006) research data, arises from the large number of shark finings and unrecorded landings.

Only a few of the approximately 1200 species of chondrichthyans are commercially targeted, but many do fall prey as by-catch. However the number caught as by-catch is hard to predict because of limited identification or unreported numbers (Barker and Schluessel, 2005). Francis *et al.* (2001) reported that between 1988-89 and 1997-98 207,205 fish (including tuna) and invertebrates were caught in tuna longline fisheries in New Zealand. Almost half (47.3%) of these were elasmobranchs. Records for Japanese and Korean vessels combined, recorded between 1979-80 and 1987-88 an annual catch of 78,000 *Prionance glauca*. However the reported numbers increased to 97,000 for the last five years

(Francis *et al.*, 2001). In the West Indian Ocean, tuna fisheries had an increase in pelagic oceanic shark by-catch of 1124 tonne from 1985 to 1994 (Romanov, 2002). Bonfil (1994b) estimated a worldwide incidental by-catch of 8.3 million elasmobranchs (232,425 metric tonnes), which is three times as much as reported by FAO as the total world catch in 1991. Graham *et al.* (2001) conducted a study of the abundance of elasmobranch by-catch species from the upper Australian New South Wales coast over 20 years and found a 80% decrease in mean catch rates. together with the history of the fishery and the biology of the species, Graham *et al.* (2001) suggested that the elasmobranch stocks was historically low. In the south Australian shark fishery, *H. portusjacksoni* is one of the four most important by-catch species (Walker *et al.*, 2005).

Long-established shark-control programs involving beach meshing have existed off the coast of New South Wales, Australia since 1937 (Stevens *et al.*, 2000). This presents another threat to shark populations. Pollard (1996) reported a 3.8% (n = 369) catch rate of the protected and endangered (IUCN Shark Specialist Group, 2003) *Carcharias taurus* from the NSW beach meshing programme between 1950 to 1990, with a decline in catch rate after the late 1980s. Krogh (1994) reported catch rates of *Sphyrna* spp., *Carcharhinus* spp., *Squatina australis*, *Heterodontus* spp., *Carcharodon carcharias*, *Galeocerdo cuvier*, *C. taurus*, *Notorynchus cepedianus*, *Alopias* spp., *Isurus oxyrinchus* and *Orectolobus* spp. sharks from the NSW beach meshing programme over the period from 1972 to 1990. A total of 4,603 sharks were reported in the 49 nets spread from Wollongong in the south to Newcastle in the north. It is worth mentioning that both angel and Port Jackson sharks catches were inconsistent, since data from individuals <1m long was not included in the report. Reid and Krogh (1992) reported data from the same beach meshing programme but divided the data into two categories. Firstly from 1950 to 1972 and second from 1972 to

1990, due to differences in the contract after 1972. The total catch declined from 5063 to 4715 between the two periods, with the largest decline (58.6%) on Newcastle beaches. Although *H. portusjacksoni* is not a commercially significant species and has only a 3% mortality rate from beach meshing, 647 was caught between 1950 and 2002, and between 1997 and 2001 432 kg of *H. portusjacksoni* were caught in NSW waters (NSW Fisheries, 2003)

Most sharks are slow growing and mature at a later stage in life compared to bony fish. Branstetter and Stiles (1987) recorded a growth of 15-20 cm year^{-1} for the first five years for juvenile *Carcharhinus leucas*. Growth gradually decreased to 5 cm year^{-1} for ages 5-16 years, and was less than 4-5 cm year^{-1} thereafter. *C. leucas* does not reach maturity until 13-16 years of age. Therefore, this combination of slow growth, long life span and late maturity makes this species vulnerable to overexploitation. In addition, theses characters are common to elasmobranchs. Smith *et al.* (2003) recorded a growth rate of 1.47 cm year^{-1} for an adult *Triakis semifasciata*. Female *S. acanthias*, can live for 90 years and do not mature before 50% of their total length is achieved (McFarlane and Beamish, 1987b). The gestation period of *S. acanthias* is 22 months (Ketchen, 1975), which is the longest gestation period of any species known in the phylum Chordata, the closest rival being the elephant whose gestation period is variously estimated to be 18-21 months. All this makes sharks extremely vulnerable to overfishing and the management of these resources have to take account of their life history.

Few shark fisheries have proven sustainable because of their vulnerable life history (Walker, 1998). The few populations that have been harvested sustainable have only achieved this by this long-term scientific research. For sustainability, it is crucial to collect the biological data on the species. Such data includes age, growth, reproduction and general

ecology. Moreover, if the demands from fisheries is not enough, it is unknown what the impact of future climate changes will have on elasmobranch populations (Walker, 2007). The vulnerability of elasmobranchs to climate change is dependent on life history and habitat requirements (Walker, 2007). Therefore, without the proper knowledge we might loos these animals in the future.

In addition, the effects of removing large apex predators from complex marine food webs may impact lower trophic levels (Bascompte *et al.*, 2005). Myers *et al.* (2007) investigated the effects of the diminishing numbers of large (>2 m) sharks from the east coast of the U.S.A. As the population of the larger shark species declined, their smaller elasmobranch prey increased. One of these species was *Rinoptera bonasus* which is a main predator of bivalves and scallops. This resulted in closure of the century-old North Carolina scallop fishery due to decreased catches (Myers *et al.*, 2007).

1.4 Ageing Techniques

Beginning with Reibisch`s observations on otoliths in 1899, there has been a continued and growing interest in the use of hard body structures as indicators of annual growth (Campana and Thorrold, 2001). Band pairs in calcified structures corresponding to environmental, daily, seasonal and/or annual patterns and are common in aquatic phyla. Band pairs are found in coral skeletons, bivalve shells, mammal teeth, otoliths, fin spines and vertebral centra. Annual band pairs occur in dorsal spines (Ketchen, 1975) and in calcified vertebral centra in sharks (Branstetter, 1987a).

Elasmobranchs, unlike teleosts, do not have otoliths, scales or dorsal fin rays which are used for age estimation. Instead, reliable age estimations have focused on using the vertebrae and dorsal fin spines (Cailliet and Goldman, 2004; Musick and Bonfil, 2004).

Different techniques have been produced to visualize and read the band pairs. These techniques have focused on visualizing the band pairs in the hard parts of the dorsal fin spines and the vertebral centra. There are two types of bands: (1) lighter, narrower (translucent) bands which are laid down in winter when the growth is slow, and (2) darker, wider (opaque) bands which are normally laid down during the summer period when growth is faster (Holden and Meadows, 1962; Tucker, 1985). Temporal variation in ambient phosphorus can affect the rates of mineralization of the cartilage. High concentrations of calcium phosphate will make rapid mineralization of the extracellular matrix, making the formation of hyper-mineralised band pairs (narrow) possible, while low concentrations of calcium phosphate will slow down the mineralization of the extracellular matrix making the formation of hypo-mineralised band pairs (wide) (Walker *et al.*, 1995).

Little is known about the age and growth of elasmobranchs because many species are difficult to sample, of relatively large size, highly mobile and exhibit seasonal migration. Nevertheless, several methods of age estimation have been developed for elasmobranchs. Length frequency analysis has been used by Ketchen (1975), Francis and Stevens (2000), Dykhuizen and Moller (1992), Francis and Francis (1992) and Simpfendorfer (1993). Often, this kind of analysis is coupled with tag-recapture studies as conducted by Natanson *et al.* (1999), Simpfendorfer *et al.* (2000), Cailliet *et al.* (2001), Morita and Matsuishi (2001) and Natanson *et al.* (2002). These two approaches are limited due to the slow growth rates exhibited by most elasmobranch and sampling difficulties. Moss (1972) used tooth replacement rates to estimate growth rates, however this technique provides only rough estimates, as the rates vary among individuals and species. Other techniques that have been used include the developmental state of secondary sex characters by Johnson and Horton (1972) and embryonic growth rates by Ketchen (1972), Holden

(1974) and Francis (1981). Dorsal spines have been examined by Ketchen (1975), Maisey (1979), McFarlane and Beamish (1987b), Tanaka (1990), Machado and Figueiredo, (2000), Avsar (2001), Jones and Ugland (2001) and Clarke *et al.* (2002a), and were found to have band pairs. Most elasmobranchs do not have dorsal spines or they are worn down as they age, which makes the applicability of this technique limited. However the age of several shark species had been successfully determined from this method (Appendix A).

Band pairs deposited in calcified vertebral centra are useful tools for age estimation of elasmobranchs. Ridewood (1921) first described these bands pairs in his review of calcification processes. Haskel (1949) first suggested that these band pairs could be useful in age estimation studies. Urist (1961) and Applegate (1967) provided further morphological evidence that these band pairs were common among sharks and rays. Several authors have since developed and used various techniques to visualize these band pairs in both whole and sectioned vertebrae. These techniques include; alcohol immersion, xylene impregnation, histology, x-ray spectrometry (Cailliet *et al.*, 1983a), alizarin red S staining (La Marca, 1966), crystal violet staining (Johnson, 1979), no staining (Carlson and Parson, 1997), haematoxylin staining (Correia and Figueiredo, 1997), grinding (Branstetter and Stiles, 1987), shading, decalcifying (Correia and Figueiredo, 1997), silver nitrate impregnation (Stevens, 1975), metal substitution (Gelsleichter *et al.*, 1998), and X-radiography (Simpfendorfer *et al.*, 2000; Wintner and Dudley, 2000; Simpfendorfer *et al.*, 2002b).

Most shark cartilage is un-mineralized, yet considerable deposits of calcium phosphate are found within it in the form of poorly crystallized apatite (Clement, 1992). These deposits are not located randomly however occupy well-defined sites in the chondrocranium, jaws, visceral arches, fin cartilages, claspers or mixopterygia, neural and

haemal arches, dorsal spines, and the centra of the vertebrae. Mineralization occurs on the external surface of the most superficial and youngest growth increment (Clement, 1992). Evidence has been found that the deposition of band pairs in shark vertebrae is a seasonally determined phenomenon (Clarke *et al.*, 2002a; Simpfendorfer *et al.*, 2002b; Oshitani *et al.*, 2003). The deposition of band pairs might correlate with environmental factors such as food supply, breeding behaviour, and water temperature. In addition, the body size of most sharks shows a correlation with centrum diameter. Finally the banding pattern in the centrum of the vertebrae, visible in x-rays and other techniques occurs because of density differences. It is likely that the differences between the high and low density bands is due to differences in mineralization during different growth phases (Cailliet *et al.*, 1983a).

1.5 Validation

Before the band pairs can be used for ageing, they need to be validated to ensure that they fulfil a number of criteria (Pilling *et al.*, 2000). First, the structure must grow continually throughout the life span of the individual fish. Second, the structure must show an internal increment structure. And finally, this structure must correspond to a regular time scale. Errors in ageing fish may result in mismanagement of fisheries. Estimates of natural mortality, age composition, growth parameters, and maturity schedules of individuals in populations all depend on accurate ageing. Thus, validation is the key to understanding the biology and dynamics of populations (Heifetz *et al.*, 1998).

Validation is to prove a technique that is accurate its importance has been stressed by different authors. In the early 1940s van Oosten (1941) questioned the age estimation of fishes, as this method had never been validated and was just taken for granted. Beamish and McFarlane (1983) stressed the importance of validation by comparing it to the calibration

of instruments: "Validation means providing a technique that is accurate" (Beamish & McFarlane, 1983, pp 735) and did criticized the lack of validation in ageing studies. They surveyed 500 studies published between 1907 and 1980 and found that only 63% of the studies that estimated the age of fish mentioned or attempted to validate the ageing technique, while only 3% of the studies validated all age groups in the population. Failure to validate the methods used for age estimation can cause over or under estimates of the age of the population. Age estimation is fundamental to an understanding of species biology and population dynamics, it is crucial that age estimations occur with high precision and low bias (Beamish & McFarlane, 1983).

As suggested by Beamish and McFarlane (1983) the preferable validation technique is to use a chemical dye to mark the hard parts. Injected individuals can be marked so that the number of band pairs laid down between injection and recapture can be determined and the periodicity of band pair formation validated. The combination of laboratory and field growth studies with the use of chemical markers is essential to any age validation study (Beamish and McFarlane, 1983). This technique gives the precise somatic growth information along with a direct comparison of band pair deposition whilst the animal is still at liberty (Smith, 1984). Holden and Vince (1973) were the first to validate elasmobranch vertebrae ageing methods proving that bands were formed annually in *Raja clavata* and that these bands could be used for ageing the species. Many others have since followed their recommendations and used the chemical dye oxytetracycline (Cailliet *et al.*, 1983a; Gruber and Stout, 1983; Beamish and McFarlane, 1985; Tucker, 1985; Branstetter, 1987b; McFarlane and Beamish, 1987b; Brown and Gruber, 1988; Kusher *et al.*, 1992; Walker *et al.*, 2001; Smith *et al.*, 2003). Oxytetracycline has been used since the 1950s in the study of human bone formation, and has been used since the early 1960s to validate the annual

deposition of growth bands in the hard parts of teleosts. The fluorescent oxytetracycline hydrochloride (OTC), at a dosage of approximately 25-35 mg/kg (McFarlane and Beamish, 1987a), is easily absorbed by calcifying structures and deposits at sites that are actively calcifying at the time of injection. Under ultraviolet (UV) illumination (365 nm), these sites fluoresce bright yellow against a bluish fluorescent field whereas newer growth that has occurred after the injection will not fluoresce (Smith, 1984). The disadvantage of OTC is that it is light sensitive and therefore limits the amount the sample can be illuminated before its clarity or brightness is reduced. Other markers such as alizarin red S, alizarin complexone, calcein and xylenol orange are also used to mark calcium deposits and can be distinguished because they fluoresce at different wavelengths (Officer *et al.*, 1997). Calcein has only recently been used, but has proven useful in elasmobranchs (Gelsleichter *et al.*, 1997; McAuley *et al.*, 2006). Gelsleichter *et al.* (1997) successfully used calcein as a vertebral marker on *Ginglymostoma cirratum*. At a dosage of 5 mg/kg calcein marks were revealed as bright yellow-green under blue light (470 nm) illumination.

There are other different ways of validating age estimation. Marginal Increment Analysis (MIA) uses measurements of the narrow translucent growth band to verify the annual nature of the growth band (Conrath *et al.*, 2002; Carlson *et al.*, 2003). However, samples from all months throughout the year must be collected, which in deepwater and migratory species might be impossible. Size frequency analysis is one other method of validating age estimation (Kusher *et al.*, 1992). However, this technique is difficult due to the slow growth of most elasmobranchs and is mostly used on smaller and younger size classes. Centrum Edge Analysis (CEA) (Campana *et al.*, 2006) detects differences in width and density of the outer band on the centrum edge over time in different vertebral centra caught throughout the year. It can also use the difference in calcium and phosphorus levels

at the edge of the centra (Cailliet and Radtke, 1987). Radiometric dating which uses radioactivity levels of naturally occurring isotopes from different parts of the vertebrae to estimate age had been used by some researchers (Andrews *et al.*, 2002; Campana *et al.*, 2002a; Campana *et al.*, 2006). Francis *et al.* (2007) used bomb radiocarbon assays to validate age estimates of *Lamna nasus* concluding that the age of older sharks (>20 years) could have been under-estimated by as much as 50%, underlining the importance of a full age range in age validation studies.

Squatina californica (Natanson and Cailliet, 1990) is the only well documented species that does not have annual band pairs throughout life out of the many validated shark species and amplifies the importance that this practise is utilised. Age estimations on sharks are difficult to obtain because of their mostly slow growth which makes their band pairs fine and difficult to interpret. This problem makes validation important for accurate age and growth determinations. Validation of ageing techniques has given researchers important results. McFarlane and Beamish (1985) validated the method of age estimation using the second dorsal spine. By injecting OTC, they revealed that *S. acanthias* forms one band pair each year and that the species was older, slower growing and matured later than previously thought. *G. cuvier* growth was determined with the aid of OTC-injections, and provided an independent estimate of growth by using the tag-recapture and length-frequency data (Natanson *et al.*, 1999). Their data concluded that although the centrum band pairs were laid down annually in juveniles, this did not appear to continue throughout the adult life of the shark. Therefore, band pairs underestimated the age of the shark, which highlights the importance of validating all age classes. Moulton *et al.* (1992) tried to verify the age estimates that were reported on gummy sharks and school sharks by comparing the von Bertalanffy growth curves derived from age-length data, with those derived from tag

release-recapture length-increment data. Unfortunately, this method was unsuccessful of larger adults and therefore limited the results. However they were able to highlight the limitations of using tag data for this purpose.

Although the importance of validation has been stressed the lack of validation still exists. Machado and Figueiredo (2000) adopted a technique for enhancing band pairs on thin cross-sections of *Deania calcea* without conducting a validation. Clarke *et al.* (2002a; 2002b) estimated the age of *Centriphorus squamosus* and *Deania calceus* by using the dorsal spines with the assumption that the band pairs formed annually after birth, but no validation was attempted.

Precision and accuracy should also be part of the validation process to evaluate the errors that might occur during age estimation (Cailliet and Tanaka, 1990). In addition, not only is reproducibility important, which only relates to consistency, but precision between determinations or readers will indicate the average error in ageing. Beamish and Fournier (1981) described this as the Index of Average Percentage Error (IAPE) and has together with Chang's (1982) Coefficient of Variation (CV) been applied to many elasmobranch studies (Kusher *et al.*, 1992; Simpfendorfer, 1993; Lessa *et al.*, 1999; Francis and Maolagain, 2000; Walker *et al.*, 2001; Simpfendorfer *et al.*, 2002b; Carlson *et al.*, 2003). Most of these studies have compared age estimations and readers to identify the precision between them. However, the level of precision must be identified in all determinations if it is between ageing structures such as dorsal spines and vertebral centra or between different readers. Since systematic differences (bias) can occur in all levels of age estimation it is important to quantify the bias and understand where it can be limited. Analyses such as *t*-tests and age-bias plots are effective at detecting systematic differences between readings and readers. However, Campana *et al.* (1995) concluded that only age-bias plots could

detect if one reader under-aged either young or old individuals while the other reader over-aged those same young or old individuals. Researchers should therefore use multiple verification and precision analyses when studying age estimation (Cailliet *et al.*, 2006).

A consequence of ignoring validation can contribute to underestimated ages. This will give a false growth rate and longevity of the population, which again impact all other ecological considerations for managing a population.

1.6 Growth Models

von Bertalanffy (1938) thought of an organism as a closed system that was able to grow because its input was larger than its output and wrote, "growth is the measurable increase of an organic system, produced by its assimilation of materials obtained from its environment" (von Bertalanffy, 1938, pp 181). Growth is widely dependent on external factors (nutrition, temperature, free living space) and internal factors (hormones and age). His work came to be known as the von Bertalanffy Growth Equation (VBGE).

$$L_t = L_\infty (1\text{-}e^{-K(t-t_o)}),$$

where L_t = predicted length at time t; L_∞ = theoretical asymptotic length; k = growth coefficient; and t_0 = theoretical age at zero length.

Although the von Bertalanffy equation is the most commonly employed, others have adapted the growth model for further research. Since only two of the three parameters can be calculated (L_∞ and k) if only growth data are available, a third parameter (L_0) requiring

data of known age at birth was used by Fabens (1965) to create a computer program to fit any recaptured or age-size data using the von Bertalanffy growth model.

$$L_t = L_\infty - (L_\infty - L_0)\, e^{-kt},$$

where L_t = predicted length at time t; L_∞ = theoretical asymptotic length; k = growth coefficient; and L_0 = the length at birth.

It is important to mention that the three parameters (t_0, k and L_0) adjust themselves to obtain the best fit whatever the length at birth is and therefore a growth equation should only be interpreted for those ages that the age estimation was based upon.

The von Bertalanffy parameter k is used as an index for vulnerability and is compared between species (Musick, 1999). Most elasmobranch populations have a k-value <0.1 which is considered to be an indication of being particularly vulnerable (Cailliet and Goldman, 2004).

McFarlane and Beamish (1987) found that *S. acanthias* are older, slower growing and mature later in life than previously thought. The new insight into the species meant that new strategies such as decreased catch limits increased length at capture had to be applied. Moulton *et al.* (1992) verified by tagging *M. antarcticus*, that the mean length of the females was larger than that of males at any age over 3 years and that their maximum life span was about 16 years. Their research on *G. galeus* revealed that the mean length was the same for both males and females, and that they had the same VBGE curve. This meant that different strategies related to sex had to be applied for the *M. antarcticus* but not for *G. galeus*.

Many methods have been developed to compare the precision of age estimations. Research on the north-western Atlantic *G. cuvier* has revealed that it is similar to other larger coastal carcharhinoid species in its slow growth and long life. However it matures earlier (25% of its total age) than most of the other carcharhinoid species (*Sphryna lewini*, 33-50%) (Branstetter, 1987a). This suggests that *G. cuvier* have a longer reproductive life and possibly a higher fecundity than other carcharhinoid (Natanson *et al.*, 1999) which might make it less vulnerable to pressure. Avsar (2001) revealed that *S. acanthias* in the south-eastern Black Sea had different growth rates for males and females after 5 years of age, where the growth of males decreased but females still maintained large annual growth. Avsar's (2001) research concluded that the fishing of individuals smaller than age group 5 was not economically viable for stock maintenance. Carlson and Parson (1997) found that female *Spyrna tiburo* had a lower growth coefficient ($k = 0.28$ year^{-1}) than that of males ($k = 0.69$ year^{-1}), but attained a larger maximum size. Different strategies had to be applied to the different sexes to be able to sustain the fisheries. Research conducted by Carlson *et al*. (2003) on *Carcharhinus isodon* in the northern Gulf of Mexico revealed that the species is one of the "slowest" growing of the coastal species ($k = 0.24$ year^{-1} for females and $k = 0.41$ year^{-1} for males). The study also revealed that the survival rate of the juveniles and adults are much more crucial to the whole population growth rate than to the survival rate of age-0 individuals, a feature that normally applies for "fast" growing sharks (Carlson *et al.*, 2003)

1.7 Application of Techniques

Branstetter (1987) stated that insufficient information existed to assess accurately survival rates or longevity for most shark species. In addition, the few instances where such data had been available were from fisheries or research efforts on a relatively finite population. His report suggested that further studies were needed to define accurately the biology and ecology of these common apex predators in the marine ecosystem. Stevens *et al.* (2000) used three ECOPATH models, which can only be obtained by using mortality, abundance and diet composition data for each group/species in the model, to predict the outcome of removing sharks from their ecosystems. This data can only be gained by an intense study of the ecology and biology of each of the species. Three different ecosystems (Venezuelan shelf ecosystem, Alaskan Gyre ecosystem and Hawaiian coral reef ecosystem) were used to obtain the general response that removing sharks from one ecosystem would influence the food web in a complex way. Fundamental research on age and growth is crucial to fisheries management. Moreover, although few species are of commercial value and targeted, almost all shark species are affected by some sort of fishing pressure, be it as by-catch or beach meshing. It is therefore alarming that so few shark species been aged and had their growth rates determined. Research by Walker *et al.* (1995) on the southern sharks (*M. mustelus* and *G. galeus*) resulted in benefits to the well established shark fishing industry of southern Australia. The research established a better method of ageing sharks, which allowed the fishery to better manage their resources. The research also established better data for fittings the von Bertalanffy growth model, which could help in better stock assessment management. Walker *et al.* (2001) called for the need for accuracy in the method of ageing sharks used for producing length-at-age data for fishery monitoring and stock assessment.

Using the combined age and growth models determined for *Rhizoprionodon terraenovae* in the Gulf of Mexico, Carlson and Baremore (2003) found that median age at maturity had decreased from 2.3 years in 1979-1980 to 1.4 in 1998-2001. They hypothesized that a decline in the world shark population could give *R. terraenovae* decreased intra- and/or inter-specific competition, increasing food intake and enabling faster growth.

1.8 Port Jackson Shark

Chondrichthyan fishes represent the oldest surviving group of jawed vertebrates with fossil records dating back approximately 450 million years. They have remained almost unchanged for the last 100 million years (Last and Stevens, 1994). The heterodontids occupy an isolated evolutionary position. They are more related to the hybodonts, whose general structure and bottom-feeding ecology appear to have changed little within the last 150 million years. The genus *Heterodontus* includes the only living species of sharks that date back to the Upper Jurassic period (205 million years ago (mya)). The closely related family Hybodontidae is only represented by fossils and dates from the Devonian period (416 mya) to the Cretaceous period (144 mya). The two families thus overlap in geological history. This means that the recorded lineage of heterodontid sharks is one of the more ancient of extant shark genera. The Port Jackson shark (*H. portusjacksoni*) is in the order Heterodontiformes and the family Heterodontidae. The Port Jackson shark is also called bullhead shark, oyster crusher and tabbigaw (Last and Stevens, 1994). There are 8 other recognised species in the genus occurring from the subtropical waters of the eastern Pacific of U.S.A., Mexico and Peru (*H. francisci, H. mexicanus* and *H. guoyi*, respectively), western south Pacific of Australia (*H. galeatus*), western north Pacific of Japan and China

(*H. japanicus* and *H. zebra*, respectively), north western Indian Ocean of South Africa (*H. ramalheira*) and northern Indian Ocean of Oman (*H*. sp. A) (Compagno, 2001).

H. portusjacksoni is a harmless, non-commercial, common species distributed in the southern half of the continent from Queensland to Western Australia. Adult *H. portusjacksoni* sharks are largely nocturnal in activity (Last and Stevens, 1994; Compagno, 2001). The breeding season starts in late July and early August. During this period, the mature females spend much of their time lying on the bottom of caves, trenches and gutters of shallow coastal reefs, while the males are more mobile. The mature breeding female *H. portusjacksoni* lays 12-20 (mean = 16) eggs mainly in August and September. Eggs are laid between rocks and trenches in shallow water reefs (Powter, 2007). The young emerge after 10-11 months at a length of 18-22 cm (Rodda, 2000). Some juveniles might migrate to deeper waters, particularly during summer, however most stay in nursery grounds for several years in mixed male and female groups. Sexual maturity is thought to occur at 950 mm total length females and 700 mm total length for males (McLaughlin and O'Gower, 1971). Adults move away from inshore waters towards the end of the breeding season (late September or early October), however in Sydney waters the breeding season will end when the water temperature is over 18°C. Some adults move offshore to deeper cooler water, but most migrate southwards along the coast. Most feeding occurs at night over reefs and soft substrates. Echinoderms, molluscs and crustaceans are the predominant diet of the adults. *H. portusjacksoni* use the same breeding sites each year, with a homing ability that is precise and well-developed (McLaughlin and O'Gower, 1971). It is currently not a commercially targeted species but is heavily caught as by-catch in prawn- and fish-trawls (Walker *et al.*, 2005).

Although listed as "low risk" in the Red List assessment of the World Conservation Union (IUCN) Shark Specialist Group, it is important to highlight the enormous pressure from commercial fishing this species and most chondrichthyan's experience. On three fishing trips in October and November 2005, 417 *H. portusjacksoni* were caught as by-catch by one commercial fish trawler alone (personal communication). Bonfil (1994b) estimated that sharks and their relatives are the leading group in by-catch around the world. In the late 80s-early 90s this group of fishes had a total annual by-catch rate of 260,000-300,000 tonne. Although, this value is probably higher due to high by-catch rates and un-reported landings of this fishery.

1.9 Conclusion

Sharks and rays are particularly vulnerably to overfishing because of their *k*-selected life history. Migratory patterns, oceanic and deep water habitat preferences of some species, place them outside the responsibility of both national and international bodies (Bonfil, 1994a). As fishing pressure on the world's shark population increases, the need to harvest these resources sustainably is becoming more and more important. Information on life history, longevity, growth and sexual maturity are crucial to maintaining a sustainable fishery. Sharks have no scales, and therefore cannot be aged by the use of any parts on the outer body. The only hard parts that are available for age estimation are the vertebrae and for some species the dorsal spines. The hardening of the cartilage imprints small circular growth bands, which can be used for this purpose. Although elasmobranchs have limited hard structures to be used for ageing, many different techniques have been used on different species. Some are species-specific and others are more common and can be applied to different species. Therefore, for each new shark that is aged it is important to validate

different techniques and adapt these to the species in order to find which is most efficient. Further comparison to different subpopulations may give information on the structure of the species. Ultimately, validation of any technique must be undertaken so that crucial data on population age structure is not missed.

Although, some information on the biology of *H. portusjacksoni* exists for New South Wales (McLaughlin, 1969; McLaughlin and O'Gower, 1971; Powter, 2007) tagging studies (O'Gower and Nash, 1949) and population studies (Tovar-Avila, 2006) suggest there might be more than one population. Here it is critical that knowledge of key biological parameters from the New South Wales population is estimated. Although this species is not commercially targeted, nor under any threat at the present time, some catches are retained for commercial use and the species is extremely sensitive due to its narrow geographical distribution to any influences that may change its normal lifecycle. Powter (2007) concluded that the NSW population of *H. portusjacksoni* was vulnerable to minor increases in mortality of adults using population modelling. Life-history traits as maturity, growth rates and fecundity might be the most important traits and must be clearly understood when considering the extreme pressure that commercial fishing will inflict on any fish population. Ideally the management of a population should determine life history traits before harvesting the resource (Musick, 1999).

Chapter 2

A Port Jackson shark resting on a sandy bottom covered with seagrass (photo by the author)

Chapter 2

Anatomy of the Vertebrae and Dorsal Spine of the Port Jackson Shark (*Heterodontus portusjacksoni*).

2.1 Introduction

All sharks belong to the class elasmobranchii and all have endoskeletons composed of cartilage. Most of the cartilage is un-mineralised, but at well defined sites in the chondrocranium, jaws, visceral arches, fin cartilages, claspers or mixopterygia, neural and haemal arches and vertebral centra, considerable deposits of calcium phosphate are found in the form of crystallized apatite (Clement, 1992). Elasmobranchs inhabit a calcium-rich environment and unlike terrestrial vertebrates do not need to store calcium for their metabolic requirements. This means that sharks are not required to reabsorb calcium from their skeleton in order to regulate their calcium metabolism or to grow (Officer *et al.*, 1995). Once calcified, the mineralised tissue of sharks remains permanently deposited and additional growth will only occur by apposition (Clement, 1992; Walker *et al.*, 1995). This periodic and incremental growth of shark vertebrae and other hard parts makes these tissues a potential record of the growth history that could be used as a tool in elasmobranch ageing.

Two types of vertebrae, trunk and caudal vertebrae, are found in all teleosts and elasmobranchs. Trunk vertebrae occur anteriorly from the head to the caudal fin (the trunk). Caudal vertebrae are restricted to the caudal fin or just anterior to the caudal fin. The major features of trunk vertebrae include the centrum (partly replaces the notochord), the neural arches (surrounds the spinal cord) and the ribs. The centrum includes the corpus calcareum and the intermedialia which are the articular cups and ridges, respectively. The caudal

vertebrae have the same features as the trunk vertebrae except that they bear a haemal arch (instead of ribs) that encloses the caudal artery and vein (Rosenzweig, 1988).

There is a change in the angle of the corpus calcareum close to the centre of the vertebral centra (Goldman, 2004). This angle change is usually laid down at birth and is known as the birth mark. However sometimes the change in angle can occur later in life (e.g. during the first summer) (Brown and Gruber, 1988) and is referred to as a post-natal growth mark (Branstetter, 1987a). Some shark species have also shown pre-birth growth marks where evidence suggests this is laid down during the development of the placenta when there is a sharp increase in calcium (Branstetter, 1987a; Branstetter and Stiles, 1987).

Band pairs, composed of one translucent and one opaque band, are normally found in the corpus calcareum and the intermedialia. However they are usually more clearly seen in the more calcified corpus calcareum. These band pairs are thought to be deposited annually and therefore may be used to estimate the age of the animal (Cailliet and Goldman, 2004).

In extant sharks dorsal fin spines occur in every species in the order Heterodontiformes (bullhead sharks) and Squaliformes (dogfish sharks). Dorsal spines are not shed, unlike teeth or scales. They vary in shape and appearance however, most are made up of a cap and a stem (Maisey, 1979). The cap, covering the upper part of the dorsal spine, consists of several layers. These layers are divided up into external layers called the enamel, the often pigmented mantel, outer dentine layer and the inner dentine layer. The dorsal spines grow in diameter by the action of odontoblasts and in length by dentinogenesis at the spine base (Clarke *et al.*, 2002a). Clarke *et al.* (2002a) described the dorsal spine of *Centrophorus squamosus* where they found that only the inner dentine layer forms throughout the life of the shark. The bands from this layer were used for ageing. The

stem was composed of two distinct layers, one outer and one inner layer, which were separated by the stem primodium with canaliculi visible in both layers (Clarke *et al.*, 2002a). Clarke *et al.* (2002b) described the spine structure of the Centrophoridae, *Deania calceus*, and reported that it was deeply inserted in the body of the shark, having its base just above the vertebral column. The pigmented enamel forms in a series of bands which was used for ageing. Beamish and McFarlane (1985) described the second dorsal spine of *Squalus acanthias*. The dorsal spine was triangular in shape and consisted of three major structures: the cartilaginous interior rod, the stem and the cap. The interior of the spine is filled with a cartilage rod and surrounded by pulp tissue which degenerates towards the tip of the spine leaving a central cavity. The same cartilage that supports the base of the spine also supports the dorsal fin. The main body of the spine is called the stem and surrounds the pulp tissue. The stem consists of several layers of dentine. The cap covers the top part of the stem and consists of an inner dentine layer, a middle layer containing pigmented mantel and an outer layer of enamel. Cartilage is produced at the base of the spine and these cells (chondrocytes) are distributed within a homogeneous tissue. Upward growth occurs by the continuos deposition of dentine at the base of the stem, while outward growth results from the production of cartilage in the centre of the spine. The darkened pigment ridges in the mantle surface was defined and used to estimate the age of *S. acanthias* (Beamish and McFarlane, 1985). Maisey (1979) tried to describe the anatomy of all of the approximately 31 species of sharks possessing dorsal spines. However, the anatomy of the dorsal spines has only been properly been described for *S. acanthias* (Maisey, 1979; Beamish and McFarlane, 1985; Tanaka, 1990).

There are still anatomical features of the dorsal spine that authors have not agreed upon. One such feature is the number of dentine layers in the stem. Many authors suggest

that there are two layers of dentine, one inner and one outer (Maisey, 1979; Clarke *et al.*, 2002a; Clarke *et al.*, 2002b; Braccini, 2006) while others report a third middle dentine layer (Holden and Meadows, 1962; Beamish and McFarlane, 1985; Tanaka, 1990; Irvine *et al.*, 2006a). While *S. acanthias* is the only species that have been extensively studied and therefore described by different authors to possess both two and three dentine layers, it is of major importance to determine the number of dentine layers if sectioned dorsal spines are to be used for age estimation so not to over- or underestimate the age.

The aims of the present study were to (1) describe the macroscopic anatomy of the vertebral centra and dorsal spines of *Heterodontus portusjacksoni* and (2) to determine whether these structures are potentially useful for ageing. Aim (2) will be approached by testing for relationships between features of each structure and somatic growth and by confirming (or not) the macroscopic existence of visible band pairs in each structure.

2.2 Materials and Methods

2.2.1 Sampling

H. portusjacksoni were taken as by-catch by commercial fishing boats operating in the Newcastle (New South Wales) region (32° 55′05″*S*, 151°45′37″*E*). All sharks were measured to the nearest 1 mm and sexed. Total length (T_L) was measured from tip of the snout to the posterior tip of the caudal fin and fork length (F_L) was measured from the tip of the snout to the subterminal notch in the caudal fin, both were measured to the nearest mm (Plate 2.1). All measurements were made over the curve of the body using a flexible measuring tape with the shark flat on its abdomen. This measuring technique was adopted to minimise the stress and handling time on the animals. F_L was used throughout the remaining study instead of T_L because of damage to the caudal fin.

All sharks were also weighted (T_W). Sharks weighing less than 3000 g were weighed to the nearest 1 g using a digital scale (Chyo, MS 3300c), while sharks weighing more than 3000 g were weighed to nearest 100 g using a analogue scale (Avery, 3551-CUB). All sharks were measured and weighed while still alive to minimize the error of shrinkage and weight loss. Sharks were stored frozen at -20˚C until required.

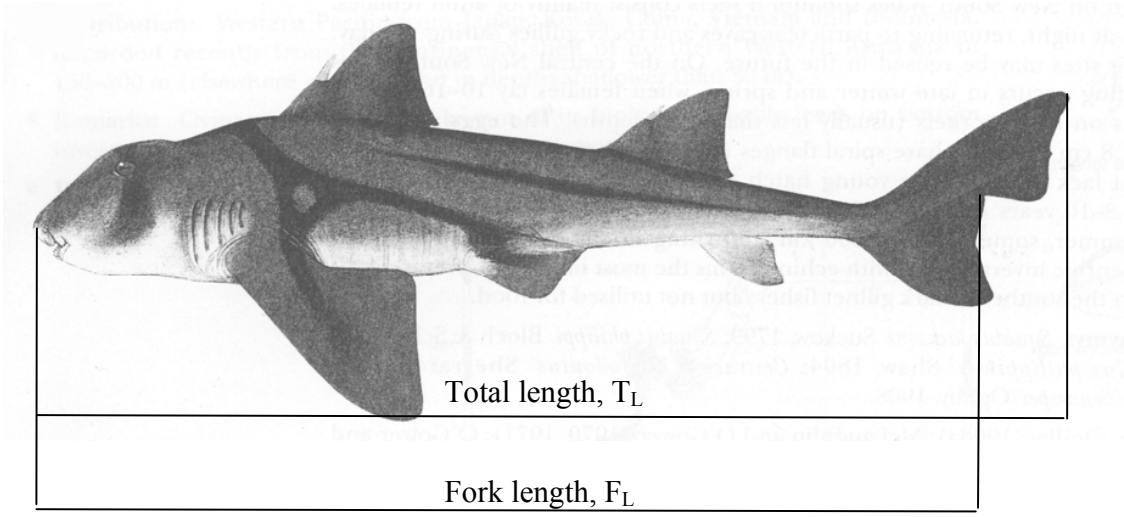


Plate 2.1: The line measurements on the *H. portusjacksoni* laying flat down on it abdomen showing total length (T_L) and fork length (F_L) measurements.

2.2.2 Preparation of Vertebral Centra

Sharks were defrosted and the vertebrae and dorsal spines were removed by first cutting through the skin with a scalpel along the mid line from behind the eyes to the end of the caudal fin. A cut was then made between the second dorsal spines and the second dorsal fin and through the vertebral column. Care was applied to not damage the vertebral centra. A dorsal cut through the neural arch was made along the column. Then the ribs were cut on

both sides of the centrum and the remaining vertebrae were removed. The whole vertebral column was removed from 36 individuals of both sexes ranging from 225 to 1195 mm F_L. Excess tissue was removed from vertebrae by cutting and scraping without damaging the centrum. Uncalcified cartilage was removed from between the two articular cups using a scalpel. The vertebrae were then soaked in 6% sodium hypochlorite solution for 10-120 minutes depending on size. Vertebrae were washed in running tap water for 30 minutes and air dried. Vertebral diameter (V_D) and length (V_L) were measured with a vernier calliper to the nearest 0.02 mm (Plate 2.1a and b). Vertebral weight (V_W) was measured to nearest 0.01 g. Vertebrae were then stored in specimen containers.

Fork length (F_L) was plotted towards vertebral diameter (V_D) to determine the relationship between somatic growth and vertebral growth. Analysis of covariance (ANCOVA) was done to test for differences between the sexes in the relationship between F_L and V_D.

A range of different techniques were tested for cleaning the vertebrae: 2% NaOH (5-15 hr), 4% NaOH (10-55 hr), 3% Papain (20 hr), 5% Papain (20-24 hr), 5% nitric acid (4 min), and 8% formic acid (10-25 hr). None of these techniques gave any reasonable results and were time consuming. An attempt was made to enhance the appearance of vertebral band pairs in whole vertebrae by decalcifying the centra with formic acid and applying standard haematoxylin and eosin staining method (Officer *et al.*, 1995; Machado and Figueiredo, 2000) were tried. Scanning Electron Microscopy (SEM) was also used to attempt to visualise band pairs.

2.2.3 Preparation of Dorsal Spines

After defrosting, dorsal spines was boiled in tap water for 2-5 min (Jones and Ugland, 2001). All excess tissue was removed by forceps and the dorsal spines were air dried. Three measurements were taken of the dorsal spine: (1) external spine length (E_{SL}) was measured on the anterior edge of spine and was the total length of cap from the spine tip (apex) to the base (Watson and Smale, 1999), (2) total spine length (T_{SL}) was the total length of the spine from the tip of cap to the end of the stem. This was also measured on the anterior edge of the spine, and (3) spine base width (S_{BW}) was measured as the total diameter of the stem base (Plate 2.12). All measurements were performed using a vernier calliper to nearest 0.02 mm. S_{BW} was used for all analyses since this was the only measurement that is not effected by wear and can therefore be retrieved from both juveniles and adults.

2.3 Results

None of the different decalcification periods, sectioned thicknesses or SEM gave any indication that the cell structure or cell density differed throughout the corpus calcareum during the life of the animal. Therefore giving no indication of cell difference in the growth layers of the vertebral centra.

For comparison with other studies T_L was estimated from F_L:

Sexes combined: $T_L = 8.19 + 1.08\ F_L$ ($r^2 = 99.8$, $F_{1,621} = 268183.3$, $p < 0.0001$)

Female: $T_L = 8.15 + 1.08\ F_L$ ($r^2 = 99.9$, $F_{1,300} = 232259.9$, $p < 0.0001$)

Male: $T_L = 8.23 + 1.08\ F_L$ ($r^2 = 99.6$, $F_{1,319} = 896181.2$, $p < 0.0001$)

2.3.1 Vertebral Centra

2.3.1.1 Number of Vertebrae

The total number of vertebrae in the vertebral column varied from 109 to 128 (mean ±SE = 115.6 ±3.79, n = 36). The total number of trunk vertebrae ranged from (39.86 ±0.69, n = 7). The total number of caudal vertebrae ranged from 69 to 88 (76 ±3.79, n = 36). There was no relationship between shark length and the total number of vertebrae (Figure 2.1).

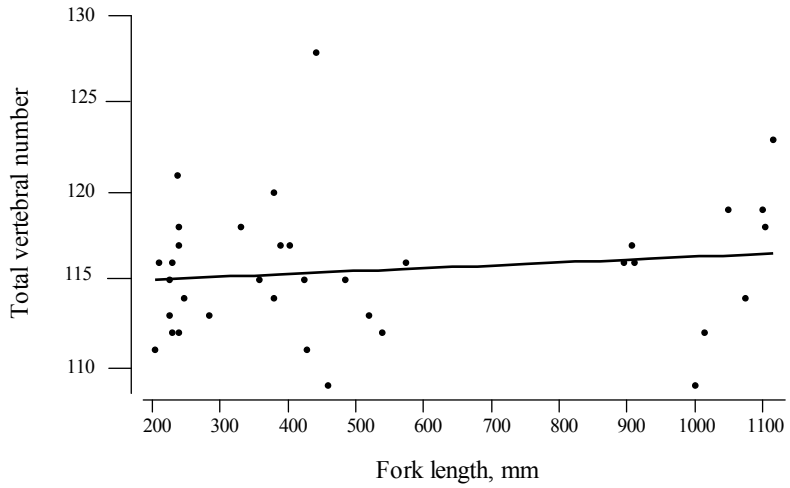


Figure 2.1: Relationship between *H. portusjacksoni* fork length (F_L) and the total number of vertebrae in the column. Total number of vertebrae = 114.71 + 0.002(F_L), r^2 = 1.9%, $F_{1,34}$ = 0.66, p = 0.42.

Vertebrae numbers 10, 20, 30, 40 and 50 (counting posteriorly) were tested for ageing. Vertebra 10 was positioned between the head and the first dorsal fin. Vertebra 20 was positioned approximately under the middle of the first dorsal fin. Vertebra 30 was positioned between the first dorsal fin and the second dorsal fin. Vertebra 40 was

positioned just posterior to the second dorsal fin. Vertebra 50 was positioned mid-way between the second dorsal fin and the tail.

2.3.1.2 Gross Anatomy

Trunk vertebrae consist of a centrum, 2 ribs, 4 neural arches, basiopophyses and neural foramen (Plate 2.2). The centrum has an anterior and posterior articular cup on each side.. The neural arch is positioned dorsally on the centrum. It consists of four plates and is made up of hard cartilage, but is not calcified (Plate 2.2). The neural foramen runs through all vertebrae and surrounds the spinal cord. The ribs are attached on each lateral side of the vertebrae through the basiopophyses. These are positioned at an angle pointing slightly posteriorly from the centra. Both basiopophyses and the ribs are made up of the same cartilage as the neural arch. Between the ridges and surrounding each vertebra is non-calcified cartilage giving the vertebrae their flexibility (Plate 2.3). The non-calcified cartilage is enriched with blood vessels (Plate 2.4).

There is a drastic change in centrum size at vertebra number 40, where the caudal vertebrae begin. V_D of vertebral number 40 is 3.45 ±1.23% smaller than centra number 38, but V_L is 31.1 ±5.91% smaller (n = 7).

Caudal vertebrae from *H. portusjacksoni* consist of a centrum, neural and hemal arches. The neural arch and the centrum have the same features as in the trunk vertebrae. The hemal arch surrounds the haemal foramen which contains the superficial caudal artery and the basial caudal vein (Plate 2.5).

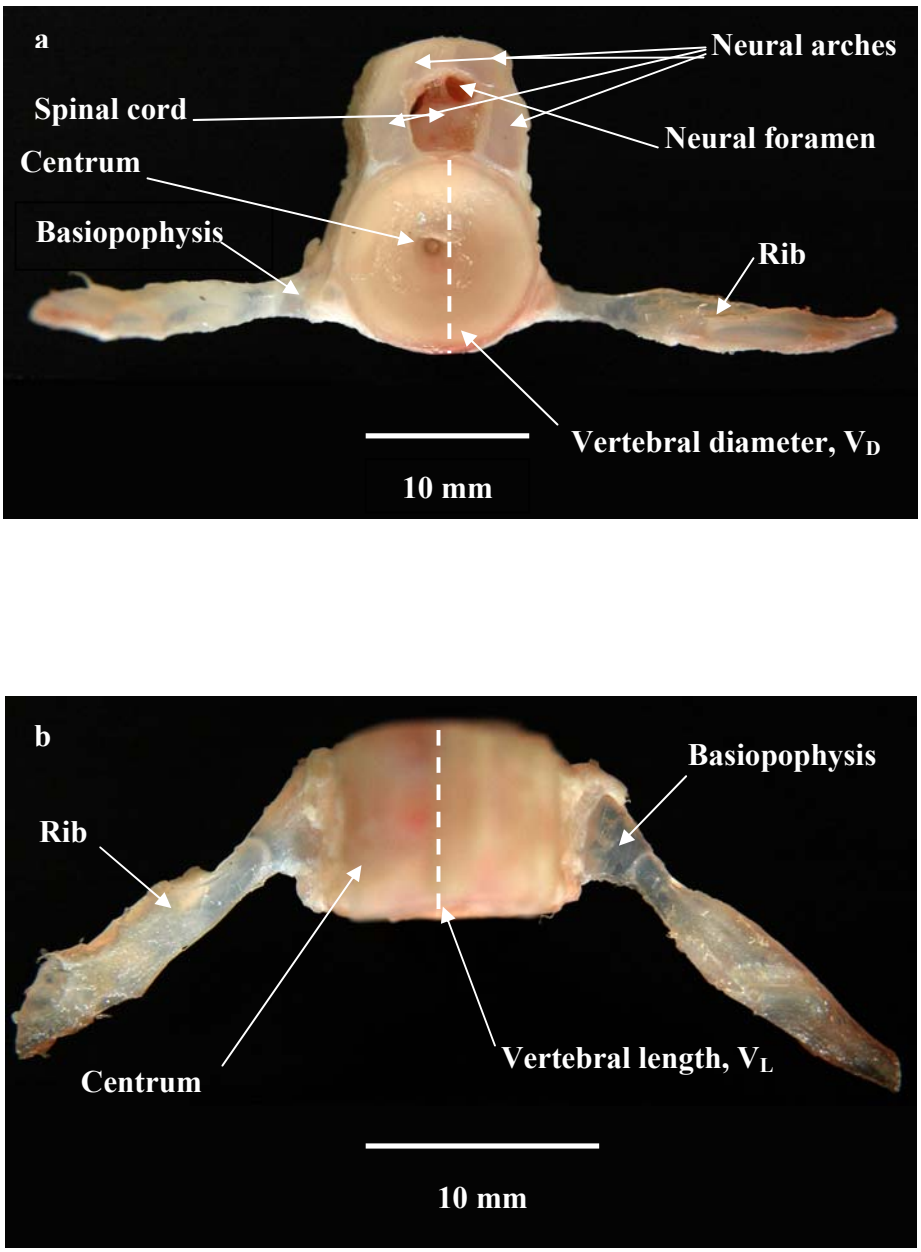


Plate 2.2: Anterior view (**a**) and dorsal view (**b**) of trunk vertebral number 27 from an 832 mm F_L male *H. portusjacksoni*, showing all the trunk vertebra features and the positions of the two measurements (vertebral diameter and vertebral length).

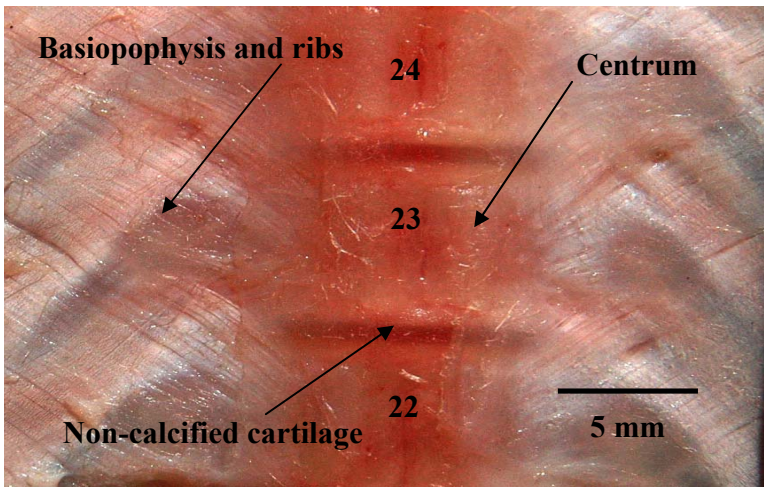


Plate 2.3: Dorsal view of trunk vertebra number 22 to 24 from an 832 mm F_L male *H. portusjacksoni,* showing the vertebrae with connective tissue in situ.

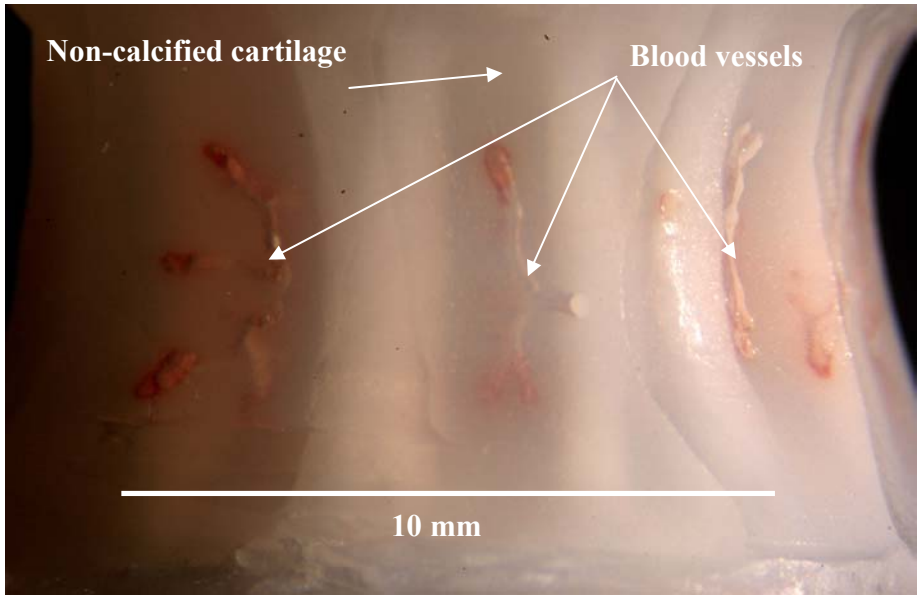


Plate 2.4: Dorsal view of trunk vertebra number 30 from a 968 mm F_L female *H. portusjacksoni* showing blood vessels in the non-calcified cartilage.

Several ridges arranged as buttresses intermedialia connect the two articular cups. The articular cups are made of heavily mineralised cartilage and are deeply curved giving the centrum an hour glass shape (Plate 2.6). Both articular cups show lighter and darker band pairs when viewed upon from the anterior side. When these two articular cups are sectioned longitudinally they are referred to as the corpus calcareum and contain band pairs (Plate 2.7).

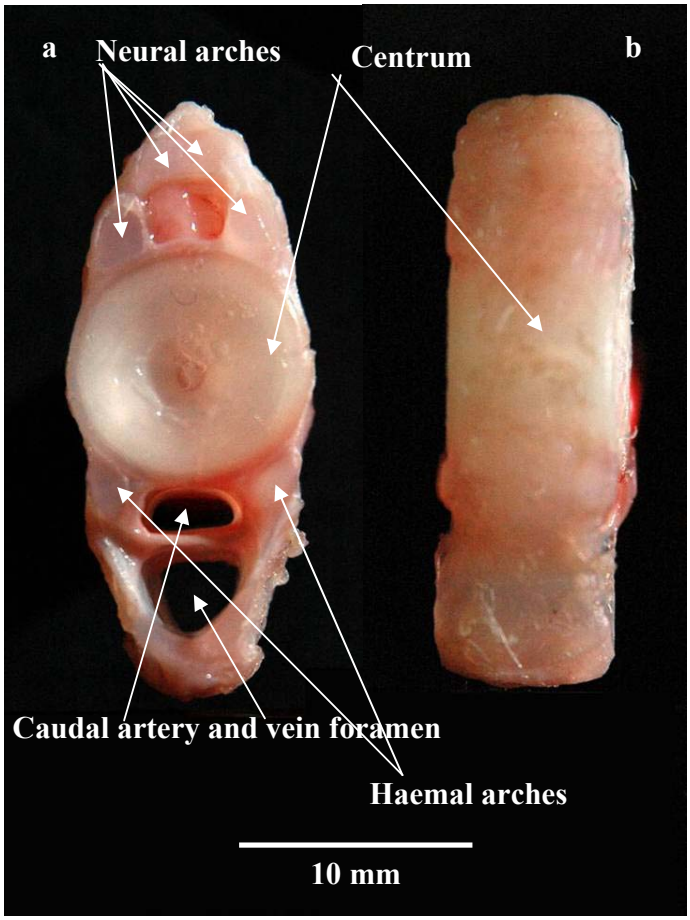


Plate 2.5: Anterior (**a**) and dorsal (**b**) view of caudal vertebrae number 61 from an 832 mm F_L male *H. portusjacksoni,* showing the caudal vertebrae features.

Each centrum has a distinctive pattern of ridges in the intermedialia. These ridges are of calcified cartilage and decrease in number from anterior to posterior in the column (Figure 2.2). The ridges are either single- or multiple-based (Plate 2.8). There was a significant positive relationship between in the number of ridges and the length of shark (Figure 2.3).

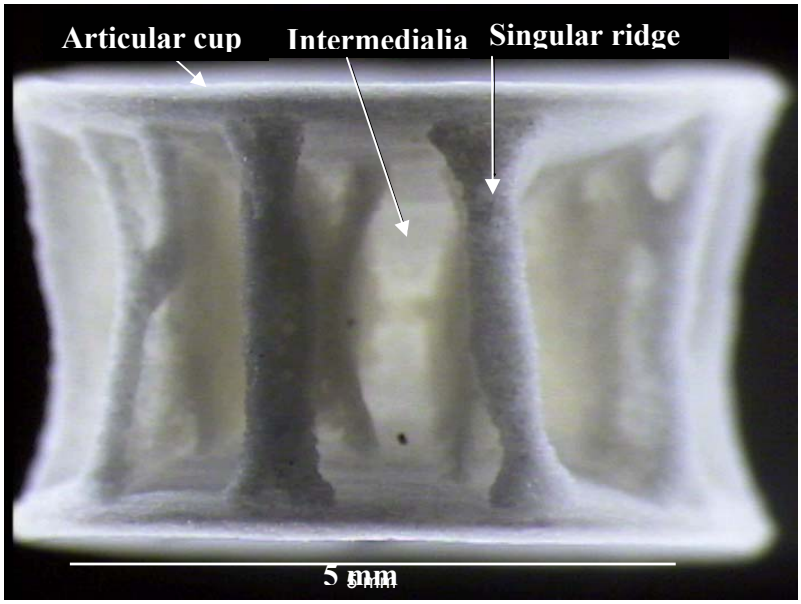


Plate 2.6: Dorsal view of trunk vertebra number 20 from a 475 mm F_L female *H. portusjacksoni* showing the calcified ridges between the two articular cups

The shape of the centrum changes along the column. Vertebrae 1 and 2 are different in shape than the rest of the vertebrae in the column. The anterior articular cup of vertebra number 2 is smaller in diameter than the posterior articular cup (Plate 2.9).

Some individuals have hollow centrums, where some notochordal material still remains and some have solid centrums were no notochordal material remains (Plate 2.10). 95% of juveniles (225 to 550 mm F_L) had hollow centrums, whereas only 10% of adults (835 to 1197 mm F_L) had hollow centrums.

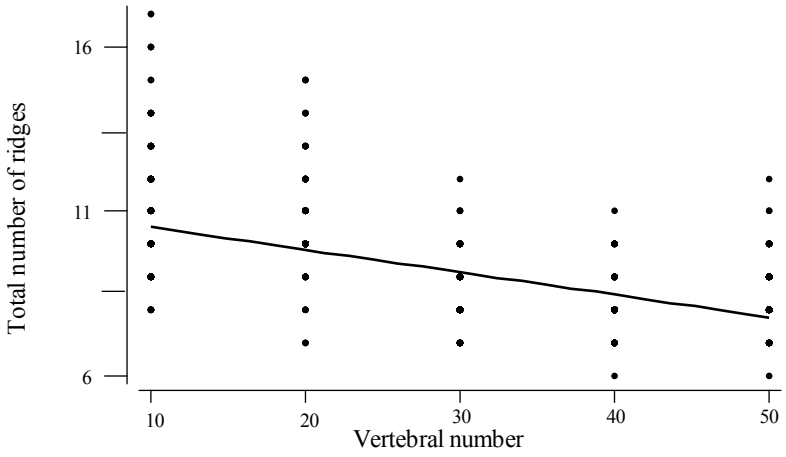


Figure 2.2: The change in number of ridges of *H. portusjacksoni* throughout the column. Total number of ridges = 11.2 – 0.07(vertebra number), r^2 = 39.8%. $F_{1,1098}$ = 726.0, p < 0.001.

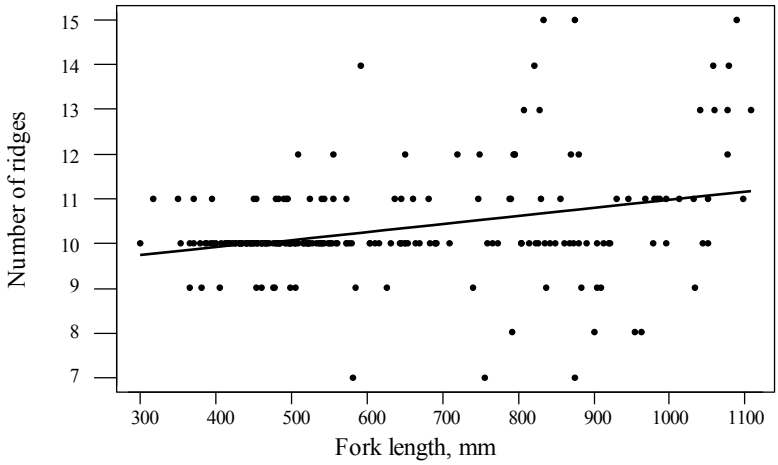
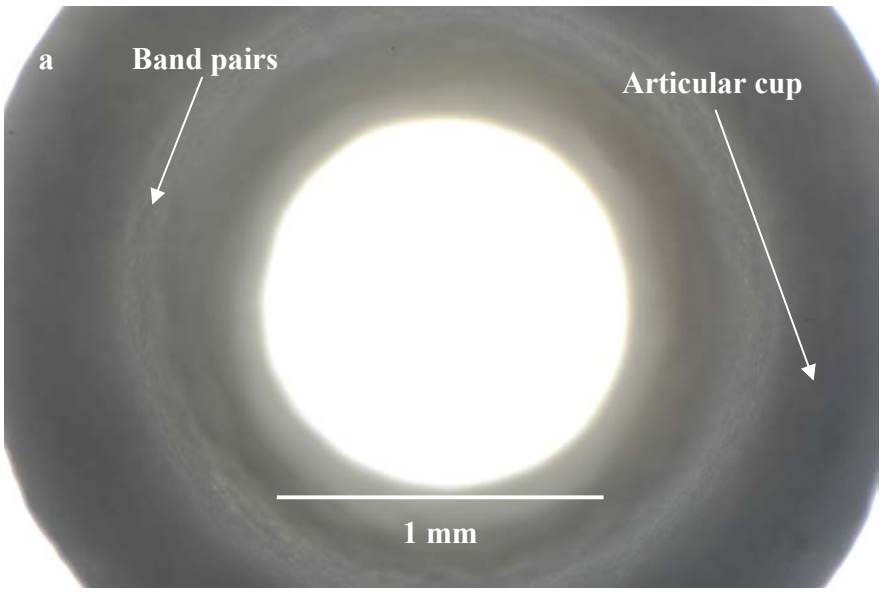


Figure 2.3: Relationship between number of ridge in the intermedialia of vertebra centra number 20 and *H. portusjacksoni* length. Total number of ridges = 9.2 + 0.008(F_L), r^2 = 9.9%. $F_{1,219}$ = 24.1, p < 0.001.



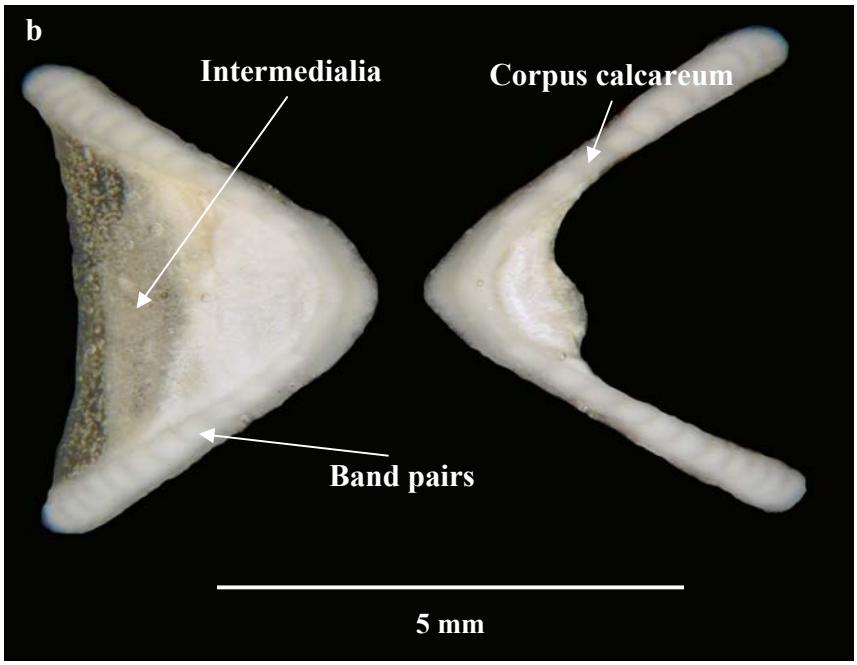


Plate 2.7: Anterior view of trunk vertebra number 20 from a 277 mm F_L female *H. portusjacksoni* showing the calcified articular cup with its lighter and darker band pairs (**a)**, and (**b**) lateral view of a longitudinal section of a vertebral centra from a 683 mm male *H. portusjacksoni* showing the intermedialia and corpus calcareum with its lighter and darker band pairs.

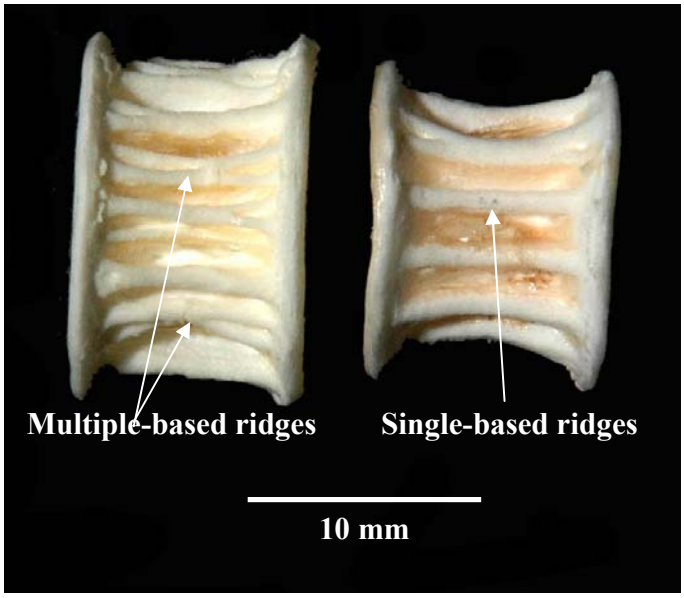


Plate 2.8: Dorsal view of two cleaned trunk vertebrae number (10 and 30) from an 1100 mm F_L female *H. portusjacksoni* showing multiple- and single-based ridges.

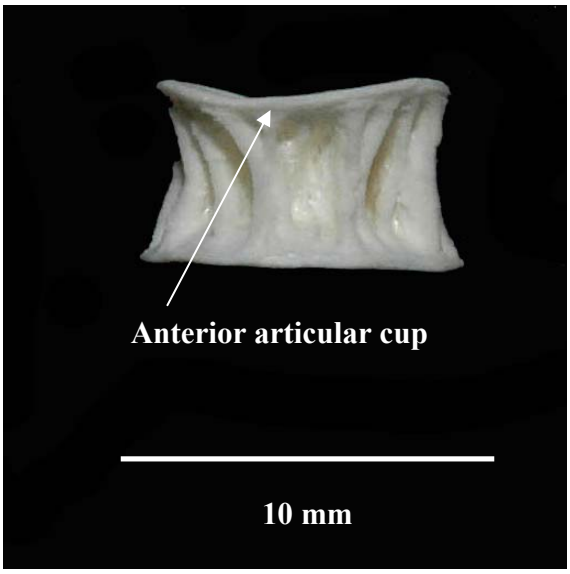


Plate 2.9: Dorsal view of centrum of vertebra number 2 from a 690 mm F_L female *H. portusjacksoni*, showing the difference in the shape and size of the anterior and posterior articular cups.

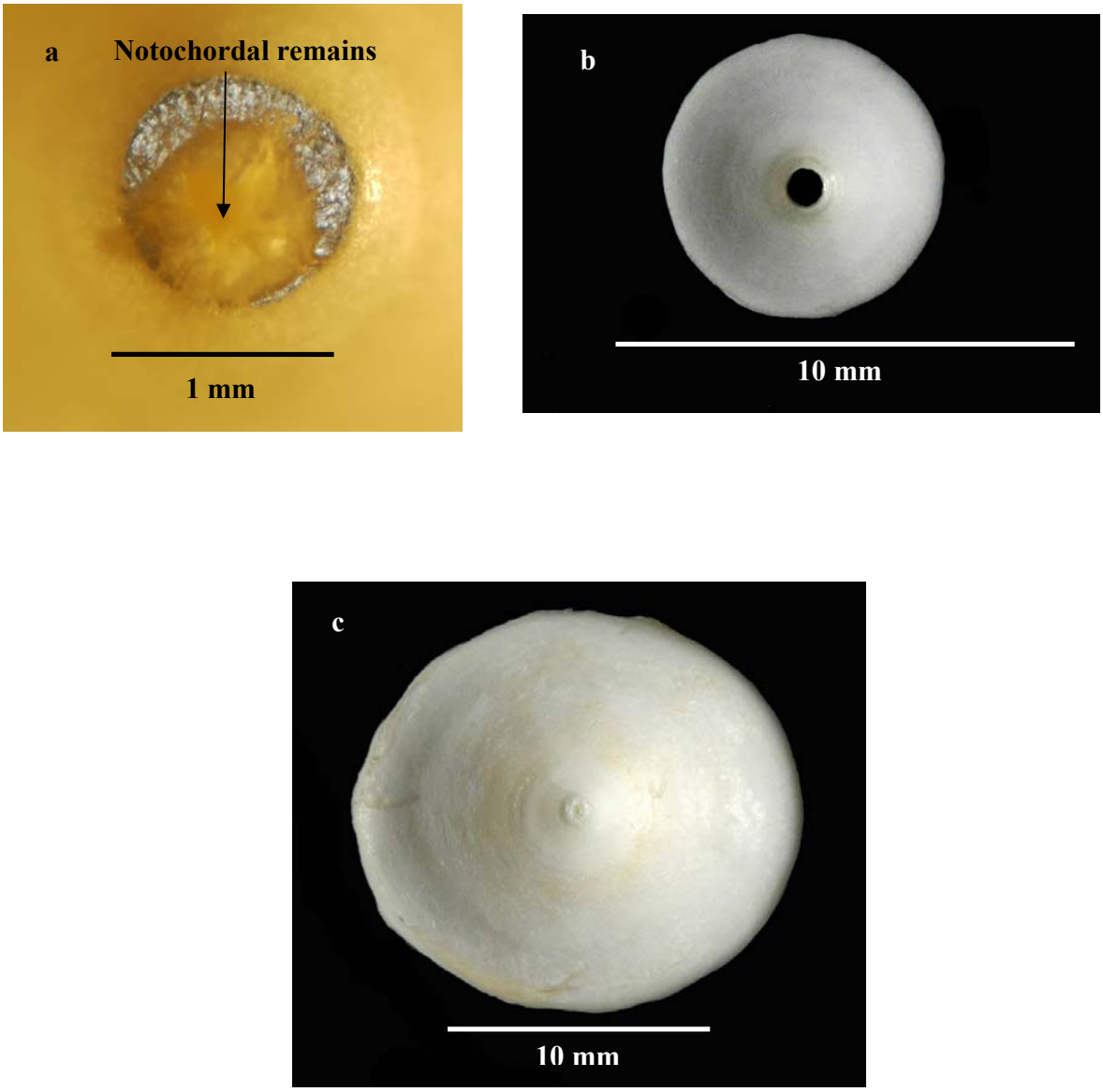


Plate 2.10: Anterior view of hollow centra trunk vertebra number 10 with notochordal remains (x 40) from a 498 mm F_L male *H. portusjacksoni* (**a**) hollow trunk vertebral centrum number 10 from a 580 mm F_L male *H. portusjacksoni* (**b**) and solid trunk vertebral centrum number 10 from an 1104 mm F_L female *H. portusjacksoni* (**c**).

2.3.1.3 Relationship to Size

Vertebral diameter (V_D) ranged from 2.40 to 16.58 mm (6.37 ±0.21, n = 302) females and 2.30 to 13.60 mm (6.53 ± 0.17, n = 321) for males. There is a strong relationship between the shark length and centrum diameter (Figure 2.4). The length and diameter of vertebral centra are significantly related (Figure 2.5). The diameter of the centrum also decreased from anterior to posterior in all *H. portusjacksoni* size range (Figure 2.6). With vertebra number 10 ranging from 2.66 to 16.70 mm (6.65 ±0.13 mm) and vertebra number 50 ranging from 2.32 to 11.4 mm (5.21 ±0.09 mm). The big variation in the values is because individuals from all size ranges were used.

The slopes of the relationships between vertebral diameter (V_D) and fork length (F_L) for female and male *H. portusjacksoni* were significantly different ($F_{1,619} = 41.17$, $p < 0.001$), and therefore data from the two sexes were separately analysed.

Both sexes showed a strong positive linear relationship between F_L and V_D.

Female: $V_D = - 1.28 + 0.0151\ F_L$ ($r^2 = 98.0\%$, $F_{1,300} = 14961.3$, $p < 0.001$)

Male: $V_D = - 0.834 + 0.014\ F_L$ ($r^2 = 97.6\%$, $F_{1,319} = 12933.9$, $p < 0.001$)

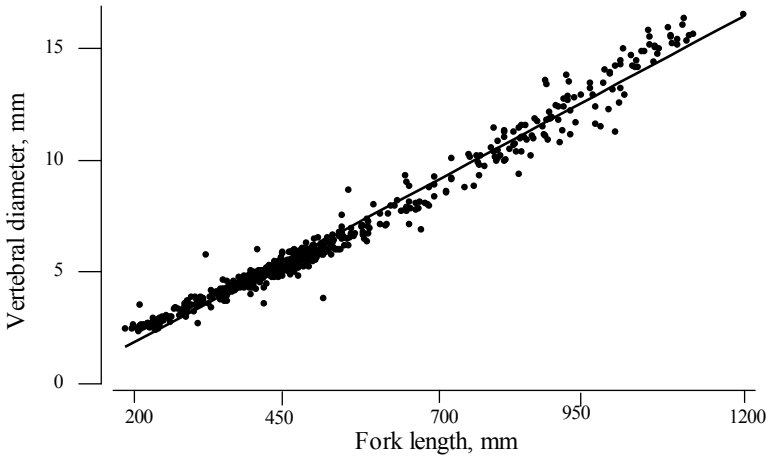


Figure 2.4: The relationship between fork length (F_L) of *H. portusjacksoni* and vertebral diameter (V_D) of vertebra centrum number 20. $V_D = -1.08 + 0.02(F_L)$, $r^2 = 97.7\%$. $F_{1,621} = 25995.6$, $p < 0.001$.

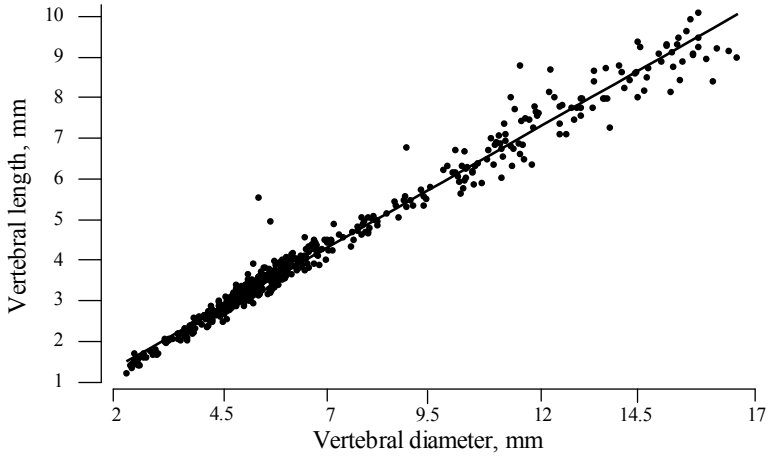


Figure 2.5: Vertebral centra number 20 shows that the vertebrae form *H. portusjacksoni* are uniform in shape throughout the size of the animal. $V_L = 0.12 + 0.6(V_D)$, $r^2 = 97.9\%$. $F_{1,529} = 24306.9$, $p < 0.001$.

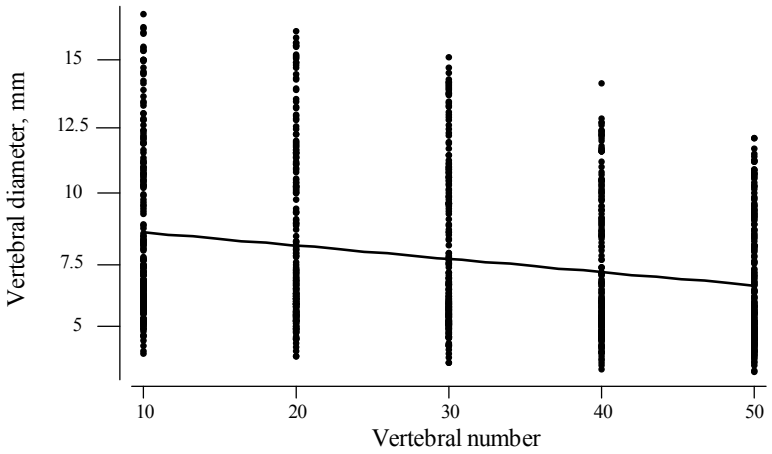


Figure 2.6: The change in centrum size from anterior to posterior in the vertebral column of *H. portusjacksoni*. Showing a decrease in vertebral centrum size from anterior to posterior. $V_L = 9.01 - 0.05$(vertebral number), $r^2 = 5.5\%$. $F_{1,1098} = 63.9$, $p < 0.001$.

2.3.2 Dorsal Spines

2.3.2.1 Length and Width

The length of the first dorsal spines (T_{SL}) ranged from 14.6 to 87.96 mm (47.5 ±16.66 mm, $n = 301$) for females and 17.8 to 80.54 mm (48.2 ±14.37 mm, $n = 319$) for males. The width of the of the first dorsal spine (S_{BW}) ranged from 2.90 to 16.82 mm (7.7 ±3.34 mm) for females and 2.56 to 15.46 mm (7.9 ±2.89 mm) for males. The length of the second dorsal spines (T_{SL}) ranged from 16.5 to 81.45 mm (43.7 ±14.41 mm, $n = 301$) for females and 16.5 to 76.1 mm (44.2 ±31.1 mm, $n = 319$) for males. The width of the of the second dorsal spine (S_{BW}) ranged from 2.22 to 13.5 mm (6.3 ±2.63 mm) for females and 2.26 to 12.36 mm (6.5 ±2.36 mm) for males. The two dorsal spines are identical in features. The

only difference is in the size of the two spines: the first dorsal spine is significantly longer (*T*-test =23.62, $p = 0.03$) but not wider ($T = 10.29$, $p = 0.062$) than the second dorsal spine.

2.3.2.2 Gross Anatomy

Each dorsal spine is positioned almost vertical and anterior to the each dorsal fin (Plate 2.11). Each spine is deeply inserted in the body of the shark, with the base positioned just dorsal to the vertebrae and connected to the neural arch with muscular tissue (Plate 2.12).

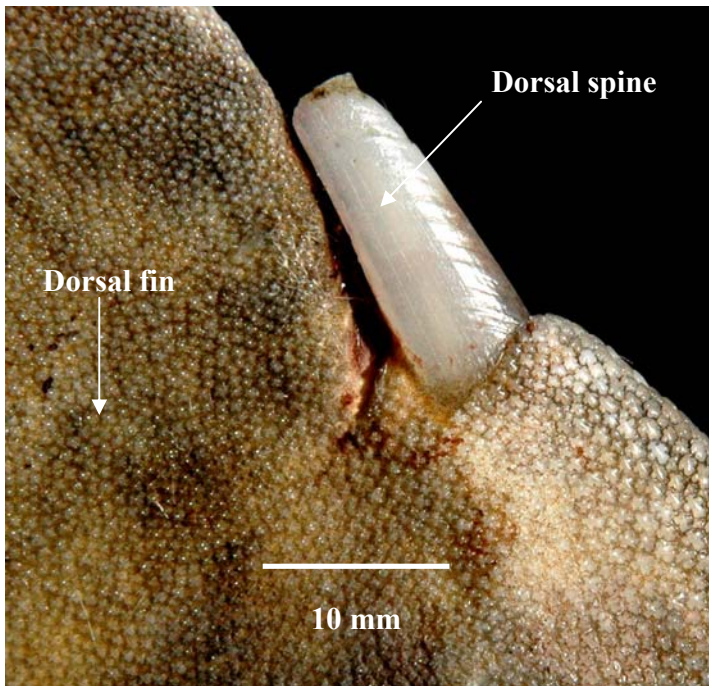


Plate 2.11: Lateral view of the position of second dorsal spine on a 968 mm F_L female *H. portusjacksoni.*

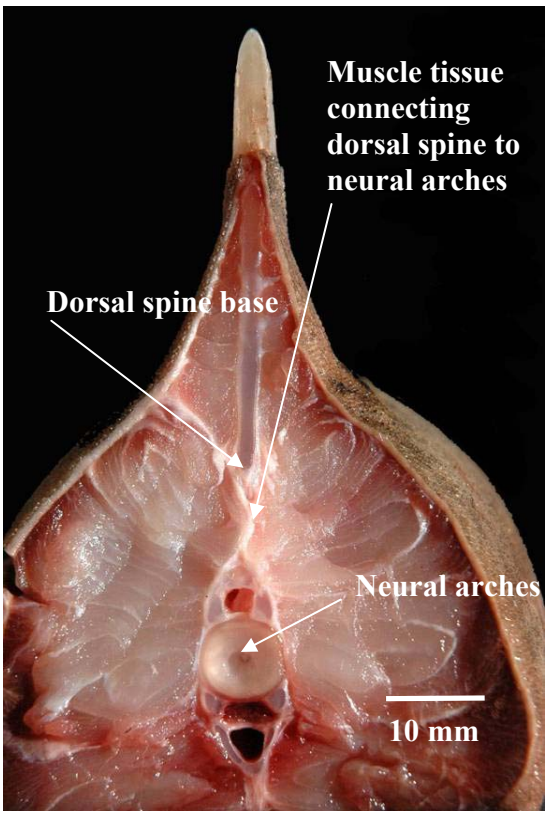


Plate 2.12: Posterior view of the internal position of the second dorsal spine from an 832 mm F_L male *H. portusjacksoni.*

The spine consists of a stem and the cap which slightly curves posteriorly (Plate 2.13). A non-calcified cartilaginous rod occurs inside the stem in the pulp cavity and is connected to the cartilage supporting the dorsal fin by muscle tissue.

The stem is long and of white/cream in colour and occupies most of the spine. The cap lies on top of the stem on the two anterior-lateral faces of the spine and is often darkly pigmented. It is often worn down to some degree in adults (35.6%), however is very sharp in juveniles (Plate 2.14). The whole stem and the lower part of the cap is inserted in the skin and only the upper part of the cap is visible.

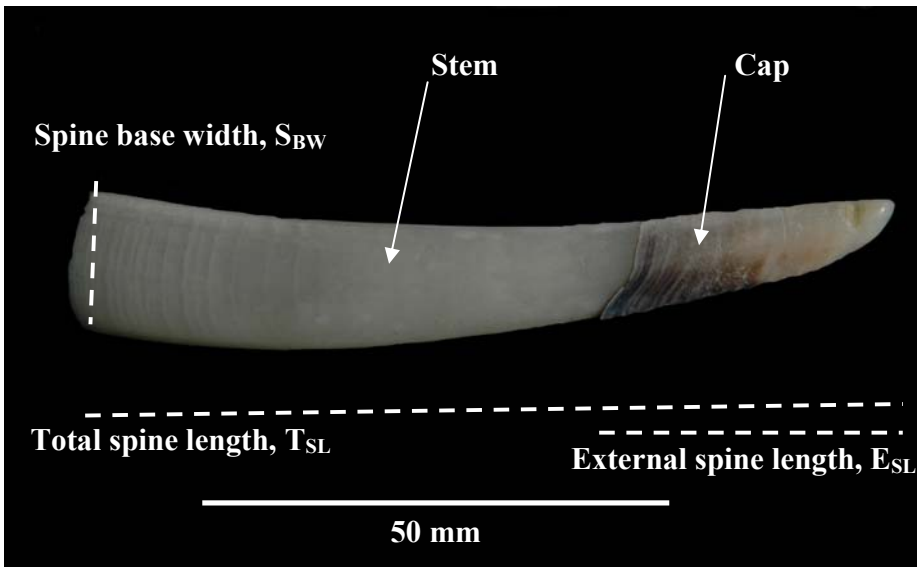
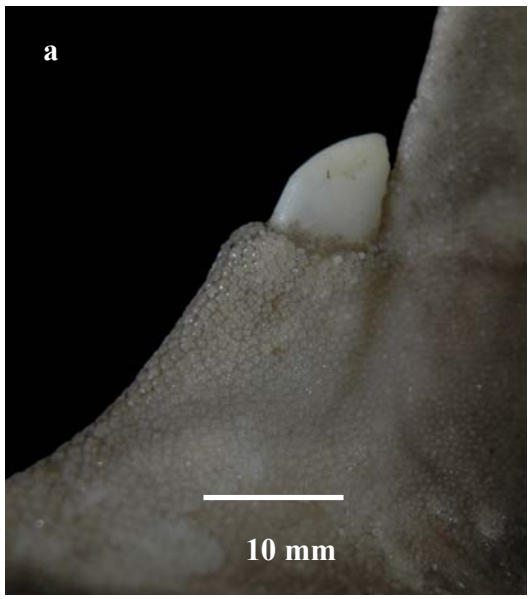


Plate 2.13: Lateral view of a cleaned first dorsal spine from a 1032 mm F_L female *H. portusjacksoni* showing the slightly backwards curling of the stem and cap with the different measurements.

The lower part of the stem is hollow and is referred to as the pulp cavity (Plate 2.15). This cavity is filled with a non-calcified cartilaginous rod which surrounds the base and posterior part of the dorsal spine and is connected to the cartilage of the dorsal fin. The lumen, the upper part of the stem (covered by the cap) is solid. The pigmented dentine layer (mantel) of the cap often extends below the lumen and continues down on the outside of the pulp cavity. The stem is triangular in cross-section with an almost oval posterior wall. It is posteriorly convex in adults but is straight in juveniles. Layers of horizontal light and dark base bands are visible on the stem (Plate 2.16). This suggests that the stem grows upwards and therefore deposits these horizontal base bands. Both the stem and the cap have several layers of dentine (Plate 2.17) which indicates diameter growth. The stem consists of two dentine layers which both shows band pairs (Chapter 4). The cap consists of outer enamel

and the thin sometimes pigmented mantel dentine layer which is penetrated by longitudinal vascular canals (mantel canals) (Plate 2.17). The cap also shows external horizontal band ridges (Plate 2.18).



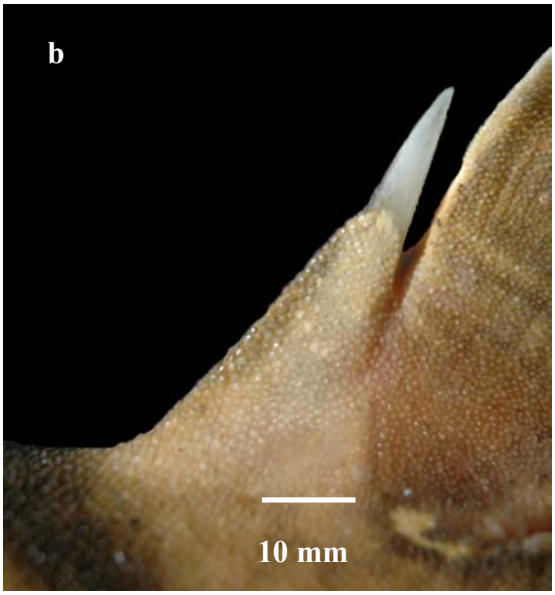


Plate 2.14: Lateral view of a worn first dorsal spine from an adult 853 mm F_L male *H. portusjacksoni* (**a**) and a sharp fist dorsal spine from a juvenile 528 mm F_L male *H. portusjacksoni* (**b**).

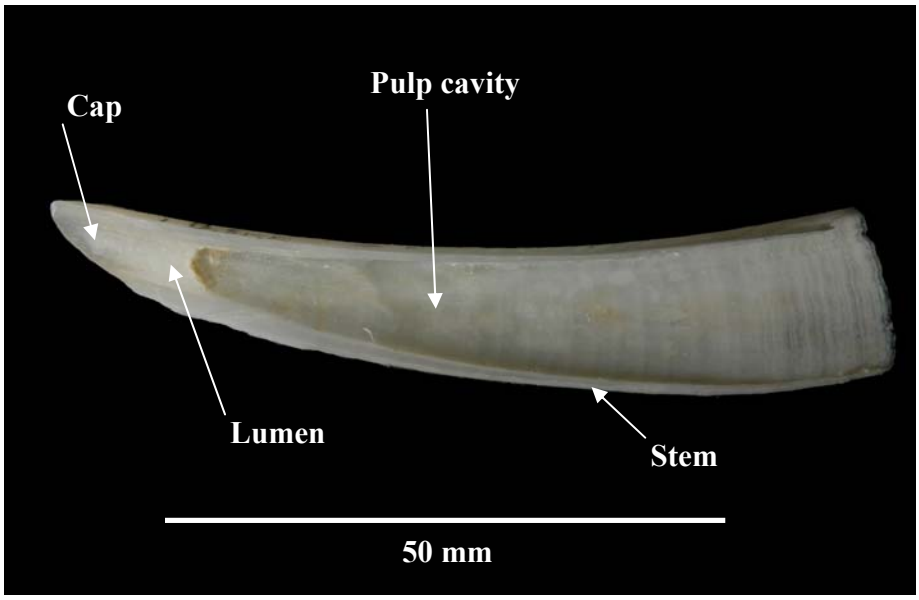


Plate 2.15: Lateral view of a transverse section of a first dorsal spine from a 1030 mm F_L female *H. portusjacksoni* showing the position of the non-calcified cartilage rod and the internal characters of the dorsal spine.

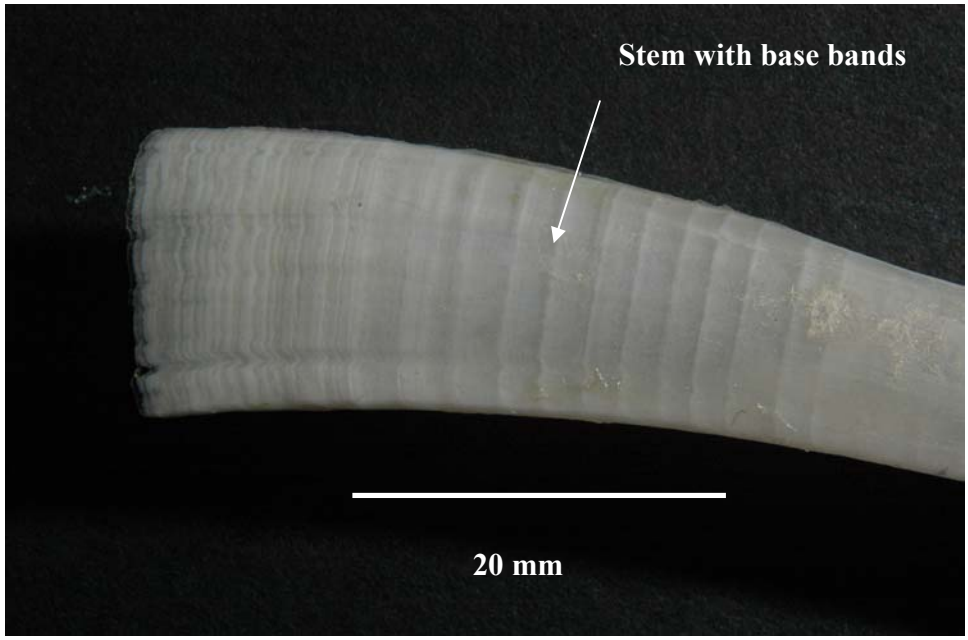


Plate 2.16: Lateral view of a first dorsal spine stem from a 1030 mm F_L female *H. portusjacksoni* showing base bands in the stem.

2.3.2.3 Relationship to Size

The spine base width of the first and second spines increased linearly with the body length (Figure 2.7). The dorsal spines also grow linearly, increasing in length when the diameter increases (Figure 2.8). However, the relationship becomes more variable with increasing size (e.g. beyond 9 mm S_{BW} for first and 7.7 m S_{BW} for second dorsal spines).

The slopes of the relationships between S_{BW} and F_L for female and male *H. portusjacksoni* were not significantly different (ANCOVA; $F_{1,617} = 0.14$, $p = 0.71$), therefore data from the two sexes were combined.

Both dorsal spines showed a strong positive linear relationship between S_{BW} and F_L.

First dorsal spine: $S_{BW} = 0.73 + 0.0137\ F_L$ ($r^2 = 95.4\%$, $F_{1,619} = 12797.87$, $p < 0.001$)

Second dorsal spine: $S_{BW} = 0.751 + 0.0109\ F_L$ ($r^2 = 95.6\%$, $F_{1,619} = 13358.45$, $p < 0.001$)

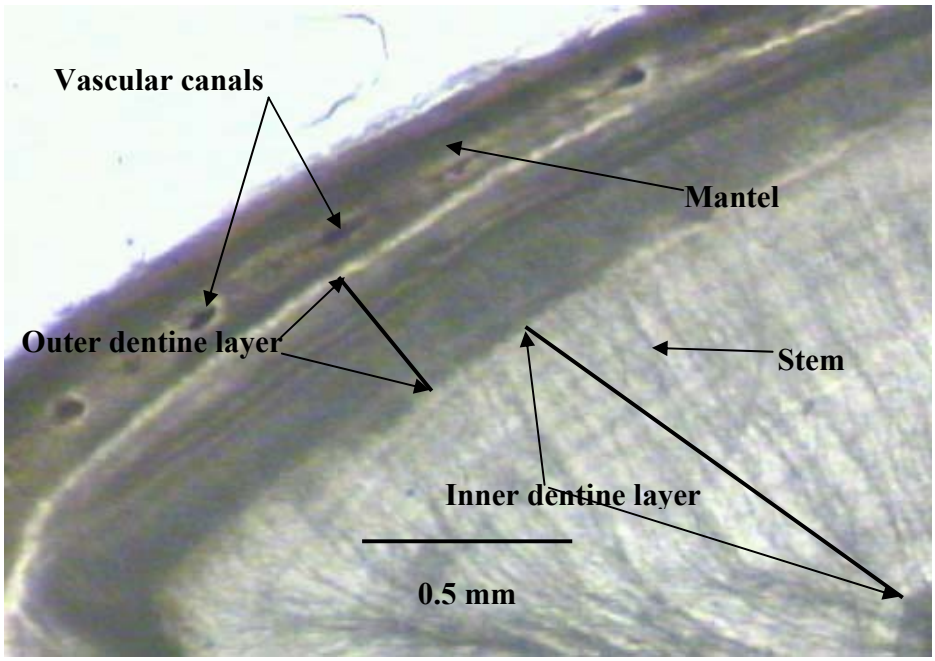


Plate 2.17: Transverse section of a second dorsal spine from a 1050 mm F_L female *H. portusjacksoni* showing the internal structures and layers.

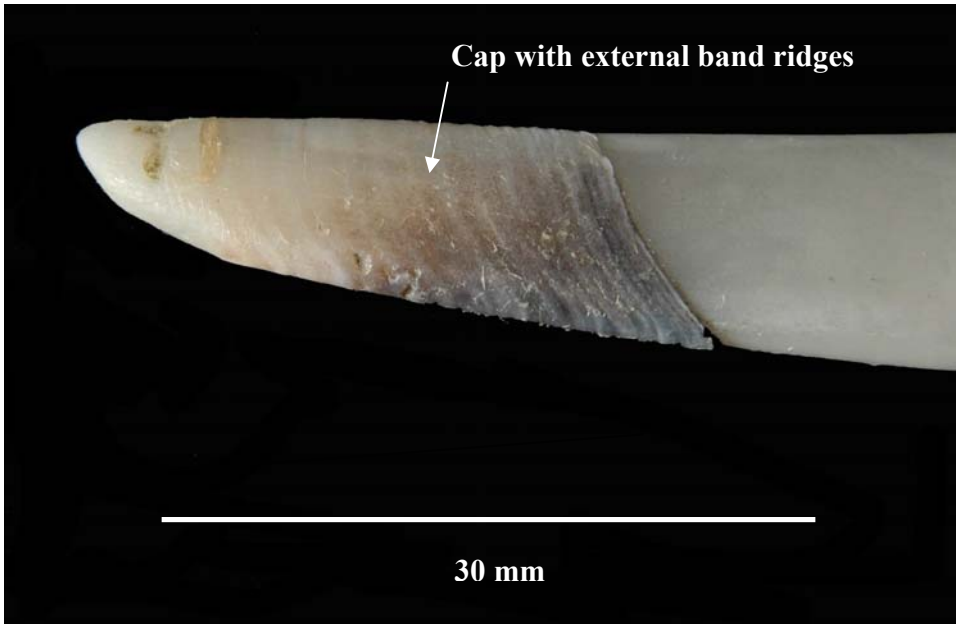


Plate 2.18: Lateral view of first dorsal spine cap with external bands from a 1032 mm F_L female *H. portusjacksoni.*

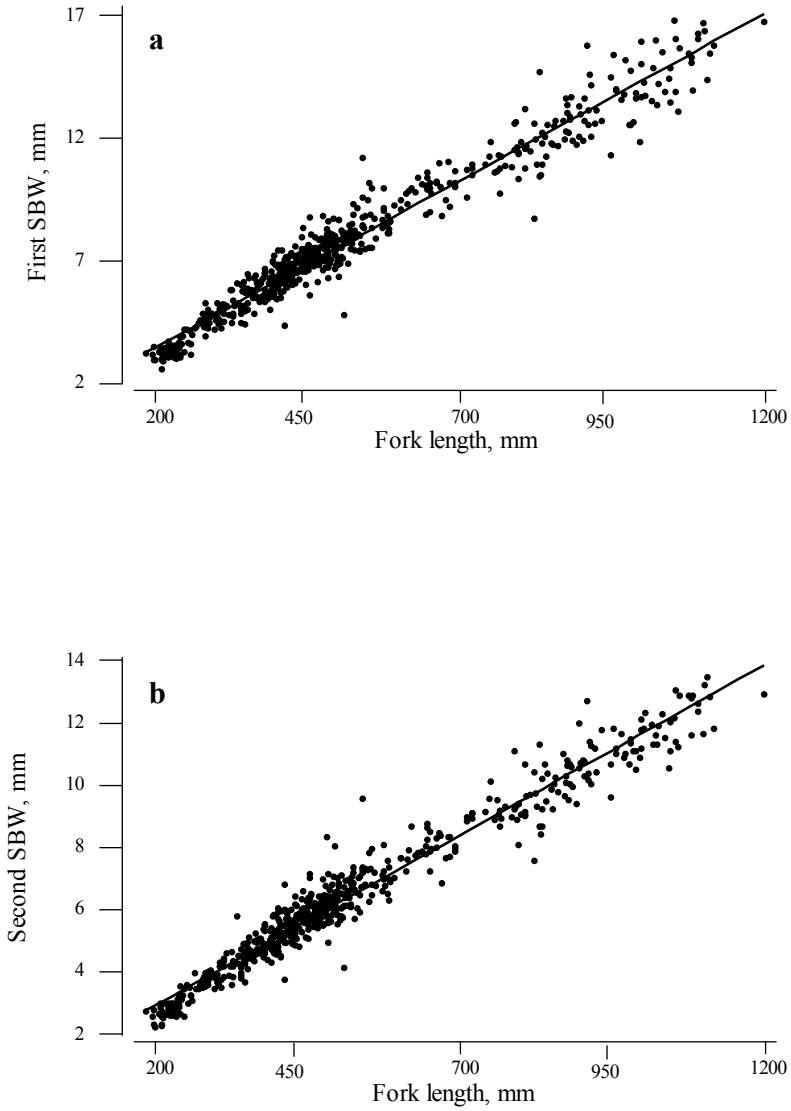


Figure 2.7: The relationship between fork length (F_L) and spine base width (S_{BW}) of *H. portusjacksoni* for the first dorsal spine (**a**) $S_{BW} = 0.73 + 0.01(F_L)$, $r^2 = 95.4\%$, ($F_{2,619} = 12797.9$, $p < 0.001$) and second dorsal spine (**b**) $S_{BW} = 0.75 + 0.01(F_L)$, $r^2 = 95.6\%$, ($F_{2,619} = 13358.5$, $p < 0.001$). ($n = 620$).

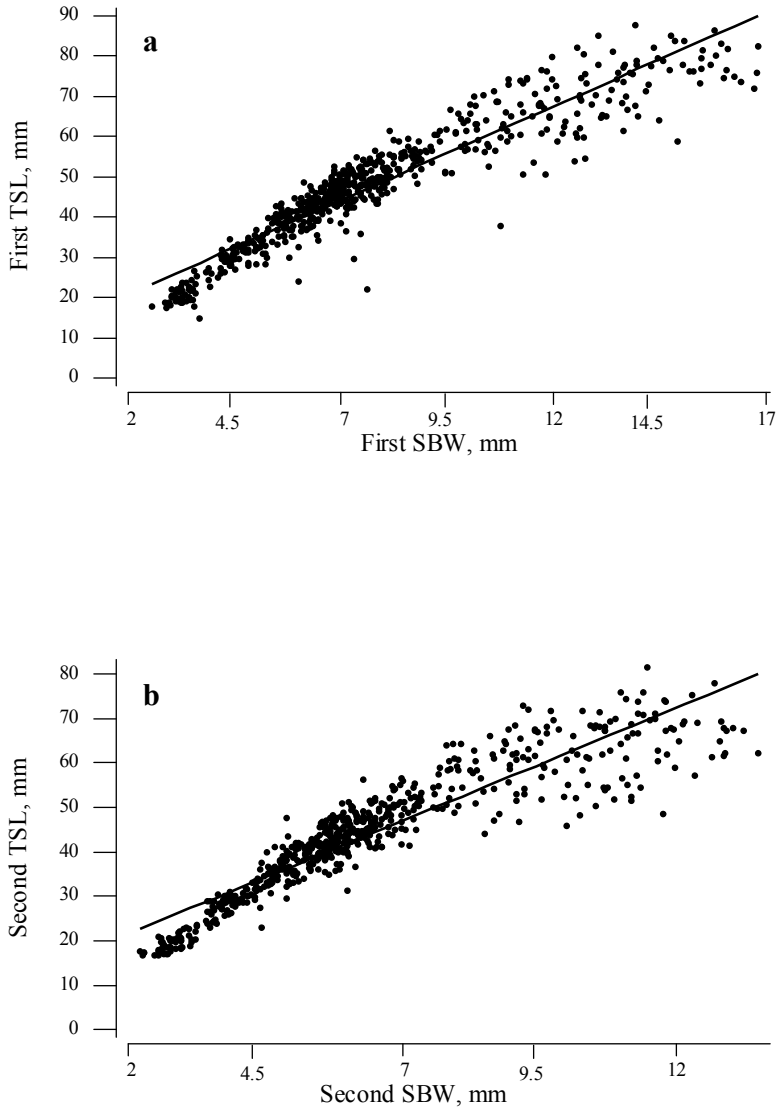


Figure 2.8: The relationship between spine base width (S_{BW}) and total spine length (T_{SL}) of *H. portusjacksoni* for the first dorsal spine (**a**) $T_{SL} = 11.48 + 4.67\ (S_{BW})$, $r^2 = 88.0\%$, ($F_{1,619} = 4543.6$, $p < 0.001$) and second dorsal spines (**b**) $T_{SL} = 11.48 + 5.07\ (S_{BW})$, $r^2 = 84.6\%$, ($F_{1,619} = 3413.4$, $p < 0.001$).

2.4 Discussion

2.4.1 Vertebral Centra

H. portusjacksoni vertebral centra tend to have the same features as most of the other shark species studied so far. Stark (1844) described the vertebrae of species of dogfish in the family Squaliformes. The two cup-shaped centra appear to be of similar appearance to *H. portusjacksoni*, however Stark (1844) described them with a "large rounded aperture" which was a feature in all centra. In this study, only smaller individuals of *H. portusjacksoni* had this feature. He also described the centra of being almost hexagonal in shape, which is not found in *H. portusjacksoni* centra. However they were almost perfectly circular in shape. The last feature that differs is the ridges between the two articular cups. He described the ridges as plates and that there are only six of them in each vertebrae (Stark, 1844). No other study has described the ridges between the two articular cups and there seems to be differences between Squaliformes and *H. portusjacksoni* in there number. Although Stark (1844) did not describe where in the vertebral column he did his examination, it appears that the vertebral centra between of Squaliformes and *H. portusjacksoni* are of similar appearance.

Rosenzweig (1988) described the anatomy of the *S. acanthias*. The skeletal system of this species, which was described as a representative species of sharks, resembles the vertebral centra of *H. portusjacksoni*. Although Rosenzweig (1988) did not describe some of the features in the clean centra however, focused on the vertebral column, the gross anatomy of the vertebrae of the two species appears to be very similar. The vertebrae of the Alopiidae, *Lamna nasus*, are also very similar in external appearance (Natanson *et al.*, 2002). Clement (1992) found blood vessels in only the mineralised parts of the vertebrae of *Prionace glauca* and *Squatina squatina angelus*. In this study, blood vessels were only

visible in the non-mineralised cartilage surrounding the ridges. Although many elasmobranchs differ in phylogeny and ecology the macroscopic anatomy of the vertebral centra shows many common features.

Francis and Maolagain (2000) described a change in vertebral length (from trunk to caudal vertebrae) near the pelvic fin, however did not indicate in which vertebral number this change occurred. Compagno (2001) reported that *H. portusjacksoni* had a total of 114 vertebrae. This study showed a more flexible number ranging from 109 to 128, with a mean of 116. Variation in total number of vertebrae was reported in the order Heterodontiformes ranging from 103 to 123 throughout the 9 species. This is relatively low compare to the order Lamniformes (mackerel sharks) and Orectolobiformes (carpet sharks) that have total vertebral count of 109 to 477 and 117 to 243, respectively (Compagno, 2001).

Diameter of vertebral centra of *H. portusjacksoni* increased as length increased. A positive relationship between vertebral centra and somatic growth has also been reported in other elasmobranchs species such as *Carcharhinus leucas, Carcharhinus porosus, L. nasus, Rhizoprionodon taylori, Mustelus canis, Carcharhinus falciformis, P. glauca* and *Carcharodon carcharias* (Stevens, 1975; Branstetter and Stiles, 1987; Simpfendorfer, 1993; Lessa and Santana, 1998; Wintner and Cliff, 1999; Conrath *et al.*, 2002; Natanson *et al.*, 2002; Oshitani *et al.*, 2003), where all successfully used vertebral centra to age the individuals. The existence of band pairs in the corpus calcareum on vertebral centra in elasmobranchs have been reported by many authors (Ketchen, 1975; Moulton *et al.*, 1992; Natanson *et al.*, 1999; Watson and Smale, 1999; Wintner and Cliff, 1999; Lessa *et al.*, 2004), with the few exceptions having dorsal spines (Clarke *et al.*, 2002a; Braccini, 2006; Irvine *et al.*, 2006a). This suggests that vertebral centra are potentially useful as an ageing structure.

2.4.2 Dorsal Spines

Dorsal spines of *H. portusjacksoni* had similar macro anatomy as most other shark species studied so far. Maisey (1979) described in detail the morphogenesis of the dorsal spine in the squalid and heterodontid sharks. He reported that the dorsal spines of the heterodonts and squalid sharks were very similar in macroscopic anatomy. Irvine *et al.* (2006a) reported that the first dorsal spine was more than twice the size of then that of the second dorsal spine in *Etmopterus baxteri*, which is a larger difference that found in this study. Beamish and McFarlane (1985) described the second dorsal spine of *S. acanthias*. All features described in *S. acanthias* were very similar to the features found in *H. portusjacksoni* in this study. The only feature not described was the slight posterior curvature of the spines in adults which was found in this study. This posterior curvature was described as a general feature in most Squalidae, however dorsal spines of Heterodontidae were described as being almost vertical (Clarke and Irvine, 2006). Clarke *et al.* (2002b) described the dorsal spine of *Deania calceus*. Their description also matched what was found in *H. portusjacksoni*. Ketchen (1975) and Tanaka (1990) described the structure of the dorsal spine in *S. acanthias* and *C. acus*, respectively. Both species showed wearing of spines similar to *H. portusjacksoni*. Braccini *et al.,* (2007) reported 11% of *Squalus megalops* had worn spines. This was a much lower number than observed in *H. portusjacksoni* (35.6%), although might be explained by the different behaviour in the two species. Adult *H. portusjacksoni* was often found under rocks and crevices that would contribute to the wear of the dorsal spines. In contrast the dogfish lives on open sandy substrates (personal observation).

Width of the dorsal spine base of *H. portusjacksoni* increased as length increased. A positive relationship between dorsal spines and somatic growth has also been reported in other elasmobranchs species such as *S. acanthias, Centrophorus acus, C. squamosus, C. crepidater, E. baxteri, D. calceus* and *S. megalops* (Ketchen, 1975; Beamish and McFarlane, 1985; Tanaka, 1990; Clarke *et al.*, 2002a; Clarke *et al.*, 2002b; Irvine *et al.*, 2006a; Irvine *et al.*, 2006b; Braccini *et al.*, 2007). This suggests that dorsal spines are potentially useful as an ageing structure.

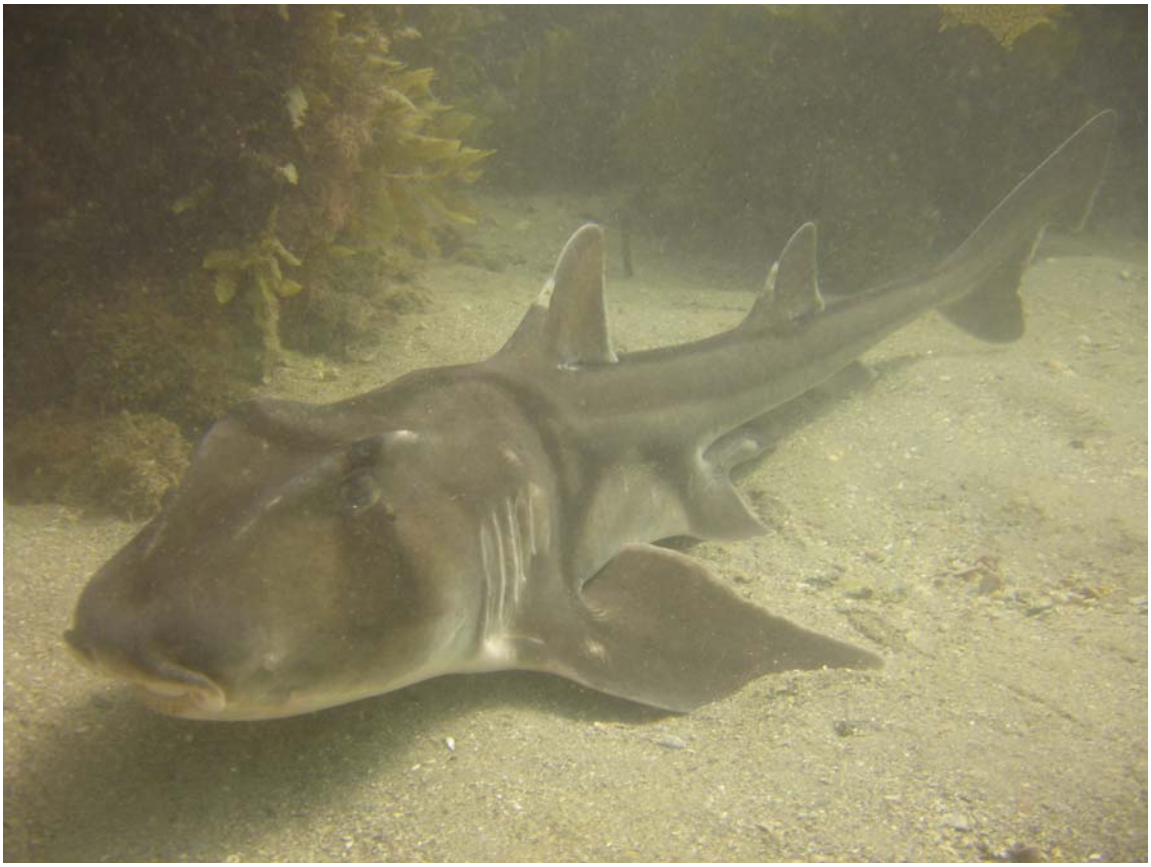
Base bands were visualised on the stem of the dorsal spines of *H. portusjacksoni*. However, these bands was not used for age estimation because of the difficulties and time consuming process of removing the cap to visualise the early bands (Irvine *et al.*, 2006b). According to Tanaka (1990), no bands were found on the surface on both the first and second dorsal spines in *C. acus*. Irvine *et al.* (2006a; 2006b) however, reported base bands on the dorsal spine stem of *Centroselachus crepidater* as a method to estimate age the species which might also apply to *H. portusjacksoni*.

2.5 Conclusion

Their macroscopic anatomy of *H. portusjacksoni* appears to be very similar to the vertebral centra and dorsal spines described for other elasmobranch species. The structures had anatomical characteristics indicating that they could be used for age estimation with both vertebral centra and dorsal spines growing throughout the life of the animal.

Band pairs could be visualised in the surface of the articular cups of whole vertebral centra and in the corpus calcareum of sectioned vertebral centra, ass well on the surface of the dorsal spine cap and in the dentine layers of sectioned dorsal spines. It was therefore established that both these structures could be used for age estimation.

Chapter 3



A male Port Jackson shark resting on a sandy bottom in Jervis Bay (photo by the author)

Chapter 3

The Use of Vertebrae for Age Estimating the Port Jackson Shark (*Heterodontus portusjacksoni*).

3.1 Introduction

Using vertebral structures to estimate the age of shark species has been successful for 51 shark species, including Carcharhiniformes such as *Galeocerdo cuvier* (Natanson *et al.*, 1999), *Prionance glauca* (Lessa *et al.*, 2004) and *Mustelus antarcticus* (Moulton *et al.*, 1992), Lamniformes such as *Carcharodon carcharias* (Wintner and Cliff, 1999), and Squaliformes including *Squalus acanthias* (Ketchen, 1975) and *Squalus megalops* (Watson and Smale, 1999) (see Appendix A for more species). Although it is one of the few techniques developed to estimate the age of elasmobranchs, vertebral ageing is subjected to many sources of variation including sampling bias, size, preparation and ageing techniques. There are several methods to quantify and limit the sources of variation including a large sample size within all age-classes and accuracy which can be investigated using bias pots and Coefficient of Variation (CV) (Chang, 1982; Campana *et al.*, 1995). However, without validation the direction of bias and precision is unknown (Beamish and McFarlane, 1983).

Validated studies are gaining more weight and are now an important technique applied to most elasmobranch age estimations. Davenport and Stevens (1998) used tetracycline injections in wild *Carcharhinus tilstoni* and *Carcharhinus sorrah* to validate the formation of annual band pairs. Branstetter and Musick (1994) suggested that *Carcharhinus taurus* formed two annual band pairs in the vertebral centra. However, further research using chemical injections concluded that only one band pair was

incorporated in the vertebral centra and that the differences might be due to the different ageing techniques used between the two studies (Goldman *et al.*, 2006). Similar results was found for the Alopiidae, *Isogomphodon oxyrinchus*, which was thought to deposit band pairs twice a year (Pratt and Casey, 1983). However, studies using bomb carbon techniques and chemical markers have confirmed that also this species deposits annual band pairs (Campana *et al.*, 2002b; Natanson *et al.*, 2006). However, one species (*Squatina californica*) does not incorporate annual band pairs, but seems to have a relationship between growth and band pair deposits (Natanson and Cailliet, 1990). So although it seems to be a trend in elasmobranchs to deposit annual band pairs, the need to validate each species still exists. Although becoming less frequent, one still finds age studies that either lack validation or have only used Marginal Increment Analysis (MIA) as a validation technique (Rossouw, 1984; Sminkey and Musick, 1995; Lessa and Santana, 1998; Carlson *et al.*, 2003; Oshitani *et al.*, 2003; Izzo, 2005). As reported by Lessa *et al.* (2006), MIA can give high biases if the sampling technique and life history is not properly developed, and should be considered a supplementary analysis to injected chemical tags (Cailliet, 1990; Cailliet and Goldman, 2004).

Position of vertebral centra selected for age estimation is often related to the size of the vertebral centra. The larger the vertebral size, the more space between band pairs and therefore easier to distinguish between band pairs (Officer *et al.*, 1996). The largest vertebral centra and is often found in the anterior region of the first dorsal fin in the vertebral column (Francis and Maolagain, 2000). Natanson and Cailliet (1990) tested whether the number of band pairs differed throughout the vertebral column *S. californica* from all size-classes. They concluded that vertebra number 12-14 gave the highest band pair counts and was therefore used for ageing. Similar results were reported of *M.*

antarcticus and *Galeorhinus galeus* where the thoracic region of the vertebral column gave higher band pair counts than both cervical and pre-caudal regions (Officer *et al.*, 1996). However, this was not the trend for *I. oxyrinchus*. Natanson *et al.* (2006) found that the difference between vertebrae was never more than one band pair and therefore concluded that that all regions in the vertebral column could be used for age estimation.

There are two well defined methods used to estimate the age of elasmobranchs. One of those methods is the "older" using whole vertebral centra (Rossouw, 1984; Carlson and Parson, 1997; Davenport and Stevens, 1998). Band pairs are counted under reflected light and used for age estimation (Stevens, 1975). The other "newer" method uses thinly sectioned vertebral centra (Branstetter and Stiles, 1987; Sminkey and Musick, 1995; Lessa and Santana, 1998; Natanson *et al.*, 2002; Piercy *et al.*, 2007). Band pairs are visible on the "bow-tie" section in the intermedialia and corpus calcareum, however due to the often lesser calcified intermedialia region, age estimation often uses only the band pairs visible in the corpus calcareum (Goldman, 2004). Although both methods are well developed, few have investigated which is more accurate to estimate the age of elasmobranchs. MacNeil and Campana (2002) compared counts from whole and sectioned vertebrae from *P. glauca* and reported no difference in band pair counts between the two methods. Similar results have been reported for *M. antarcticus* and *G. galeus* (Moulton *et al.*, 1992). However, Musick and Bonfil (2004) recommended using sectioned vertebral centra when estimating ages of elasmobranchs because of the tendency of tightly grouped band pairs in older individuals.

There are no published studies on the use of vertebral centra for age estimation on the New South Wales population of *Heterodontus portusjacksoni*. Tovar-Avila (2006) reported differences in length-frequency composition, mass-length relationship and length-

at-maturity among *H. portusjacksoni* collected from Victorian waters. The reported age range for females were between 3 and 35 years and were between 2 and 28 years for males using dorsal spines (Tovar-Avila, 2006). His research suggests that their might be differences in population structures and therefore also age and growth compositions might differ. It is therefore important to estimate the age for the New South Wales population to determine any geographical differences in the species.

Although age estimation studies have been applied to many elasmobranch species, the different life histories and techniques used to gain this knowledge are often species-specific. Therefore the aim of this study was to investigate the use of vertebrae (whole and sectioned) to estimate the age *H. portusjacksoni*. The specific objectives for each method were to (1) confirm the presence of band pairs, (2) validate the annual formation of band pairs, (3) determine whether position on the vertebral column influences band pairs, and (4) determine which method is most suitable for ageing.

3.2 Materials and Methods

3.2.1 Sampling

H. portusjacksoni were sampled from the by-catch of commercial prawn and fish trawlers operating from Newcastle, Australia (32° 55′05″*S*, 151°45′37″*E*) between September 2003 and June 2006 (Plate 3.1) when only adults where compared. In July 2004, samples were taken from trap fishing located on the Central Coast, Australia (33°26′50″*S*, 151°26′58″*E*). Samples were taken from all months of the year (except January where no sharks were captured), although the catch-frequency was highest in the winter months of July to October (Figure 3.1). A total of 1580 sharks were sampled from a depth range of 7-100.6 m (mean = 35.8 m). Slightly more males were captured (Chi-Square Test = 1.01), with a

female to male ratio of 0.95:1. Sampling occurred two to four times a month depending on weather conditions. A representative range of sizes from each catch were retained. In Newcastle, where the majority of the samples were taken from, trawls were operating from 32°47′596″*S*, 152°04′307″*E* to 33°10′228″*S*, 151°43′892″*E*. Mesh size for prawn trawls were 45 to 50 mm and 90 to 150 mm for fish trawls. From September 2003 to November 2003 only one prawn trawler was used for the sampling. After July 2004 only one fish trawler, *Avalon Star*, was used for the sampling.

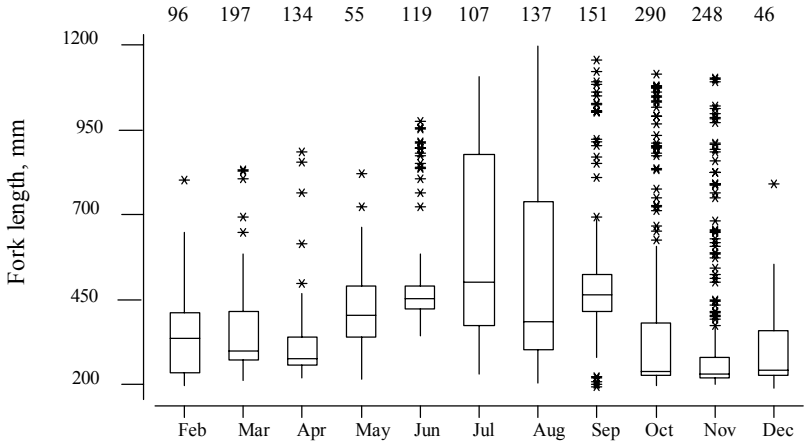
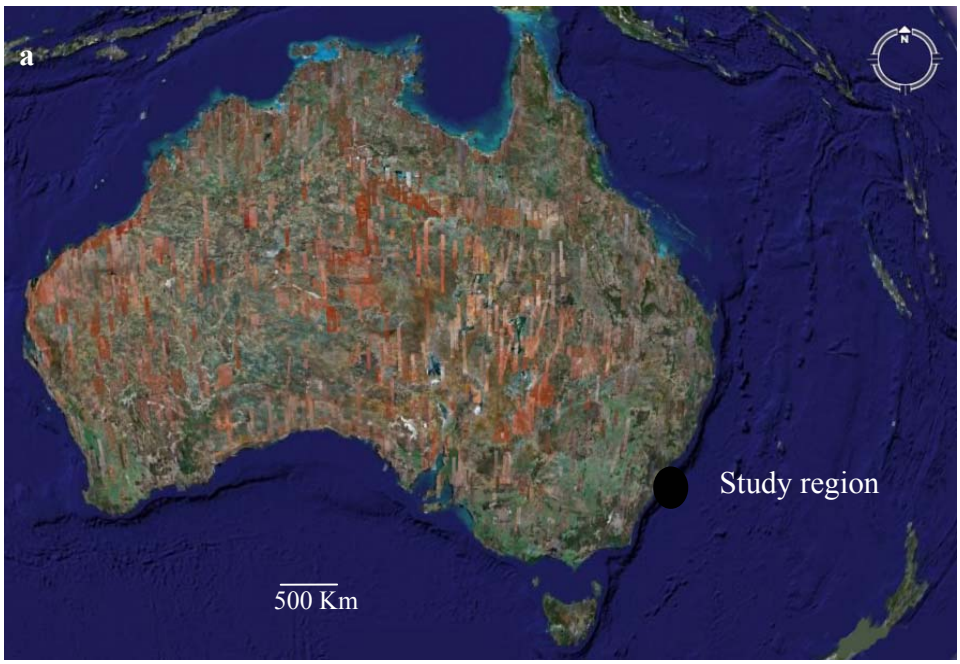


Figure 3.1: The size of each monthly catch in fork length (F_L) with bars showing median and 95% confidence interval with outliers. Total numbers of individuals caught each month are also shown. No samples collected in January.

All sharks were measured and sexed. Animals not being used for further analysis were fin-clipped, to avoid re-measurements, and released. Sharks that were kept for future analysis and age estimation were weighted (T_W) (n = 652). Complete details of the sampling design are described in Chapter 2 of this thesis.

3.2.2 Preparation of Vertebrae

Ten vertebrae were removed, cleaned in a 6% sodium hypochlorite solution and stored in specimen containers. Vertebral centra were selected from 5 parts of the vertebral column. Vertebrae 10 from the region anterior to the first dorsal fin (Natanson *et al.*, 2002); vertebrae 20 from just below the first dorsal fin (Lessa and Santana, 1998; Conrath *et al.*, 2002); vertebrae 30 from the region between the two dorsal fins (Stevens, 1975; Officer *et al.*, 1997); vertebrae 40 from just below the second dorsal fin; and vertebrae 50 from posterior to the second dorsal fin. Vertebrae were embedded in epoxy resin (Struers Epofix, Copenhagen, Denmark) under vacuum for 5 min. Longitudinal sections (~ 400 µm) were cut using a low speed saw (Accutom Struers, Copenhagen, Denmark) equipped with a diamond edge wafering blade (Pace Technologies, Arizona, USA). Sections were glued to glass slides and sealed with cover slips. Vertebrae 9, 19, 29, 39 and 49 were not sectioned and were stored dry in specimen jars for whole examination. Vertebral diameter (V_D) was measured between the two articular cups and vertebral length (V_L) was measured over one articular cup (Plate 2.1). These measurements were plotted against F_L to determine if vertebral growth was proportional to somatic growth and could therefore be used for age estimation. Complete details of the vertebral preparation are described in section 2.2 of this thesis.



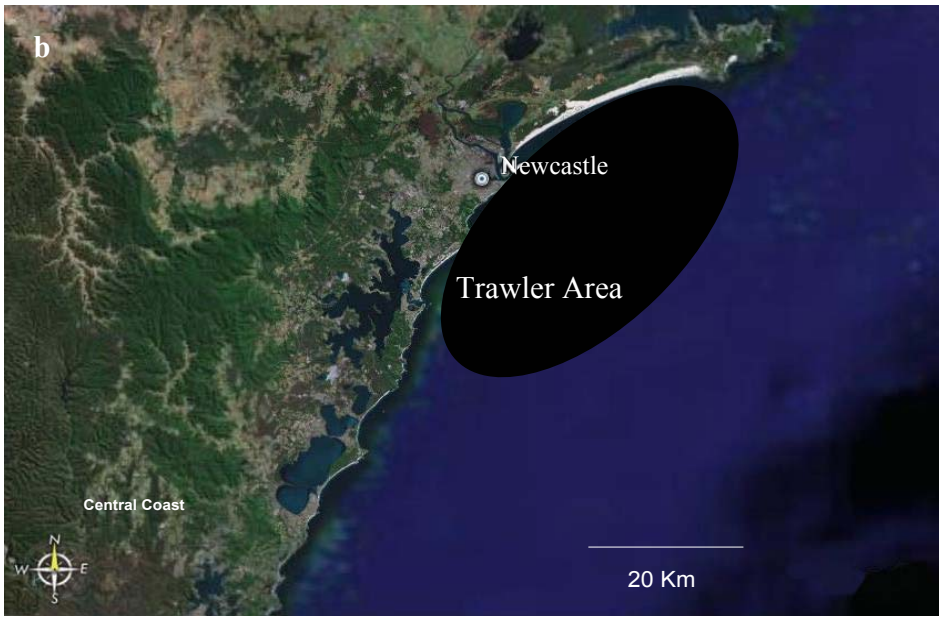


Plate 3.1: Map (**a**) indicating the study region on the Australian map and map (**b**) showing the main trawling area outside Newcastle. Maps of collection areas reproduced from Google Earth, 2007.

Staining techniques were applied to enhance the appearance of band pairs in sectioned and whole vertebrae. Sectioned and whole vertebra were stained with alizarin red S (La Marca, 1966; Gruber and Stout, 1983) and crystal violet stains (Johnson, 1979; Schwartz, 1983) between 5 minutes and 72 hours, and 20 minutes and 24 hours, respectively. Neither staining technique enhanced any band pairs in the whole or sectioned vertebrae and therefore unstained vertebral centra were used throughout the study.

3.2.3 Validation

3.2.3.1 Sampling

Live *H. portusjacksoni* were collected from Nelson Bay in the north to Jervis Bay in the south (Plate 3.2). Two juvenile sharks were caught by trap fishing from Nelson Bay (32°43′05″*S*, 152°08′42″*E*) in May 2004. From July to September 2004, 19 adult sharks were collected using SCUBA at Fairlight (33°48′02″*S*, 151°16′32″*E*). Between September 2004 and September 2005 10 juvenile sharks were collected from commercial trawl fishing operating from Newcastle (32°55′05″*S*, 151°45′37″*E*). In March 2006 4 egg capsules with year-0 neonates were collected from Jervis Bay (35°02′24″*S*, 150°40′33″*E*). All 35 sharks were housed in Oceanworld Manly Aquarium facilities.

3.2.3.2 Maintenance

All sharks were tagged, weighed and measured upon arrival at Oceanworld Manly Aquarium. Neonates and juveniles up to 500 mm F_L were kept in separate 300 L tanks with open system flow, while adults were kept in the 5.5 million L shark tank. Natural water temperature and light conditions were maintained to mimic the external environment.

Plate 3.2: Map over validation collection area from Jervis Bay in south to Nelson Bay in north (reproduced from Google Earth, 2006).

Neonate and juvenile sharks were weighed to the nearest 1 g using a digital scale (Chyo, MS 3300c). Adult sharks were weighed to the nearest 100 g using an analogue scale (Wedderburn, SA2356S), while being held in a mesh sling. Neonates and juveniles were tagged with t-bar tags and adults with cattle-ear tags (Roto-tags), inserted in the uncalcified part of the base of the first dorsal fin anterior to the dorsal spine. The open wound was treated with iodine after tagging and all wounds healed after three weeks. Sharks were classified as neonate if they had a visible umbilical scar. Juveniles were classified as not having an umbilical scar and were between 210 and 500 mm F_L. Adults were classified as 500 mm F_L or greater.

Adult sharks were hand-fed by divers at Oceanworld Manly Aquarium on each Monday, Wednesday and Friday. On each Tuesday, Thursday and Saturday they were fed by surface dropping. On Sundays there was no feeding. All adults were fed a mixture of large mussels, pilchards (Clupeidae), prawns (Penaeidae), blue-nose whiting (*Sillago ciliata*), squid (Loliginidae), and yellowtail (*Trachurus novaezelandiae*). Juveniles were hand-fed daily except Sundays. All juveniles were fed a mixture of pilchards (Clupeidae), prawns (Penaeidae), common shore crab (*Carcinus maeneas*), squid (Loliginidae) and pilchards (Clupeidae). All sharks were cared for by Oceanworld Manly Aquarium staff.

3.2.3.3 Chemical Markers

In the first year of captivity the length and weight of every shark was measured every three months and every six months thereafter. Intramuscular injections of oxytetracycline (OTC) (Walker *et al.*, 2001; Smith *et al.*, 2003) and calcein (Gelsleichter *et al.*, 1997) were used to validate the growth of bands in captive *H. portusjacksoni.* OTC was used for its popularity and calcein for its ability not to fade under light. After one week in quarantine 31 sharks (neonates, juveniles and adults) were given intramuscular injections at the base of the first dorsal spine, of a isotonic elasmobranch saline (0.9%) solution dissolved with 25 mg/kg OTC (Sigma-Aldrich) as recommended by McFarlane and Beamish (1987a). Each maturity stage was given its own dosage, with 5 neonates given a dosage of 1mg/10mL, 7 juveniles were given a dosage of 1mg/50mL, and 19 adults were given a dosage of 1mg/100mL. This minimised the amount injected in each individual. After three months at liberty, 26 of these sharks were given a second intramuscular injections at the base of the first dorsal spine with a solution of isotonic elasmobranch saline (0.9%) and 5mg/kg calcein (Sigma-Aldrich) as recommended by Gelsleichter *et al.* (1997). The calcein injections were given for each

maturity stage with the dosage 1mg/10mL and 1mg/25mL in 7 juvenile and 19 adults, respectively. The injection of OTC was repeated six months after the first injection on 3 juveniles and 19 adults. To find the birth mark, 4 egg capsules (2 males and 2 females) were collected and immersed in a 250 mL/L calcein solution for 8, 16, 24 and 30 hours at time of birth (Table 3.1).

Sharks were kept captive for a maximum of 25 months before euthanased by a standard procedure (The American Veterinary Medical Association, 2001). Individual sharks were immersed in a bath containing 250 mg/L benzocaine hydrochloride for at least 10 minutes after opercular movements cease or deceased. Sharks were stored at -20˚C until dissection. The vertebrae were removed under dimmed light since OTC is a light sensitive chemical. Vertebrae were then cleaned and stored in black containers. Vertebrae were embedded and sectioned as previously described (section 2.2 of this thesis). A dissecting microscope (Leica Mz 12) equipped with a digital camera (Axiocam HRC, Zeiss), UV filter (360 nm) and blue filter (470 nm), were used to visualise the fluorescent injection in the vertebral centra. To determine the periodicity of band pair formation a straight line was fitted to test the hypothesis that band pairs are deposited annually. The number of completed band pairs after the initial post-OTC injected date were counted and the slope of the regression between the number of post-OTC band pairs and time at liberty was calculated (Simpfendorfer *et al.*, 2002b).

3.2.3.4 Marginal Increment Analysis and Centrum Edge Analysis

Marginal Increment Analysis (MIA) (Natanson *et al.*, 1995; Conrath *et al.*, 2002) was performed to validate the annual band pair formation in the centra using Marginal Increment Ratio (MIR) with the formula:

$$MIR = MW/PBW,$$

where MW is the distance from the last translucent band to the edge of the corpus calcareum and PBW is the distance between the two last translucent band pairs (Plate 3.3).

The distance from the last translucent band to the edge of the centra was measured and divided by the width of the last complete band pair to determine the marginal increment ratio. This method was used instead of the more popular MIR = $(VR - R_n)/(R_n - R_{n-1})$ method described by Natanson *et al.* (1995) after the recommendation of Cailliet *et al.* (2006). Distances were measured on sectioned vertebral centra from digital pictures and UTHSCSA Image Tool software. One-way analysis of variance (ANOVA) was used to test for differences in MIR among months.

Table 3.1: Schedule of injections of oxytetracycline (OTC) and calcein in 35 captive Port Jackson sharks (*H. portusjacksoni*). Open values did occur in date of injection and final measurements because of unexpected death.

Sex	Captured		Date of injection				Survival (months)	Euthanasia			
	F_L (mm)	T_W (g)	OTC	Calcein	OTC	Calcein		Date	F_L (mm)	T_W (g)	Age (years)
M	190	70	29-Sep-05				12.48	4-Oct-06	475	1020	0.17
F	197	64	29-Sep-05				1.94	17-Nov-05			0.25
F	205	66	29-Sep-05				1.94	17-Nov-05			0.25
F	210	93	20-May-06				4.56	4-Oct-06	313	214	0.17
F	215	93	29-Sep-05				1.94	17-Nov-05			0.25
F	215	84	20-May-06				4.56	4-Oct-06	288	200	0.17
F	217	76	29-Sep-05				1.94	17-Nov-05			0.25
M	221	88	20-May-06				4.56	4-Oct-06	280	166	0.17
M	225	95	20-May-06				4.56	4-Oct-06	320	217	0.17
F	338	293	4-Nov-04				2.83	27-Jan-05	340	320	1.42
F	352	530	7-May-04	5-Aug-04	5-Nov-04	25-Feb-05	12.36	10-May-05	520	1530	2.75
F	365	366	4-Nov-04	4-Feb-05			23.04	4-Oct-06	620	2305	2.17
M	389	547	23-Sep-05	1-Dec-04	23-Mar-05		6.36	4-Apr-05	510	707	2.66
M	407	533	4-Nov-04	4-Feb-05			5.04	4-Apr-05	480	604	2.66
F	413	565	7-May-04	5-Aug-04	5-Nov-04	25-Feb-05	12.36	10-May-05	535	1451	2.75
M	446	724	4-Nov-04	4-Feb-05			6.24	10-May-05	510	1420	2.75
M	850	6000	14-Sep-04	1-Dec-04	18-Mar-05		24.84	4-Oct-06	963	7800	17.17
M	912	7400	14-Sep-04	1-Dec-04	18-Mar-05		8.04	3-May-05	935	6500	16.75
F	945	10200	2-Sep-04	1-Dec-04	4-Mar-05		25.32	4-Oct-06	1065	14300	26.17
F	991	11000	25-Aug-04	25-Nov-04	25-Feb-05		26.04	4-Oct-06	1033	14100	19.17
F	1004	10600	8-Sep-04	1-Dec-04			6.02	4-Mar-05	975	13000	22.58
F	1006	11500	14-Sep-04	1-Dec-04	18-Mar-05		24.84	4-Oct-06	1067	15100	22.17
F	1026	12100	8-Sep-04	1-Dec-04	7-Mar-05		25.08	4-Oct-06	1040	20300	26.17
F	1033	12400	25-Aug-04	25-Nov-04	25-Feb-05		11.04	9-Jun-05	997	8700	19.91
F	1035	9500	2-Sep-04	1-Dec-04	4-Mar-05		25.32	4-Oct-06	1070	14400	18.71
F	1038	13200	25-Aug-04	25-Nov-04	25-Feb-05		26.04	4-Oct-06	1048	14800	20.17
F	1038	11900	2-Sep-04	1-Dec-04	4-Mar-05		25.44	4-Oct-06	1058	13400	29.17
F	1048	11800	14-Sep-04	1-Dec-04	18-Mar-05		24.84	4-Oct-06	1013	14800	22.17
F	1060	12500	14-Sep-04	1-Dec-04	18-Mar-05		24.84	4-Oct-06	1095	13900	25.17
F	1075	13200	2-Sep-04	1-Dec-04	4-Mar-05		25.32	4-Oct-06	1052	12900	26.17
F	1083	14600	5-Aug-04	5-Nov-04	4-Mar-05		26.16	4-Oct-06	1110	16000	19.17
F	1085	13000	14-Sep-04	1-Dec-04	18-Mar-05		7.92	1-May-05	1057	11700	21.75

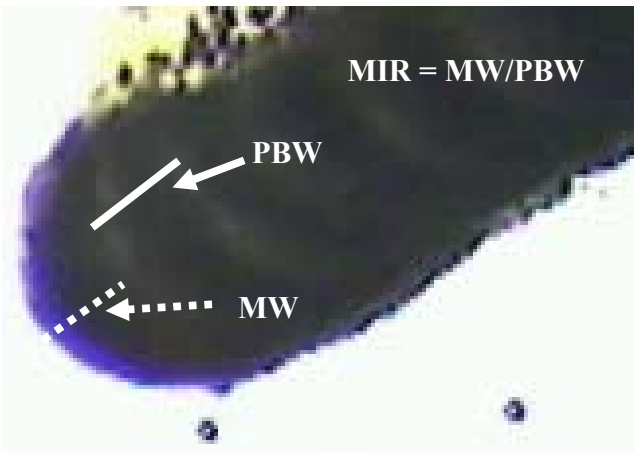


Plate 3.3: Showing the measurements for marginal increment ratio (MIR) where MW = marginal width and PBW = previous band width.

Centrum Edge Analysis (CEA) was also performed to validate the formation of annual band pairs (Holden and Vince, 1973; Goldman, 2004). CEA was performed by recording the presence or absence of translucent bands on the edge of the corpus calcareum using sectioned vertebral centra. If the last fully formed band was translucent, it was recorded and plotted against month.

3.2.4 Ageing

The relationship between vertebral centra and length of female and male *H. portusjacksoni* were significantly different and therefore the sexes were separated. Complete details of the analysis are described in section 2.3.1.3 of this thesis.

3.2.4.1 Techniques and Methods

The first reading conducted by the author scored all samples for their readability (Table 3.2). Any sample with a readability score < 3 was eliminated from further analyses. Each vertebra was read twice. If the two readings differed by > 2 band pairs, a third reading was conducted. If the third reading was within 2 band pairs from one of the previous two readings, the third reading and the previous reading which was within 2 bands of the third reading were used for further analysis. If the third reading differed by > 3 band pairs from either of the previous readings, the vertebra was not used for subsequent age estimation.

Table 3.2: Readability score assigned to each vertebrae (modified from Officer *et al.* (1996).

Readability score	Description
5	Band pair counts unambiguous with exceptionally clear bands
4	Band pair counts unambiguous but bands of diminished clarity
3	Two band pair counts possible but estimated count is most likely
2	More than two interpretations possible; band pair count is best estimated
1	No band pair counts possible; unreadable

An annulus consisted of one opaque and one translucent band pair. The opaque and translucent bands were described using transmitted light. The opaque band was wide and the translucent band was narrow. Vertebral band pairs (one opaque and one translucent) were counted on two occasions (three months apart) without knowledge of the size, sex or previous estimates of age. This was achieved by labelling each vertebra with a random number (unrelated to sex, length and date of capture) and randomising the reading order on each occasion.

True age was calculated as the number of total band pairs - birth band + number of months between date of birth (estimated to August 1st as described in upcoming validation

section 3.3.2 of this chapter) and capture. The original von Bertalanffy growth equation was fitted to the age data:

$$L_t = L_\infty (1 - e^{-k(t-to)}),$$

where L_t is the length at time t, L_∞ is the theoretical asymptotic size, t_0 is the theoretical age at zero length, and k is the rate at with L_∞ is achieved.

3.2.4.2 Whole Vertebrae

Whole vertebral centra were read by first dipping in water and then counting band pairs under a dissecting microscope (Nikon SMZ645) with reflected light. Only opaque and translucent band pairs that were visible and unbroken around the whole vertebrae were counted. The first translucent band was determined to be the birth mark.

3.2.4.3 Sectioned Vertebrae

A randomised selection of 300 sectioned vertebral centra was aged using a dissecting microscope (Nikon SMZ645) with transmitted light. Only translucent band pairs that were visible and whole through the corpus calcareum were counted. The angle change was determined to be the birth mark, and the first translucent band was formed during the first winter (see section 3.3.2 in this chapter).

3.2.5 Analyses

3.2.5.1 Influence of Vertebra Number on Age Estimation

A sub-sample of 30 animals was aged twice using sections of vertebrae 10, 20, 30, 40 and 50. One-way ANOVA was used to test for difference between vertebral number in mean band pair counts. The Coefficient of Variance (CV) (Chang, 1982) was calculated for each vertebrae in both readings. For each vertebra (position) the CV was summed and compared between the different vertebrae (positions). The lowest sum, and therefore the highest consistency of interpretation, determined which vertebrae (position) were best suited for age estimation.

3.2.5.2 Comparison of Whole and Sectioned Vertebral Centra

The accuracy of whole and sectioned vertebral centra was evaluated separately from estimates of within-reader bias and precision of each method. Within-reader bias was calculated to test for any differences between the two readings and to determine which of the readings aged higher, using the following formula (Officer *et al.*, 1996):

$$(1) \qquad (Rd_1 - Rd_2) / ((Rd_1 + Rd_2) / 2),$$

where Rd_1 and Rd_2 is the band pair counts recorded in reading one and two, respectively, for each vertebrae.

A *t*-test was used to test the hypothesis that the average difference between the two readings was not significantly different from zero (Officer *et al.*, 1996).

An age-bias plot between the two different readings was used to detect any systematic difference within the readings (Campana *et al.*, 1995). The two readings used for the age-bias plot was the once which where within 2 band pairs of each other.

Within-reader precision was estimated from the Coefficient of Variance (CV) to determine the consistency of the interpretation of band pairs, using the following formula (Chang, 1982):

(3) $$CV_j = 100 \times (\sqrt{\sum [(x_{ij} - x_j)^2/(R(R - 1)]/ x_j),$$

where x_{ij} is the *i*th age estimation of the *j*th shark, x_j is the mean age of the *j*th shark and *R* is the number of times each shark is aged.

The upper limit for CV was set at 20% for each vertebra (Wintner and Cliff, 1999). Any vertebrae with CV >20% were excluded from subsequent analyses.

Analyses (1) to (3) were all modified by substituting the readings (Rd_1 and Rd_2) with readings from whole and sectioned vertebral centra, respectively. This was performed to test for differences between whole and sectioned vertebrae readings to determine which method best described the age of *H. portusjacksoni*. Analysis of covariance (ANCOVA) was calculated to test for differences between the methods slops of the regression line for age versus length.

3.3 Results

3.3.1 Relationship Between Length and Vertebral Diameter

One-way ANOVA showed no significant difference in mean number of band pairs among the five vertebrae ($F_{4,145} = 0.07$, $p = 0.99$). There was little difference in CV among vertebrae 10, 20 and 30 however, CV of vertebrae 40 and 50 were higher (28.38, 27.57, 28.38, 35.1 and 32.07% for vertebrae 10, 20, 30, 40 and 50, respectively). Vertebrae 20 was therefore chosen for its accessibility and bigger size (Figure 2.6)

3.3.2 Validation

Incorporation of OTC and calcein varied in the 35 individuals (Plate 3.4). Four neonates (197 to 217 mm F_L) injected with OTC and euthanased 49 days later showed no fluorescent band, while an juvenile (338 mm F_L) injected with OTC and euthanased 84 days later showed a strong fluorescent band with additional post-growth. Calcein fluorescent bands were visible in the vertebral centra from a juvenile (407 mm F_L) after just 59 days post-injection.

Due to some unexpected deaths, the time in captivity varied between 137 and 789 days (Table 3.1). Vertebral centra from a 520 mm F_L female injected with OTC on May 7th and November 5th 2004 and calcein on August 5th 2004 and February 25th 2005 and euthanased on May 10th 2005, showed that opaque band pairs were laid down during the faster summer growth, while translucent band pairs were laid down during the slower winter growth. This was observed in the other injected sharks ($n = 20$). There was a significant positive linear relationship between the number of completed band pairs incorporated post-injection and years in captivity (Figure 3.2), indicating that band pairs are formed annually.

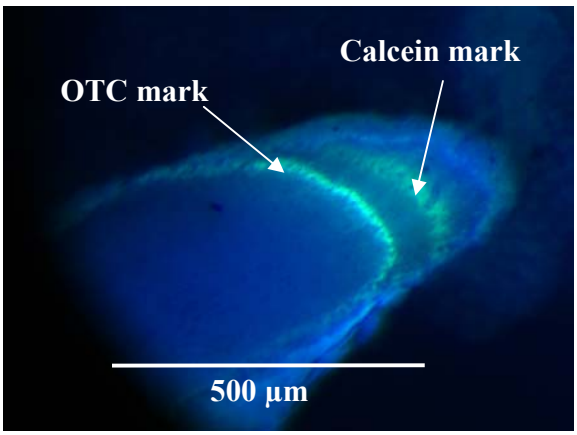


Plate 3.4: A sectioned vertebral centra photographed under UV-light 360 nm with one OTC fluorescent mark and one calcein fluorescent mark incorporated three months apart from a 510 mm F_L male *H. portusjacksoni* injected on November 4th 2004 (OTC) and February 4th 2005 (calcein), and euthanased 95 days later.

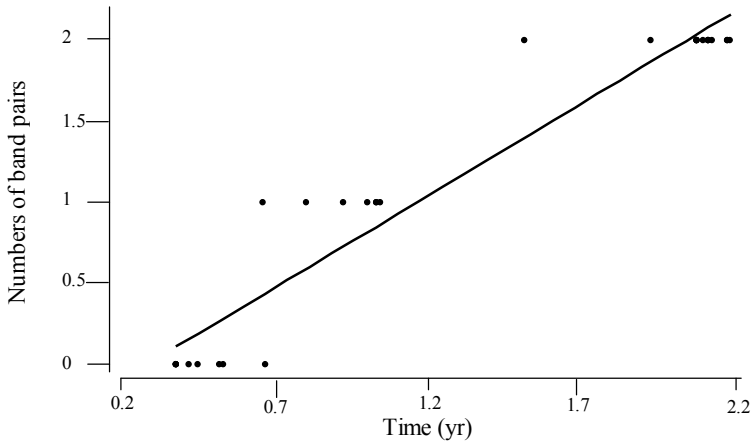


Figure 3.2: Number of completed band pairs incorporated post-injection of OTC or calcein in 35 sectioned vertebral centra from *H. portusjacksoni* showing an annual cycle of band pair formation. Number of band pairs = -0.32 + 1.14(Time), r^2 = 92.9%, $F_{1,29}$ = 382.2, $p < 0.001$.

All neonates immersed in calcein immediately after birth showed a clear fluorescent mark in their sectioned vertebrae (n = 4) (Plate 3.5). This fluorescent mark was incorporated just beyond the angle change, which confirms the hypothesis that the change of angle in the vertebral centra occurs at the time of birth and the first translucent band is formed during the first winter. With this in mind and considering the frequency of neonates caught in the trawls (Figure 3.1) the time of birth is regarded as being 1st August

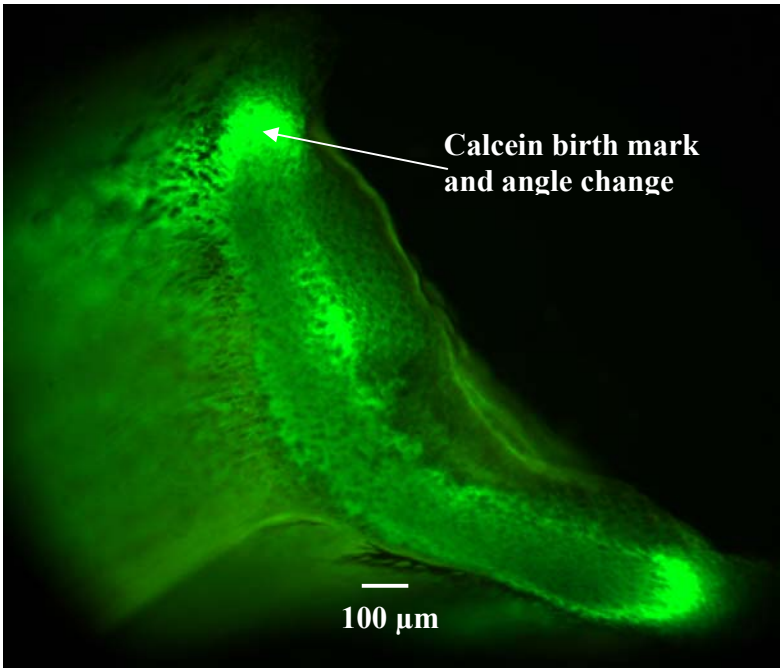


Plate 3.5: The birth mark in a sectioned vertebra from a 280 mm F_L female *H. portusjacksoni* immersed in calcein for 24 hours at time at birth and euthanased 137 days later.

Marginal increment analysis was performed on 207 individuals ranging from 200 to 1078 mm F_L. Marginal increments showed clear peaks in the months of early spring to early summer (i.e. September to December). This gives a clear indication that the narrow

translucent band is laid down in the vertebral centra in winter. The MIR then dropped from December to August giving the wider opaque band in the summer months (Figure 3.3).

Centrum edge analysis was performed on sectioned vertebral centra of 298 individual ranging from 197 to 1115 mm F_L. CEA confirmed the results given by the MIR, showing a clear rise in the presence of translucent bands on the growing vertebral edge in early spring to early summer (i.e. September to December) (Figure 3.4). Translucent bands were absent from the growing vertebral edge from February to August. This result together with MIA strengthens the conclusion that translucent band pairs are laid down and are able to be visualised on the growing edge of vertebral centra in early spring to early summer.

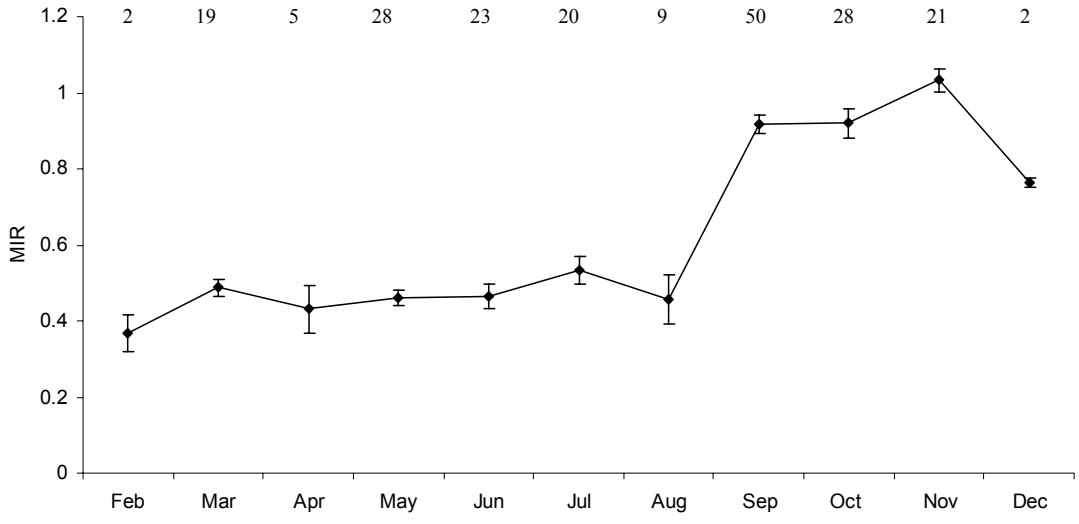


Figure 3.3: Annual trend in median Marginal incremental Ratio (MIR) growth of *H. portusjacksoni* vertebral centra over a 12 month period. Standard error bar are shown with sample size provided for each month.

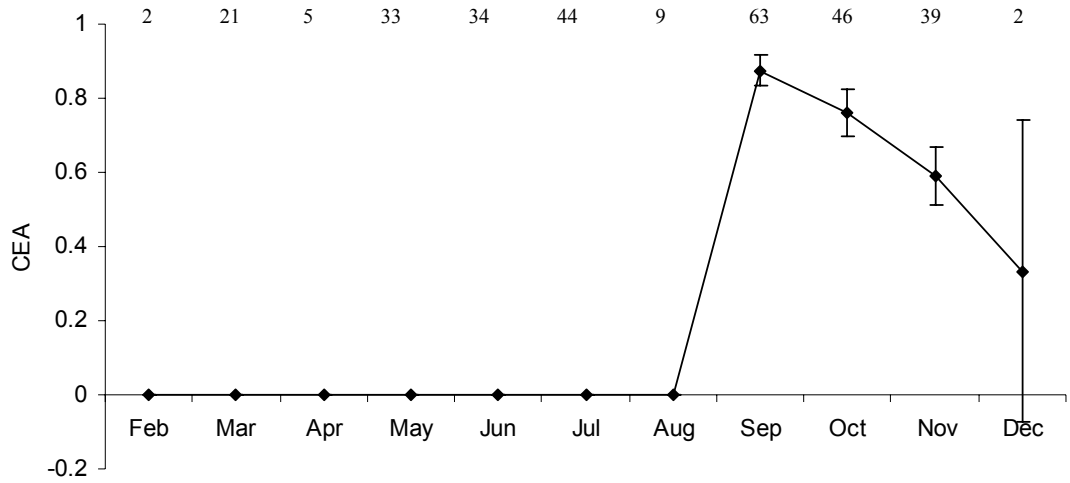


Figure 3.4: Annual trend in median Centrum Edge Analysis (CEA) in vertebral centra of *H. portusjacksoni* over a 12 month period. Standard error bars are shown with the sample size provided for each month.

3.3.3 Whole Vertebrae

A total of 600 whole vertebrae were initially examined. Of the 600 individuals a total of 76.3% had a readability score, CV and band pair precision allowing further analysis. Of these, 210 females (197 to 1197 mm F_L) and 248 males (211 to 1003 mm F_L) were successfully used for age estimation. The first translucent band was considered to be the birth mark and was withdrawn from the final real age (Plate 3.6).

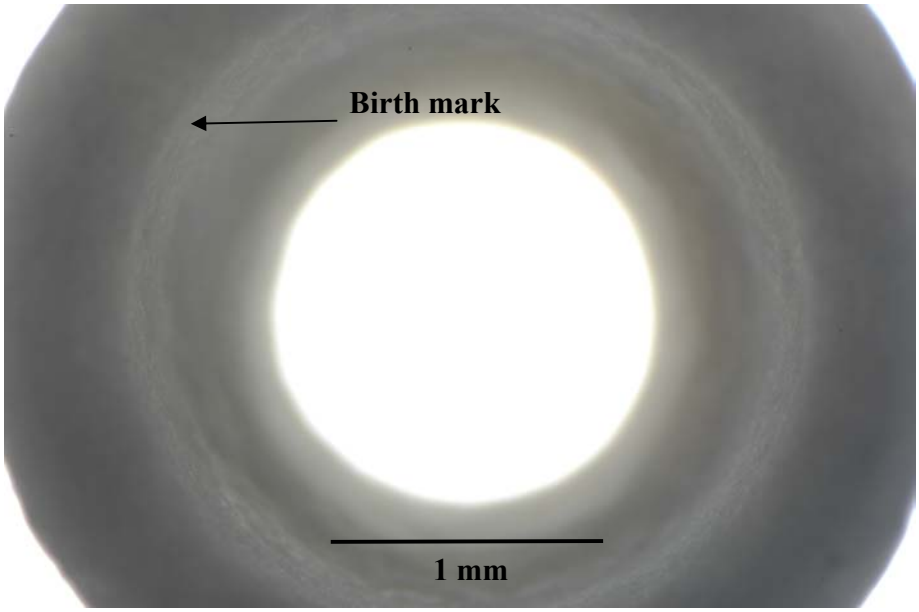


Plate 3.6: Anterior view of centra number 20 showing the birth mark (x 100) of a 277 mm F_L male *H. portusjacksoni*.

3.3.3.1 Female

Ages ranged from 0 (197 mm F_L) to 19 (1098 mm F_L) years. Female *H. portusjacksoni* from 197 to 374 mm F_L (mean ±S.E. = 272 ±12.6 mm) showed no band pairs and were assigned to the 0+ age class. The relationship between age and length was high (r^2 = 83.6%) although it decreased with age and had an asymptotic length which were unrealistically high than the actual data (Figure 3.5). The average bias between readings was 0.015 ±0.014. Average bias was not significantly different from 0 (T = 0.88, p = 0.38). The age-bias plot showed no systematic variation in bias and little variation outside the 1:1 relationship line for individuals younger than 9 years (Figure 3.6), indicating that there was no difference between band pairs in the first and second reading. The average between-reading precision (CV) was 6.85%.

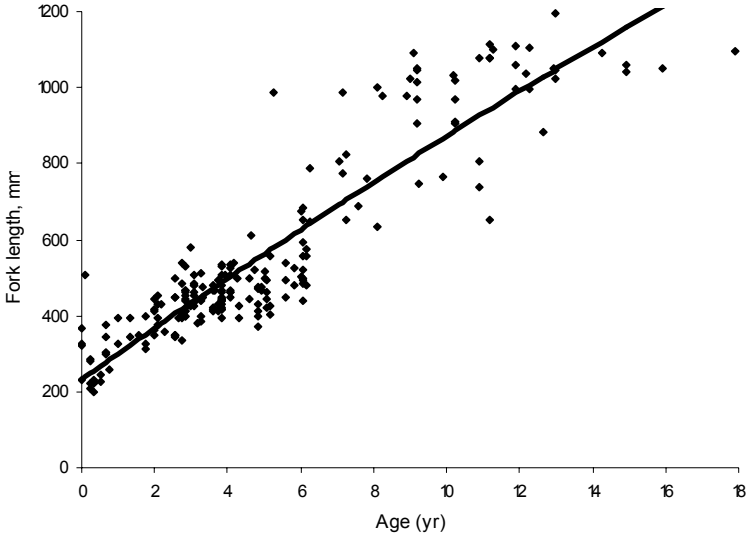


Figure 3.5: The relationship between age and fork length in female *H. portusjacksoni* (*n* = 210) based on band pairs in whole vertebrae. L_∞ = 5522.6 mm F_L, k = 0.013 year^{-1} and t_0 = -3.34 years.

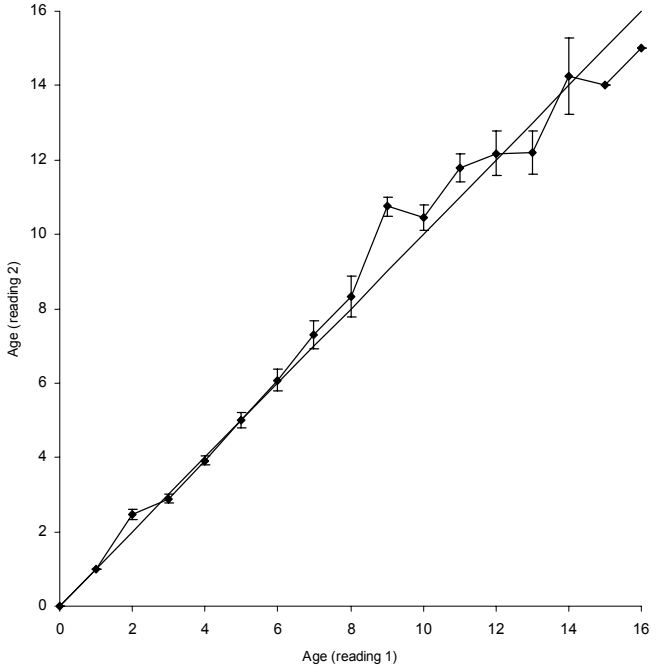


Figure 3.6: An age-bias plot with standard error and a 1:1 relationship line for female *H. portusjacksoni* comparing the ages estimated using whole vertebral centra in the first and second reading (*n* = 210).

3.3.3.2 Male

Ages ranged from 0 (211 mm F_L) to 16 (984 mm F_L) years. Male *H. portusjacksoni* from 211 to 341 mm F_L (271 ±8 mm) showed no band pairs and were assigned to the 0+ age class. The relationship between age and length was high (r^2 = 99.7%) although it decreased with age and gave an asymptotic length which was higher than the actual data (Figure 3.7). The average bias between readings was -0.007 ±0.013. Average bias was not significantly different from 0 (T = -0.57, p = 0.57). The age-bias plot showed a small systematic variation in bias between 5 and 10 years with the second reading ageing higher. However, there was little variation outside the 1:1 relationship line for individuals younger than 11 years (Figure 3.8), indicating that there was no difference between band pairs in the first and second reading. The average between-reading precision (CV) was 6.78%.

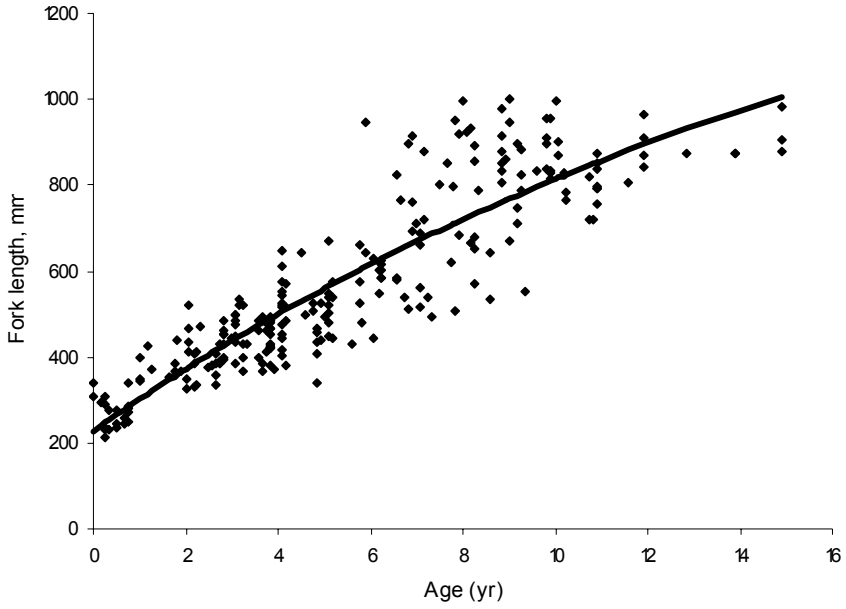


Figure 3.7: The relationship between age and fork length in male *H. portusjacksoni* (n = 248) based on band pairs in whole vertebrae. L_∞ = 1657.6 mm F_L, k = 0.053 year^{-1} and t_0 = -2.82 years.

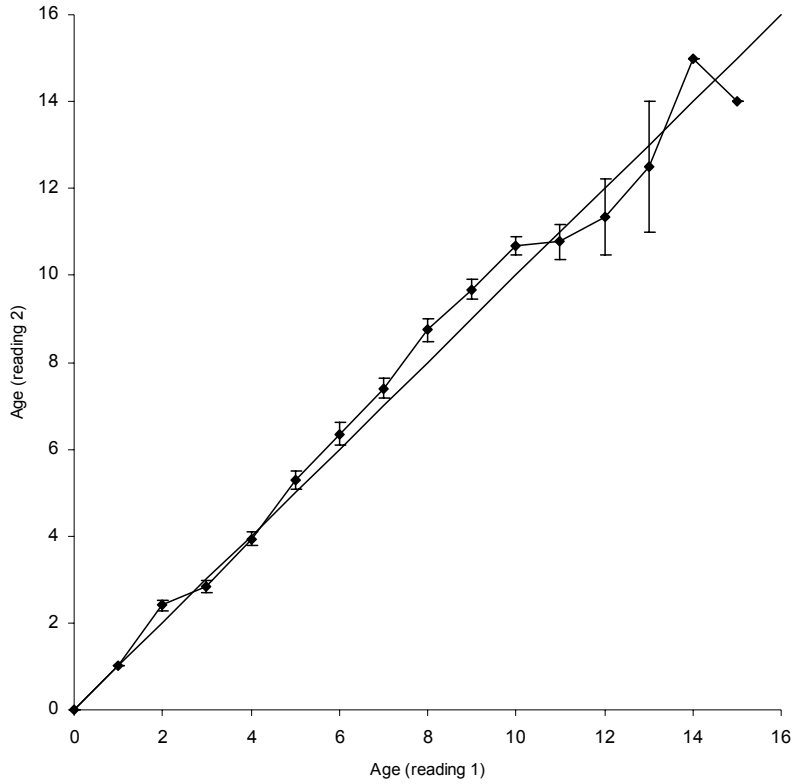


Figure 3.8: An age-bias plot with standard error and a 1:1 relationship line for male *H. portusjacksoni* comparing the ages estimated using whole vertebral centra in the first and second reading (n = 248).

3.3.4 Sectioned Vertebrae

Of the 300 individuals aged, 99.3% had a readability score, CV and band pair precision allowing for further analysis. A total of 132 females (210 to 1115 mm F_L) and 166 males (197 to 1003 mm F_L) were successfully used for age estimation. The first translucent band after the angle change was validated to be the birth mark and was withdrawn from the final real age (Plate 3.7).

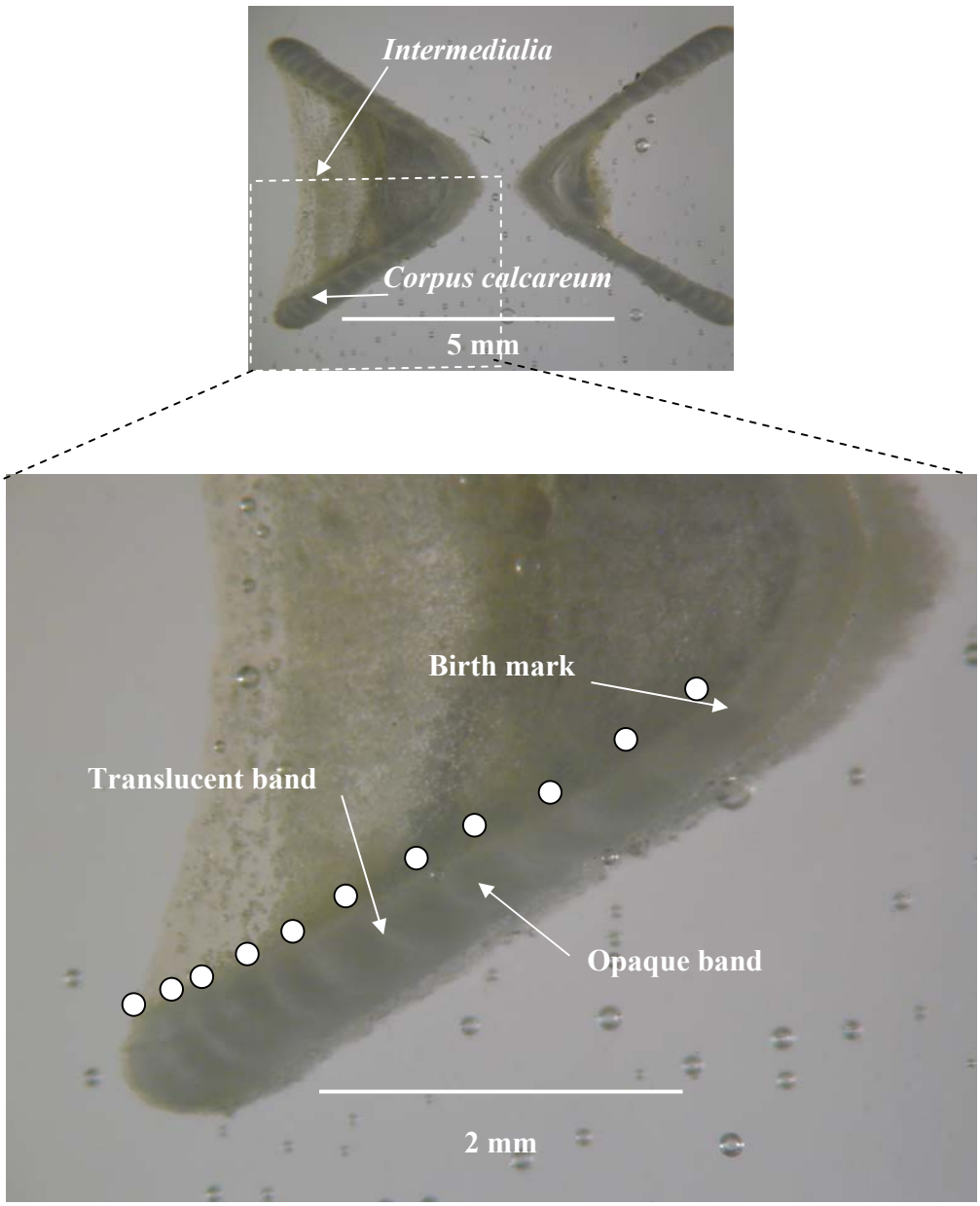


Plate 3.7: A lateral view of a sagittal section of a vertebral centra from a 683 mm male *H. portusjacksoni* showing a real age of 10. Open circles show the translucent bands.

3.3.4.1 Female

Ages ranged from 0 (210 mm F_L) to 33 (1090 mm F_L) years. Female *H. portusjacksoni* ranging from 210 to 345 mm F_L (252 ±18 mm) showed no band pairs and were assigned to the 0+ age class. The relationship between age and length was high (r^2 = 94.1%) and gave an asymptotic length which was similar to the actual data (Figure 3.9). The average bias between readings was 0.005 ±0.005. Average bias was not significantly different from 0 (T = 0.51, p = 0.61). The age-bias plot showed no systematic variation in bias and little variation outside the 1:1 relationship line (Figure 3.10), indicating no variation between the two readings. The average between-readings precision (CV) was 0.86%.

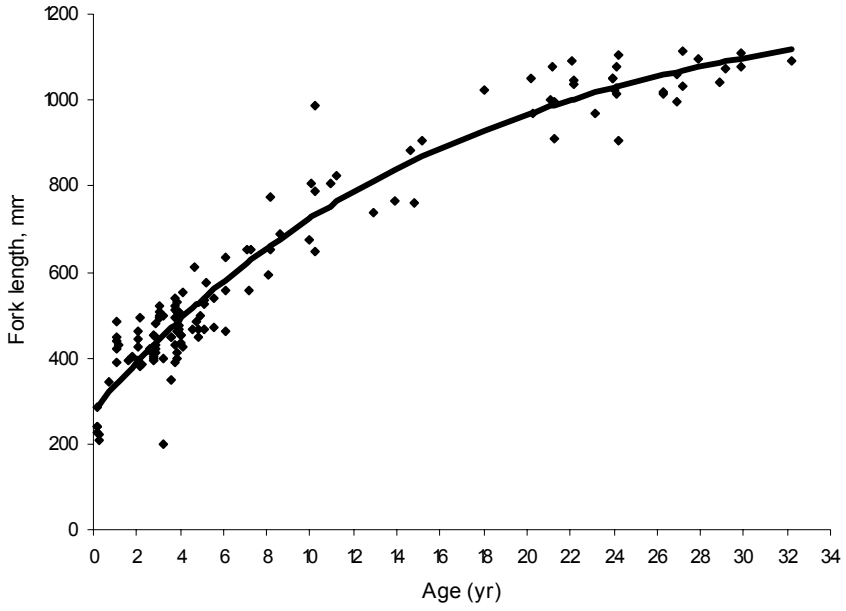


Figure 3.9: The relationship between age and fork length in female *H. portusjacksoni* (n = 125) based on counts of band pairs in sectioned vertebrae. L_∞ = 1252.7 mm F_L, k = 0.061 year^{-1} and t_0 = -4.07years.

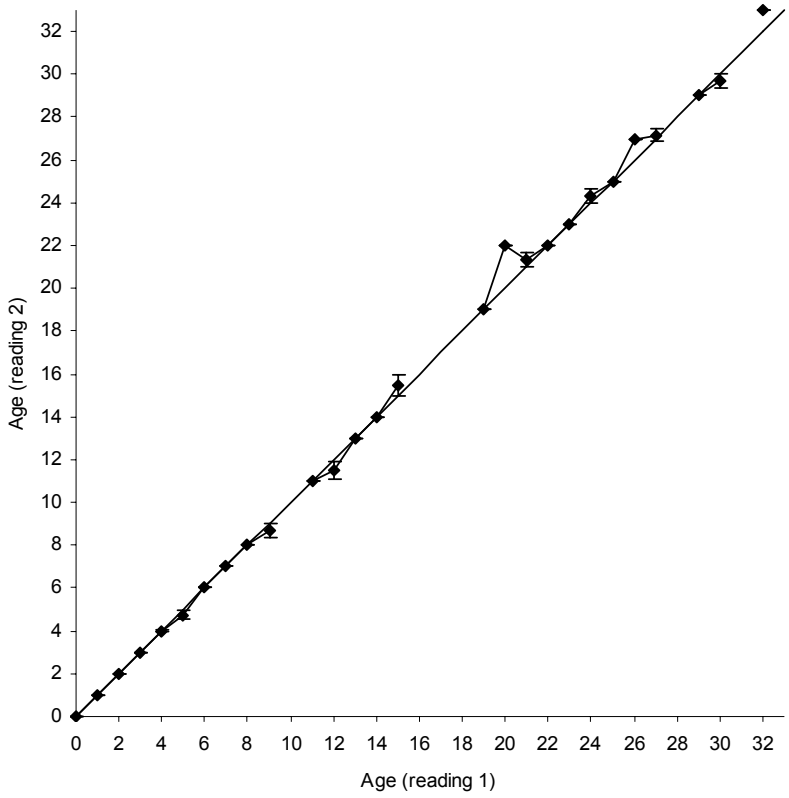


Figure 3.10: An age-bias plot with standard error and a 1:1 relationship line for female *H. portusjacksoni* comparing the ages estimated using sectioned vertebral centra in the first and second reading (n = 132).

3.3.4.2 Male

Ages ranged from 0 (197 mm F_L) to 25 (904 mm F_L) years. Male *H. portusjacksoni* ranging from 197 to 330 mm F_L (254 ±21 mm) showed no band pairs and were assigned to the 0+ age class. The relationship between age and length was high (r^2 = 90.1%) and gave an asymptotic length which was similar to the actual data (Figure 3.11). The average bias between readings was 0.008 ±0.005. Average bias was not significantly different from 0 (*T*

= 1.44, $p = 0.15$). The age-bias plot showed no systematic variation in bias and little variation outside the 1:1 relationship line (Figure 3.12), indicating no variation between the two readings. The average between-readings precision (CV) was 1.34%.

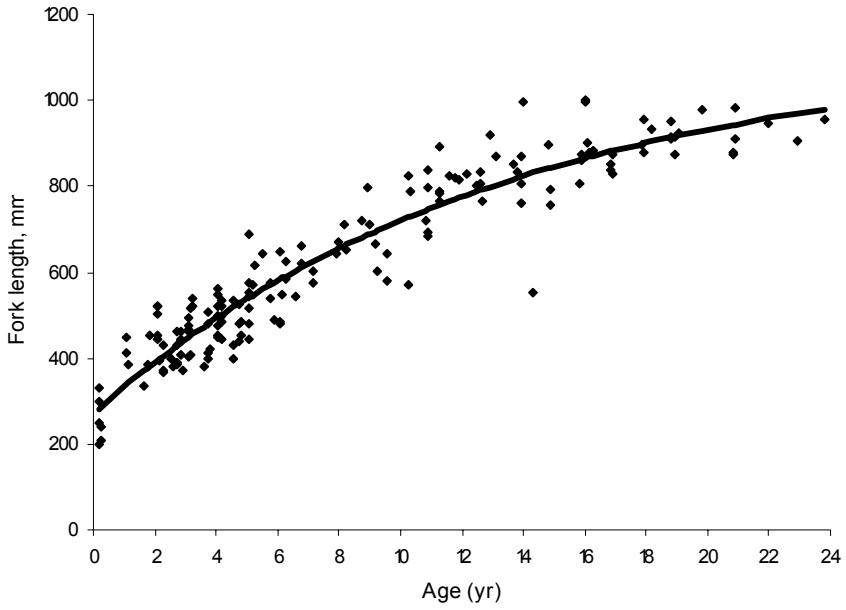


Figure 3.11: The relationship between age and fork length of male *H. portusjacksoni* ($n = 165$) based on counts of band pairs from sectioned vertebrae. $L_\infty = 1118.5$ mm F_L, $k = 0.076$ year^{-1} and $t_0 = -3.58$years.

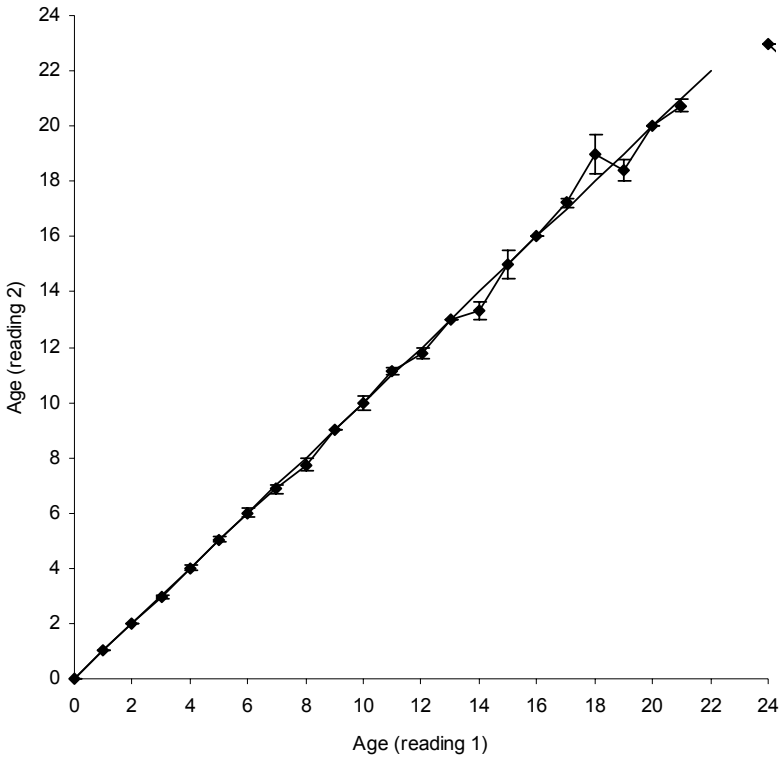


Figure 3.12: An age-bias plot with standard error and a 1:1 relationship line for male *H. portusjacksoni* comparing the ages estimated using sectioned vertebral centra in the first and second reading (n = 166).

3.3.5 Comparison Between Methods

3.3.5.1 Female

The accuracy of age estimation derived from whole and sectioned vertebral centra was compared from 118 vertebrae. For whole vertebral centra age ranged from 0+ to 18 years (6.4 ±0.35 years) and for sectioned vertebral centra age ranged from 0+ to 32 years (10.1 ±0.86 years). When comparing the relationship between age and length, sectioned vertebral centra had a higher relationship than whole vertebral centra and also better reflected the

data with whole vertebral centra indicating no asymptotic length. There was a significant difference between the two methods (ANCOVA; $F_{1,335} = 303.35$, $p < 0.001$). The average bias between methods was -0.158 ±0.05 which was significantly different from 0 ($T = -3.73$, $p < 0.001$). This indicated that whole vertebrae returned lower ages than sectioned vertebrae. This was supported by the age-bias plot indicating that whole vertebral centra aged individuals younger than 3 years higher than sectioned vertebrae. However, sectioned vertebral centra aged individuals older than 8 years higher than whole vertebrae (Figure 3.13). It also indicates that as individuals get older the age difference between the two methods increases. The average precision (CV) between methods was 92.32%.

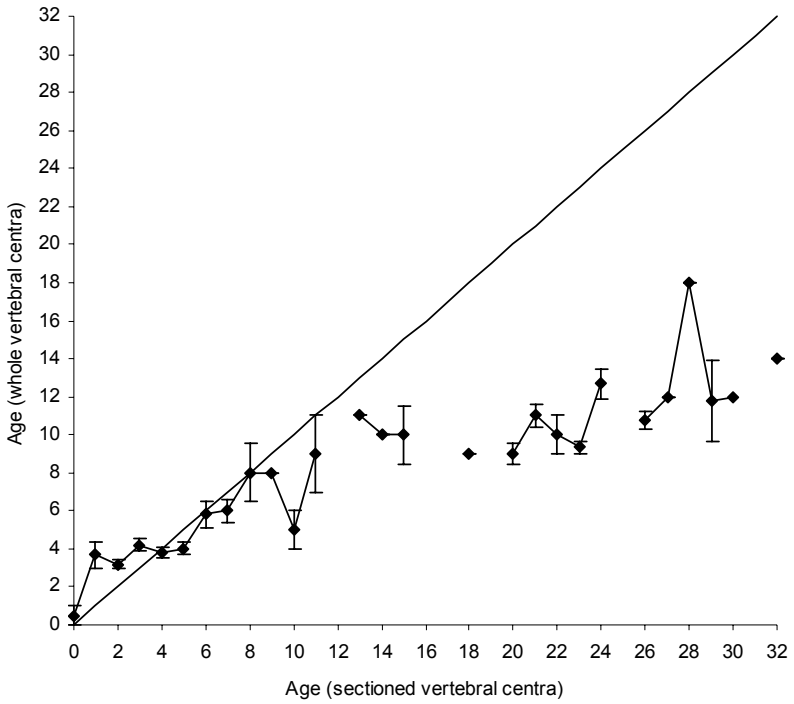


Figure 3.13: An age-bias plot with standard and a 1:1 relationship line for female *H. portusjacksoni* comparing the ages estimated using sectioned and whole vertebral centra ($n = 118$).

3.3.5.2 Male

The accuracy of age estimation derived from whole and sectioned vertebral centra was compared from 158 vertebrae. For whole vertebral centra age ranged from 0+ to 15 years (6.5 ±0.26 years) and for sectioned vertebral centra age ranged from 0+ to 28 years (8.8 ±0.5 years). When comparing the relationship between age and length for whole vertebral centra and sectioned vertebral centra, sectioned vertebrae had a higher relationship. There was a significant difference between the two methods (ANCOVA; $F_{1,410} = 1846.46$, $p < 0.001$). The average bias between methods was -0.157 ±0.03 which was significantly different from 0 ($T = -3.27$, $p < 0.01$). This indicated that whole vertebrae returned lower ages than sectioned vertebrae. This was supported by the age-bias plot indicating that whole vertebral centra aged individuals younger than 3 years higher than sectioned vertebrae. However, sectioned vertebral centra aged individuals older than 8 years higher than whole vertebrae. It also indicates that as individuals get older the age difference between the two methods increases (Figure 3.14). The average precision (CV) between methods was 56.95%.

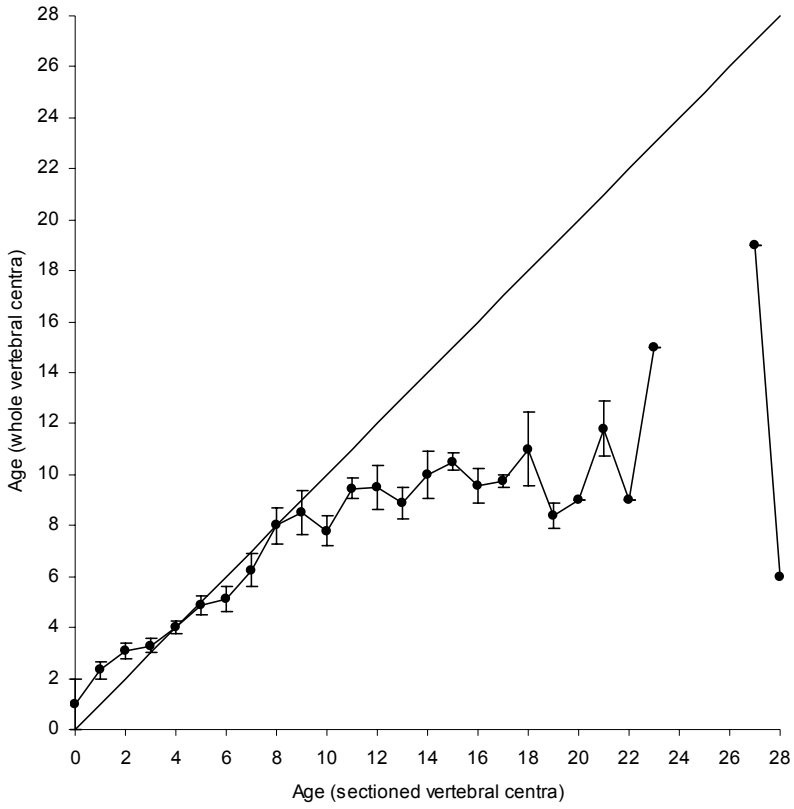


Figure 3.14: An age-bias plot with standard error and a 1:1 relationship line for male *H. portusjacksoni* comparing the ages estimated using sectioned and whole vertebral centra (n = 156).

3.4 Discussion

3.4.1 Position on the Vertebral Column

Vertebral position had no effect on the average age however, there were minor differences in CV values. The largest vertebral centra used in this study produced the largest, clearest and therefore had a higher consistency of interpretation of band pairs estimation the age of *H. portusjacksoni*, as has been reported from other elasmobranchs such as *Carcharhinus leucas, S. californica, Negaprion brevirostris, Rhinobatos annulatus, M. antarcticus* and *G.*

galeus (Ridewood, 1921; Brown and Gruber, 1988; Natanson and Cailliet, 1990; Officer *et al.*, 1996). However, no differences in age from vertebrae in the different regions of the vertebral column has been reported for *I. oxyrinchus* (Natanson *et al.,* 2006). They reported that smaller vertebral centra was more difficult to count, however no difference in band pairs counts between the different regions of the vertebral column was found.

3.4.2 Validation

Three techniques were used to validate the annual formation of vertebral band pairs in *H. portusjacksoni*. As primary validation technique chemical markers such as OTC and calcein was used. Although OTC is the most popular chemical validation marker, it vanishes with exposure to light, whilst calcein is visible under normal light and has been successfully used for several elasmobranch species (Walker *et al.*, 1995; Gelsleichter *et al.*, 1997; McAuley *et al.*, 2006). Based on the successful use of calcein for validation in this study it is recommended that calcein be used for age validation in elasmobranchs.

Together with MIA and CEA the position of the injected fluorescent marks incorporated in the vertebral centra indicates an annual pattern of band pair formation, with translucent bands (narrow hypermineralised) forming during the slower growth period as occurs in the winter months. The opaque band pairs are formed during the faster period of growth during the summer months, giving wider hypo-mineralised bands. This annual periodicity tends to be the regular pattern found in most elasmobranchs (Cailliet and Radtke, 1987; Natanson *et al.*, 2006). An exception to this is *S. californica* (Natanson and Cailliet, 1990; Cailliet *et al.*, 1992) which has a closer relationship between band pair formation and T_L than time, and fast growing juveniles deposit more bands than slow growing adults.

Rodda (2000) reported that the majority of *H. portusjacksoni* juveniles hatch in the winter- spring period in South Australia. This corresponds to the time at birth and birth-mark incorporated in the vertebral centra in juveniles reported in this study. Although this study is not the first to validate the periodicity of band pair formation in this species (Tovar-Avila, 2006), it is the first to validate the periodicity across all age groups using MIA and CEA together with fluorescent dyes. Results from the present study are consistent with Tovar-Avila's (2006) findings for the southern Australian population of *H. portusjacksoni*, showing that there is no geographical difference in the incorporation of band pairs in this species. It is important to mention that neither of the studies has been able to validate across all age groups from wild *H. portusjacksoni*. Although the aquarium used in this study had natural regimes of both light and temperature, it cannot be definitively stated that there is no artificial influences on growth and therefore differences in incorporation rate with vertebral centra of wild *H. portusjacksoni* (Branstetter, 1987b).

3.4.3 Age Estimation

H. portusjacksoni was successfully aged using whole vertebrae of ages ranging from 0+ to 18 years for females, and 0+ to 15 years for males. The initial reading showed a relatively high readability score of 76.3%, indicating that using whole vertebrae was suitable for age estimation in *H. portusjacksoni*. Bias analyses for both females and males showed minor systematic difference between the two readings. However, a high consistency in the interpretation (CV) was found between the two readings. This indicates that the method is suitable for ageing *H. portusjacksoni*, but bias analysis should be considered to test for differences. When comparing the age-length curves with the actual catch data when using whole vertebrae, the age-length curves for females indicated a relatively good fit. However,

the asymptotic length was more than 4 times greater than the actual data and the relationship got weaker with ages above 8 years which might indicate that using whole vertebrae for age estimating female *H. portusjacksoni* has size limitations. The male age-length curve was more biological realistic with a greater fit and an asymptotic length which were more similar to the actual data. However, again the relationship got weaker with ages above 7 years.

Whole vertebral centra have been successfully used for age estimating *C. tilstoni, C. sorrah, C. carcharias, Alopias vulpinus, I. oxyrinchus, P. glauca, R. annulatus, Mustelus lenticulatus, M. antarcticus, G. galeus* (Stevens, 1975; Cailliet *et al.*, 1983a; Cailliet and Bedford, 1983; Cailliet *et al.*, 1983b; Rossouw, 1984; Moulton *et al.*, 1992; Davenport and Stevens, 1998; Wintner and Cliff, 1999; Francis and Maolagain, 2000). The growth parameters derived from using whole vertebral centra are not similar to other studies however, the asymptotic length and size at birth resembles that of *Squalus mitsukurii, Carcharhinus acronotus, C. cautus, C. sorrah, Mustelus canis, M. mustelus* and *Spyrna tiburo*. But when comparing the ages of this species they all have a longevity lover than reported here (Cailliet and Goldman, 2004). However since whole vertebral centra have a tendency of underestimating older individuals (Campana, 2001; Goldman, 2004) great caution should be applied when using this method for estimating the age of *H. portusjacksoni*.

Age estimation using sectioned vertebral centra was performed successfully for all ages *H. portusjacksoni* ranging from 0+ to 32 years in females and 0+ to 24 years in males. Again the initial reading showed a relatively high readability score (99.3%), indicating that the use of sectioned vertebrae is well suited for age estimation in *H. portusjacksoni*. Bias estimates for both females and males showed little difference between two readings and

there was a high consistency of the interpretation (CV). When comparing the age-length curves with the actual catch data, both females and males indicated a good fit and an asymptotic length which were similar to the actual data. Again, indicating that the method is suited for ageing *H. portusjacksoni* with the two data sets being similar.

Others have successfully used sectioned vertebrae for age estimation of *Carcharhinus porosus, Carcharhinus signatus, C. leucas, Carcharhinus falciformis, Rhizoprionodon terraenovae, Lamna nasus* (Lessa and Santana, 1998; Natanson *et al.*, 2002; Loefer and Sedberry, 2003; Oshitani *et al.*, 2003; Santana and Lessa, 2004; Neer *et al.*, 2005). The growth parameters derived from using sectioned vertebral centra are not similar to other studies however, the asymptotic length and size at birth resembles that of *S. mitsukurii, D. calcea and C. acronotus.* But when comparing the ages of these species they all have a longevity lover than reported for *H. portusjacksoni* (Cailliet and Goldman, 2004). However, with a greater longevity and a higher resemblance between data, sectioned vertebral centra is an accurate method to estimate the age of *H. portusjacksoni*.

3.4.4 Comparison of Methods

Sectioned vertebral centra appear to be the best method of estimating the age of *H. portusjacksoni* using vertebrae. Comparing the two methods (whole versus sectioned vertebral centra) both females and males showed a significant difference between the slopes of whole and sectioned vertebral ageing. The mean age estimated from sectioned vertebrae was higher than the mean age estimated from whole vertebrae. The precision was low between the two methods. Both sexes also showed a systematic difference in the age bias plot. Whole vertebral centra underestimated the true age of older *H. portusjacksoni* compared to sectioned vertebral centra and as individuals got older the age difference

between the two methods increases. Together with the age-bias plot, all analyse agree that sectioned vertebral centra on average age higher than whole vertebral centra. Tovar-Avila (2006) also reported that reading whole vertebral centra could underestimate the age of *H. portusjacksoni*. MacNeil and Campana (2002) reported similar results for *P. glauca*. They reported that whole vertebrae underestimated individuals older than 9 years of age and suggested the use of sectioned vertebrae for age estimation on adults. Moulton *et al.* (1992) used whole vertebrae for age estimation of *M. antarcticus* and *G. galeus*. However their result only showed good agreement between whole and sectioned vertebrae for sharks of small to medium length. When the two techniques were compared across all length classes, whole vertebrae underestimated the age compared to sectioned vertebral centra. One reason might be that when the growth of the centra slows down in adults, the band pair's gets too dense and are therefore hard to distinguish. While in sectioned vertebrae they might still be identify and individually counted (Campana, 2001; Goldman, 2004).

3.5 Conclusion

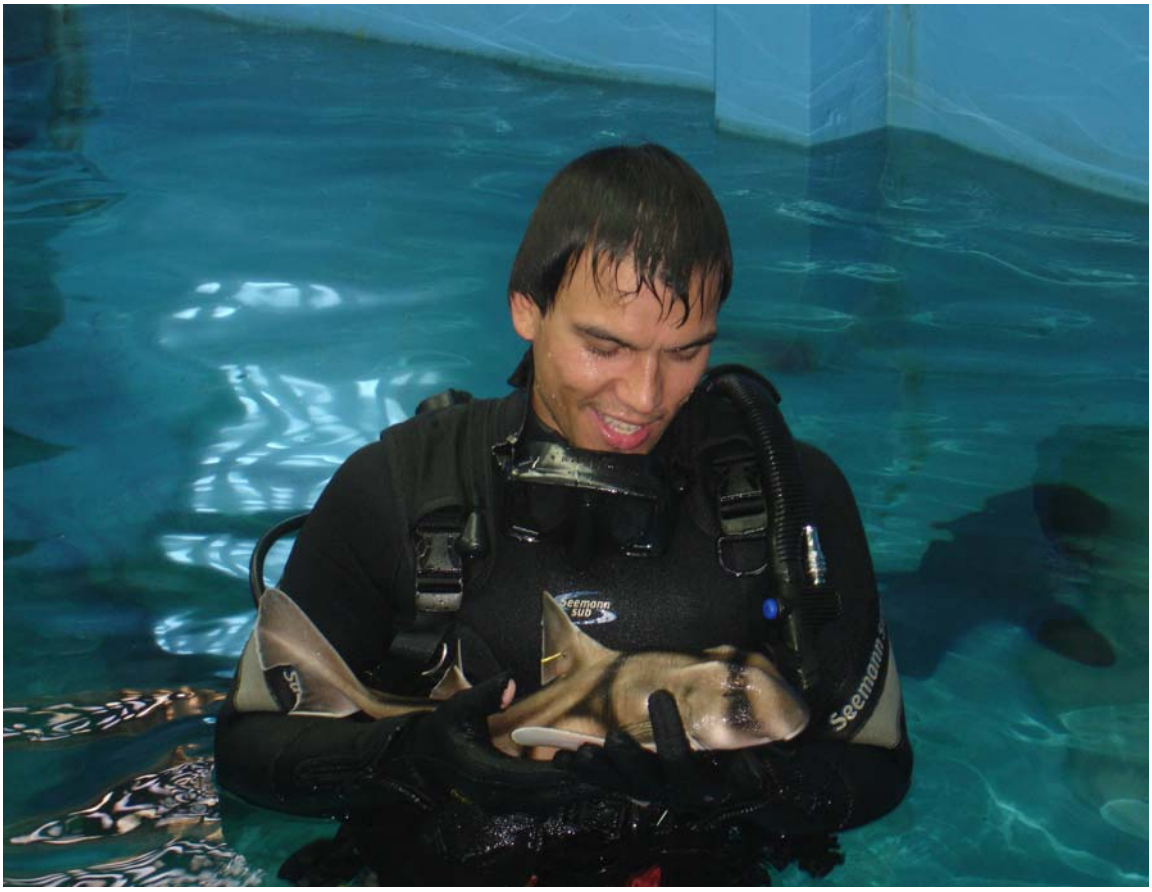
It was considered easy to identify the different band pairs in both whole and sectioned vertebral centra. Both OTC and calcein gave visible marks in the vertebral centra of *H. portusjacksoni*. They indicated that opaque bands were incorporated in the vertebral centra in the months of summer and that the translucent bands were incorporated in the months of winter.

The position on the vertebral column did not indicate any difference in bans pair counts using sectioned vertebrae. Age estimation was successfully performed on both sexes over all age classes using both whole and sectioned vertebral centra. While both methods showed individual accuracy, sectioned vertebral centra is recommended since this method

showed the highest band pair counts and therefore were less vulnerable to underestimation. Underestimation of age can have serious consequences to fisheries, since it can overestimate the potential growth of the species and therefore suggest an exploitation level which may not be unsustainable (Summerfeldt and Hall, 1987; Walker, 1998).

It is crucial that accuracy, precision and quality control is used in any age estimation and that all steps are validated for any bias (Campana, 2001). It is therefore recommended that further research on the validation of wild *H. portusjacksoni* and the differences between the two populations of *H. portusjacksoni* is undertaken.

Chapter 4



The author and one of the captive Port Jackson sharks held at Oceanworld Manly Aquarium (photo by David Powter)

Chapter 4

The Use of Dorsal Spines for Age Estimating the Port Jackson Shark (*Heterodontus portusjacksoni*).

4.1 Introduction

The search to find alternatives to the more commonly used vertebral centra for ageing elasmobranchs is leading scientists to look at the possibilities of other hard structures in elasmobranchs. Research into alternative methods is needed because vertebral centra are sometimes weakly calcified and therefore cannot be used for age estimation (Clarke *et al.*, 2002a; Braccini, 2006), and the removal of vertebrae is a lethal process. One such method is the use of the dorsal spines.

The external structure of the elasmobranch dorsal spines consists of a stem and cap. The stem is divided into two separate layers, the inner and outer dentine layer, which are separated by the primordium (Clarke *et al.*, 2002a). Growth of the stem occurs by upward and outward growth. Upward growth results from continuos deposition of dentine at the base of the stem, while outward growth is the result of cartilage production at the centre of the stem (Beamish and McFarlane, 1985). The cap is divided into an outer layer of enamel, a thin middle pigmented dentine layer called the mantel and an inner layer of dentine. Band pairs occur on both stem and cap, but are not formed in the same manner (Beamish and McFarlane, 1985).

Knowledge of age estimation from dorsal spines in elasmobranchs is scarce in the literature. The reason for this is that there are only two shark orders (Heterodontiformes and Squaliformes), including over 31 different species, that are distinguished by possessing

hard and sharp spines projecting anterior from each dorsal fin. Use of dorsal spines to estimate age has been successful for several shark species including *Squalus acanthias*, *S. blainvillei, S. mitsukurii, Deania calceus* and *Centrophorus squamosus* sharks (Holden and Meadows, 1962; Wilson and Seki, 1994; Cannizzaro *et al.*, 1995; Jones and Ugland, 2001; Clarke *et al.*, 2002a; Clarke *et al.*, 2002b) (see Appendix A for more species). However, the lack of sufficient validation has made some of the results questionable. Most of these elasmobranchs inhabit deep water and are therefore hard to sample which makes recapture rates low (Compagno, 2001). Marginal Increment Analysis (MIA) which is popular as a validation technique when using vertebral centra is very difficult, if not impossible, due to the nature of the dorsal spines (Irvine *et al.*, 2006a). For MIA to be useful, all sections have to be on the same point along the dorsal spine. This is not possible as the size of the lumen varies, even in dorsal spines of the same size, and each dorsal spine must be sectioned just below the lumen, in the pulp cavity, to be able to enhance the last band pairs.

The only validation technique that is useful is the use of chemical markers. Tucker (1985) and McFarlane and Beamish (1987b) produced one of the few successful results, validating an ageing technique that used the second dorsal spine from *S. acanthias*. They reported that each band pair was formed annually in the cap of the dorsal spine and could be used for age estimating. Not all dorsal spine-bearing elasmobranchs have been successfully aged by using dorsal spines. Braccini (2006) attempted, without success, to validate the ageing technique of *Squalus megalops* using captive animals. The animals died after five months and could therefore not be used for validation. Machado and Figueiredo (2000) established a technique including sectioning, decalcification and staining to visualize band pairs on the external dorsal spines of *Deania calcea* however, failed to validate their period of formation.

Two methods have been used to estimate age from dorsal spines. One of these methods was first described by Ketchen (1975). He used the band pairs on the external surface of the cap on whole dorsal spines to estimate the age of *S. acanthias* in British Colombia. Following Ketchen (1975) methods, Avsar (2001) used external band pairs on the cap of the first dorsal spine to estimate the age of *S. acanthias* in the Black Sea. The other method used to estimate age was the use of band pairs in the dentine layers. Beamish and McFarlane (1985) and Tanaka (1990) were some of the first to describe and use this method, using internal dentine band pairs in sectioned spines in *S. acanthias* and *Centrophorus acus*, respectively. Using internal band pairs for age estimation was again successfully applied to *C. squamosus* and *D. calceus* using both the first and second dorsal spines Clarke *et al.* (2002a, b)(Clarke *et al.*, 2002a; Clarke *et al.*, 2002b). However, none of this studies clarified which dentine layer was used for their age estimation. Tovar-Avila (2006) reported no difference in the band pair count in the outer dentine layer compared to the inner layer. Braccini (2006) found no significant difference in counts of band pairs on the enamel and the inner dentine layer of the dorsal spine of *S. megalops*. However Irvine *et al.* (2006a) concluded that in *Etmopterus baxteri*, enamel band pairs aged higher than dentine band pairs.

With few species to compare the two structures, there is limited knowledge of the relationship between the use of dorsal spines and vertebral centra to estimate the age of elasmobranchs. This may have arisen because vertebral centra in sharks possessing dorsal spines are weakly calcified (Clarke *et al.*, 2002a; Braccini, 2006; Irvine *et al.*, 2006a) which makes visualising band pairs in the corpus calcareum more or less impossible. There is no evident banding pattern in the either whole or sectioned vertebrae in *C. squamosus* (Clarke *et al.*, 2002a) or *S. megalops* (Braccini *et al.*, 2007). Fortunately this is not the case in *H.*

portusjacksoni and one can use both structures and compare the age estimation between them.

The aim of this study was to investigate the use of dorsal spines (whole and sectioned) to estimate the age of *H. portusjacksoni*. The specific objectives for each method were to (1) confirm the presence of band pairs, (2) validate the annual formation of band pair, (3) determine which method is most suitable of ageing, and (4) compare band pair counts from sectioned dorsal spines and sectioned vertebral centra.

4.2 Materials and Methods

4.2.1 Sampling

A total of 1580 *H. portusjacksoni* were sampled from commercial prawn and fish trawlers operating from Newcastle, Australia (32°55′05″*S*, 151°45′37″*E*) between September 2003 and June 2006. Samples were taken in all months except January. Sharks were measured total length (T_L), fork length (F_L) (Figure 2.1), weighed (T_W) and sexed before being frozen. Complete details of the sampling design are described in Chapter 2 and 3 of this thesis.

4.2.2 Dorsal Spine Preparation

Dorsal spines were removed, cleaned in boiling tap water and stored. External spine length (E_{SL}), total spine length (T_{SL}) and spine base width (S_{BW}) were measured to the nearest 0.02 mm using a vernier calliper (Plate 2.11). Of the 652 sharks processed for ageing, 232 (35.6%) had spines that were either worn, broken or both. Of these, 137 (59.1%) were first dorsal spines and 95 (40.9%) were second dorsal spines. Therefore, the first dorsal spine was chosen for whole examination and the second dorsal spine for sectioning since broken

spines could not be used. The second dorsal spine was embedded in polyester casting resin (Fiberglass International, Sydney, Australia). Transverse sections were cut close the interface between the lumen and the pulp cavity (see Chapter 2 for illustration), but great care was taken to include part of the pulp cavity in the section. Transverse sections (~ 400 μm) were made using a low speed saw (Accutom Struers, Copenhagen, Denmark) equipped with a diamond edge wafering blade (Pace Technologies, Arizona, USA). Sections were then washed in fresh water, mounted individually onto glass slides and sealed with cover slips. The first dorsal spine was stored dry in paper envelopes for whole (external band pair) examination. Complete details of the dorsal spine preparation are described in section 2.2 of this thesis.

4.2.3 Validation

A total of 35 *H. portusjacksoni* were collected from Nelson Bay (32°43′05″*S*, 152°08′42″*E*) in the north to Jervis Bay (35°02′24″*S*, 150°40′33″*E*) in the south from May 2004 to March 2006. All animals were housed at Oceanworld Manly Aquarium facilities. Complete details of the collection, housing, and injection of chemical markers for validation are described in section 3.2 of this thesis.

After euthanased dorsal spines were removed (see Chapter 2.2) and stored in black containers for examination. Second dorsal spines were embedded and sectioned as described above. A dissecting microscope (Leica Mz 12) equipped with a digital camera (Axiocam HRC, Zeiss), UV filter (360 nm) and blue filter (470 nm), were used to visualise the fluorescent injection in dorsal spines. The number of completed band pairs after the initial post-dye (oxytetracycline (OTC) and calcein) injected date were counted to determined the periodicity of band pair formation (Simpfendorfer *et al.*, 2002b).

4.2.4 Ageing

The relationship between dorsal spines and length of female and male *H. portusjacksoni* were not significantly different and therefore the sexes were combined. Complete details of the analysis are described in section 2.3.2.3 of this thesis.

A readability score was assigned to whole and sectioned dorsal spines (Table 3.2). The first reading scored all samples for readability. Any sample with a readability score < 3 was excluded from the rest of the experiment. If two readings differed by > 2 band pairs, a third reading was conducted. If the third reading was within ≤ 2 band pairs of one of the previous readings, those two readings were used for further analysis. If the results of the third reading differed by ≥ 3 band pairs of either of the previous readings, the dorsal spine was excluded.

True age was set as the number of total band pairs - birth band + months after date of birth (August 1st) to capture (see section 3.3.2 in this thesis for further description). The original von Bertalanffy growth equation was fitted to the age data:

$$L_t = L_\infty (1 - e^{-k(t-to)}),$$

where L_t is the length at time t, L_∞ is the theoretical asymptotic size, t_0 is the theoretical age at zero length, and k is the rate at with L_∞ is achieved.

Band pair counts on whole dorsal spines started from the base of the cap (youngest band pairs) and continued to the apex (oldest band pairs). Band pair counting was performed under a dissecting microscope (Nikon, SMZ645) with reflected light. A band pair was defined as one opaque and translucent zone, ridge or both on the external surface

of the cap (McFarlane and Beamish, 1987b) (Plate 4.3). Only band pairs that were visible and whole on both sides of the anterior cap were counted and the counting was restricted to the non-wear point to remove any bias from worn band pairs.

Sections of second dorsal spines were used for age estimation. Band pair counting started at the interface of the two dentine layers and continued towards the pulp cavity. Band pair counting was done under a dissecting microscope (Nikon, SMZ645) with transmitted light. Only translucent band pairs that were visible and unbroken in the dentine layer were counted. The inner dentine layer was used for age estimation because it was wider than the outer dentine layer and therefore it was considered easier to distinguish between band pairs (Plate 2.15). A band pair was defined as a dark (opaque) and a lighter (translucent) zone (Irvine *et al.*, 2006a).

4.2.5 Analyses

F_L was plotted towards S_{BW} to determine the proportional relationship between somatic growth and dorsal spine growth. To test for sexual differences between F_L and S_{BW} the analysis of covariance (ANCOVA) was calculated.

4.2.5.1 Comparison of Methods and Structures

A comparison of within-reader bias and precision and between-method bias and precision was undertaken to compare the accuracy of whole and sectioned dorsal spine readings.

Dorsal spine band pairs (one opaque and one translucent) in whole and sectioned dorsal spines were counted on two occasions (three months apart) without knowledge of size, sex or previous estimates of age. This was achieved by labelling each sample with a

random number (unrelated to sex, length and date of capture) and randomising the reading order on each occasion.

The same two analyses estimating bias, as used in section 3.2.5.2 were calculated to test for difference between the mean reading and the estimated age using dorsal spines. To test for the absence of random error, analysis (3) Coefficient of Variance (CV), in section 3.2.5.2 was used to determine the consistency of the interpretation of band pairs.

To test the method (whole and sectioned) and structure (dorsal spines and vertebral centra) which best described *H. portusjacksoni* age analyses (1) to (3) were all modified by substituting the readings (Rd_1 and Rd_2) with readings from whole and sectioned dorsal spines, respectively. Analysis of covariance (ANCOVA) was calculated to test for differences between the methods slops of the regression line for age versus length.

4.3 Results

4.3.1 Validation

Incorporation of OTC and calcein varied among the 35 individuals (Plate 4.1). Four neonates (197 to 217 mm F_L) injected with OTC and euthanased 49 days later showed no fluorescent band. However, a juvenile (338 mm F_L) injected with OTC and euthanased 84 days later showed a strong fluorescent band with additional post-growth. Calcein fluorescent bands were visible in the dorsal spine of a juvenile (411 mm F_L) just 59 days after injection.

There was a significant linear relationship between the number of completed band pairs incorporated after the time of injection and years in captivity (Figure 4.1) indicating that band pairs formed annually. All neonates immersed in calcein immediately after birth

showed two clear fluorescent marks in their sectioned dorsal spines (Plate 4.2) (n = 4). The first fluorescent mark was incorporated between the mantel and the external edge of the outer dentine layer in the stem of the sectioned dorsal spine while the second fluorescent mark was incorporated on the edge of the inner dentine layer towards the pulp cavity of the dorsal spine. This confirms that the dorsal spines of *H. portusjacksoni* have two dentine layers.

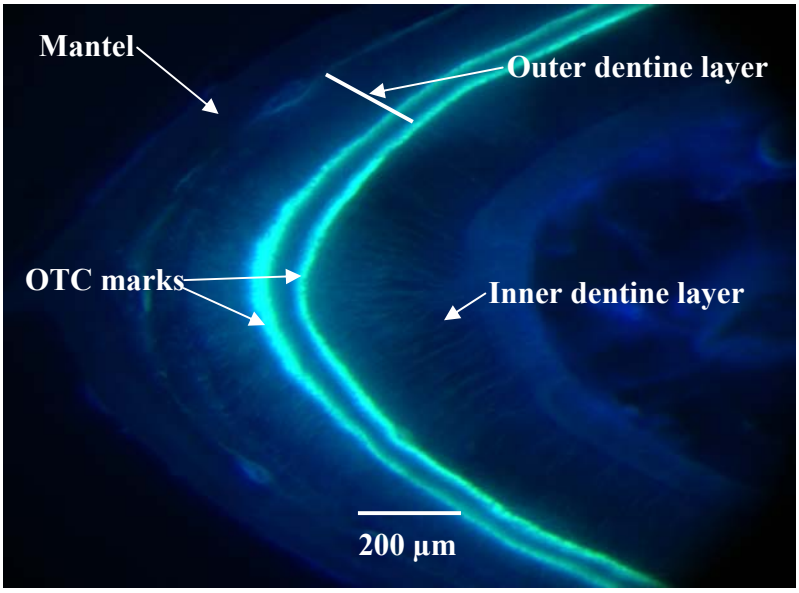


Plate 4.1: Photomicrograph of a sectioned dorsal spine and its OTC fluorescent marks in the two dentine layers from a 475 mm F_L male *H. portusjacksoni* injected on September 29th 2005 and euthanased 369 days later. OTC marks are from the same injection and are incorporated in the two different dentine layers lying internally from the mantel.

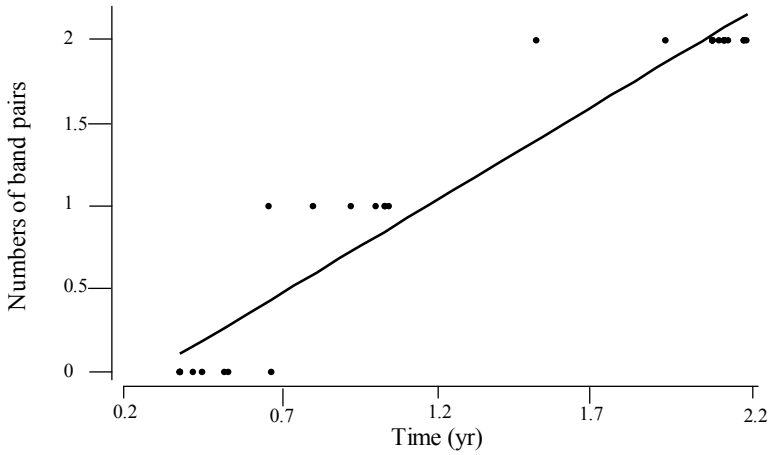


Figure 4.1: Number of completed band pairs incorporated after the time of injection of OTC or calcein in 35 sectioned dorsal spines from *H. portusjacksoni* showing pattern of annual band pair formation. Number of band pairs = -0.49 + 1.19(Time), r^2 = 96.7%, ANOVA, $F_{1,26}$ = 755.1, $p <$ 0.001.

Sharks injected in the winter months (August to September) all showed a fluorescent mark close to, or on top of, the narrower translucent band. The opposite was true for animals injected in summer (November to March), giving florescent marks in opaque bands.

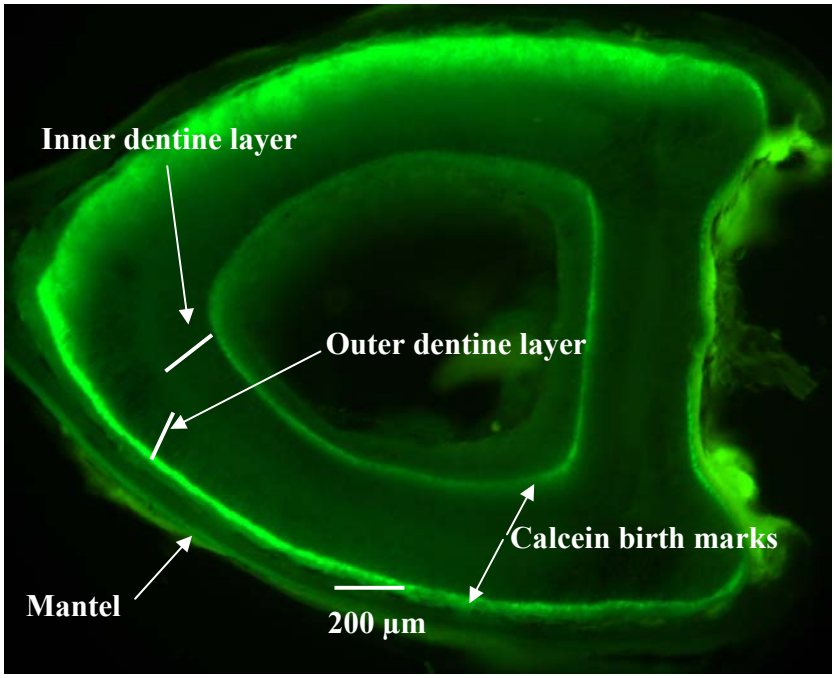


Plate 4.2: Photomicrograph of a sectioned dorsal spine with two calcein fluorescent marks incorporated in the two different dentine layers lying internally from the mantel from a 280 mm F_L female *H. portusjacksoni* immersed on May 20th 2006 and euthanased 137 days later.

The dentine layer grows inwards to the centre of the spine leaving the older growth towards the end of the layer (Plate 4.3).

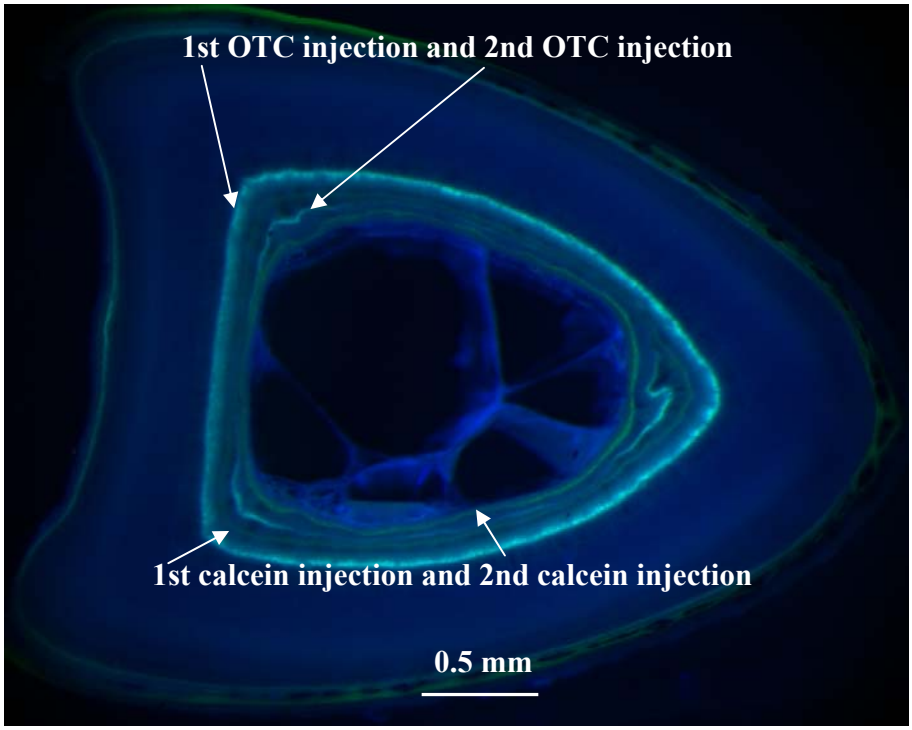


Plate 4.3: Photomicrograph of a sectioned dorsal spine and its OTC and calcein fluorescent marks in the two different dentine layer growth zones from a 413 mm F_L female H. portusjacksoni injected with OTC in May 04 and November 04, and calcein in August 04 and February 05, before euthanased in May 05.

4.3.2 Whole Dorsal Spines

A total of 615 whole dorsal spines from *H. portusjacksoni* were initially examined, and of these 72.2% had a suitable readability score, CV and band pair precision allowing further analysis. A total of 208 females (185 to 1197 mm F_L) and 236 males (196 to 1003 mm F_L) were successfully aged. The first translucent band was considered to be the birth mark and was withdrawn from the final real age. Band pairs were easily distinguished on the anterior surface of the cap (Plate 4.4).

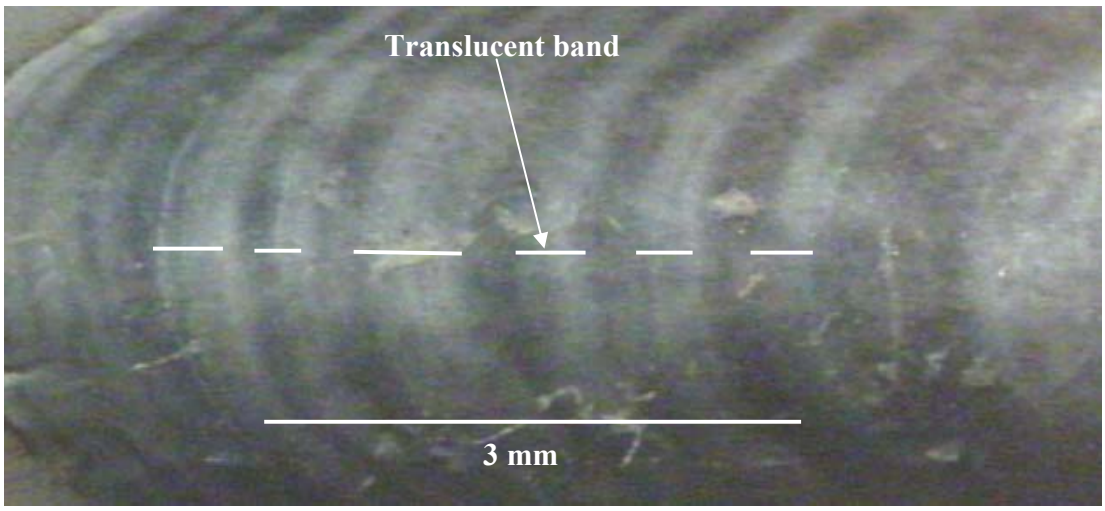


Plate 4.4: Photomicrograph of whole dorsal spine of *H. portusjacksoni* (880 mm F_L male) shows the anterior part of the enamel with its band pairs.

Age ranged from 0 (185 mm F_L) to 30 (1197 mm F_L) years for females, and 0 (196 mm F_L) to 29 (901 mm F_L) years for males. *H. portusjacksoni* ranging from 185 to 257 mm F_L (mean ±S.E. = 225 ± 4.7 mm) showed no band pairs and were assigned to the 0+ age class (Figure 4.2). The relationship between age and length had a low fit (r^2 = 58.7%) and showed great variability in individuals older than 7 years of age (shown by the wide scatter of points). the asymptotic length was also unrealistically high. The average bias between readings was -0.019 ±0.009. Average bias was significantly different from 0 (T = -2.01, p = 0.045). However, the age-bias plot indicating no variation between the two readings which was supported by the age-bias plot showed no systematic variation in bias and little variation outside the 1:1 relationship line (Figure 4.3), indicating that there was no difference between band pairs in the first and second reading. The average precision within readings (CV) was 3.45%.

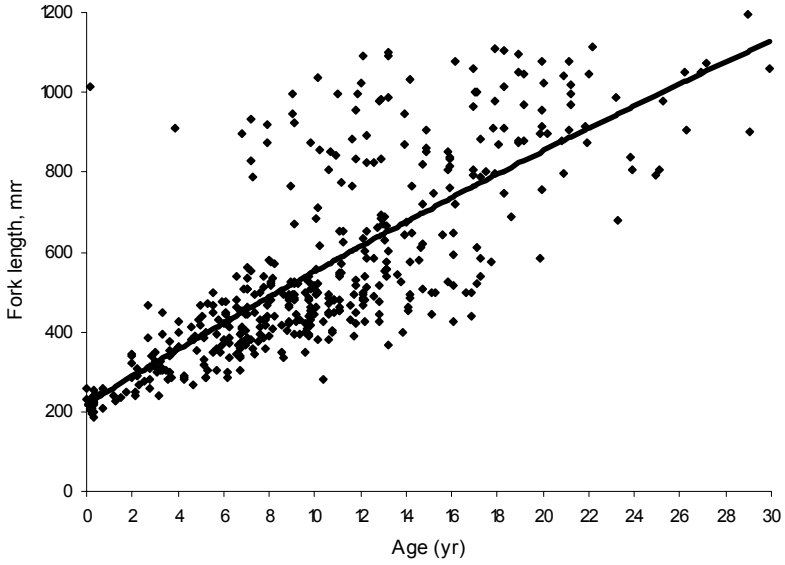


Figure 4.2: Length at age estimated from *H. portusjacksoni* first whole dorsal spine analysis for both sexes. L_{∞} = 3926.5 mm F_L, k = 0.009 year^{-1} and t_0 = -6.11 years.

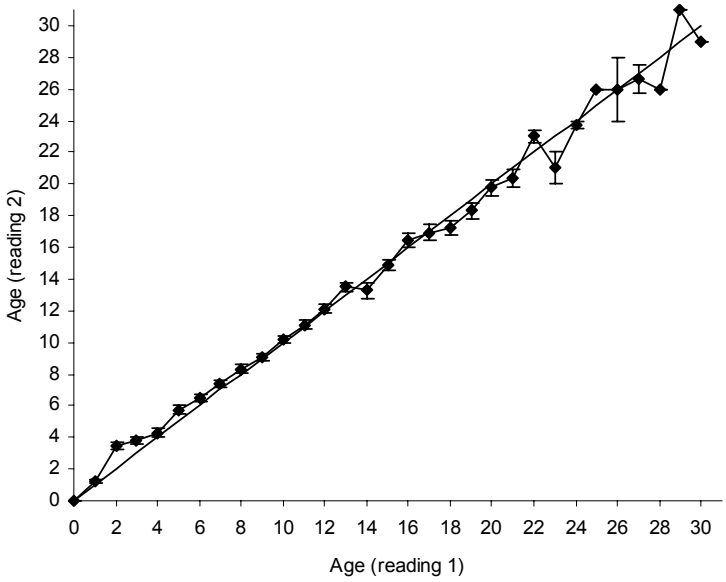


Figure 4.3: An age-bias plot with standard error and a 1:1 relationship line (sexes combined) for *H. portusjacksoni* comparing the ages estimated using whole dorsal spines in the first and second reading (n = 444).

4.3.3 Sectioned Dorsal Spines

A total of 619 sectioned dorsal spines from *H. portusjacksoni* were initially examined, and of these 82.6% had a suitable readability score, CV and band pair precision allowing further analysis. A total of 253 females (185 to 1197 mm F_L) and 258 (196 to 1003 mm F_L) males were successfully aged. The first translucent band was validated to be the birth mark and was withdrawn from the final real age (Plate 4.5).

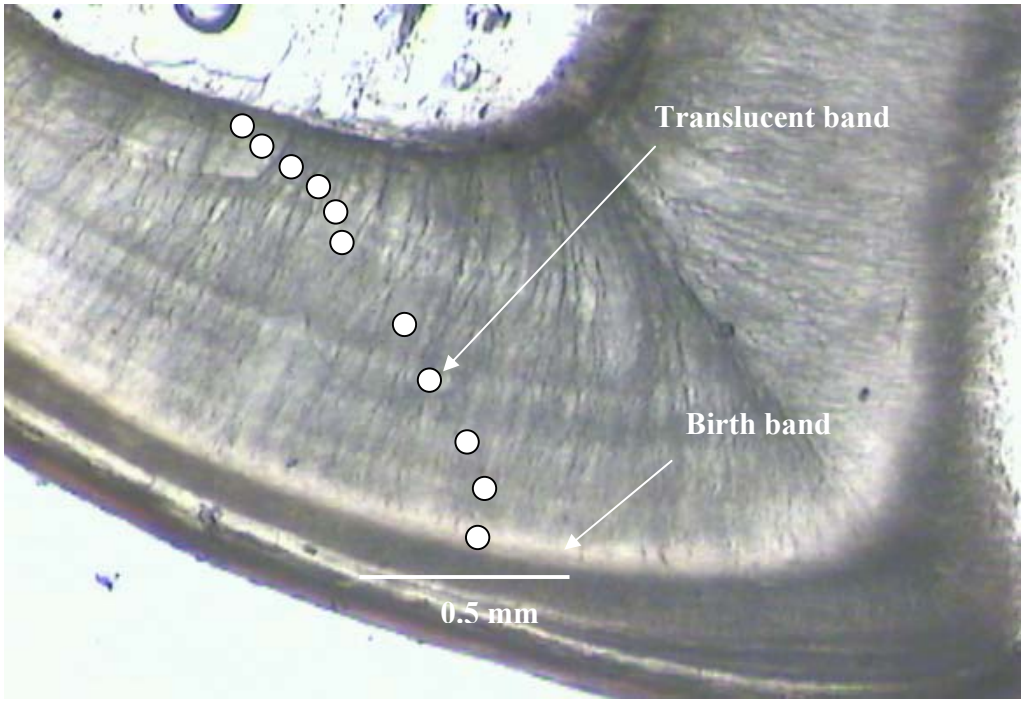


Plate 4.5: Photomicrograph of a sectioned of second dorsal spine from a 683 mm F_L male *H. portusjacksoni* showing a real age of 10. Open circles show the translucent counted bands.

Age ranged from 0 (185 mm F_L) to 29 (1020 mm F_L) years for females, and 0 (196 mm F_L) to 34 (910 mm F_L) years for males. *H. portusjacksoni* ranging from 185 to 281 mm F_L (223 ±3.4 mm) showed no band pairs and were assigned to the 0+ age class (Figure 4.4). The relationship between age and length had a low fit (r^2 = 78.9%) and showed great variability in individuals older than 5 years of age (shown by the wide scatter of points). The asymptotic length was also higher than the actual data. The average bias between readings was 0.011±0.008. Average bias was not significantly different from 0 (T = 1.32, p = 0.19). the age-bias plot indicating no variation between the two readings which was supported by the age-bias plot showed no systematic variation in bias and little variation outside the 1:1 relationship line (Figure 4.5), indicating that there was no difference between band pairs in the first and second reading. The average precision within readings (CV) was 6.69%.

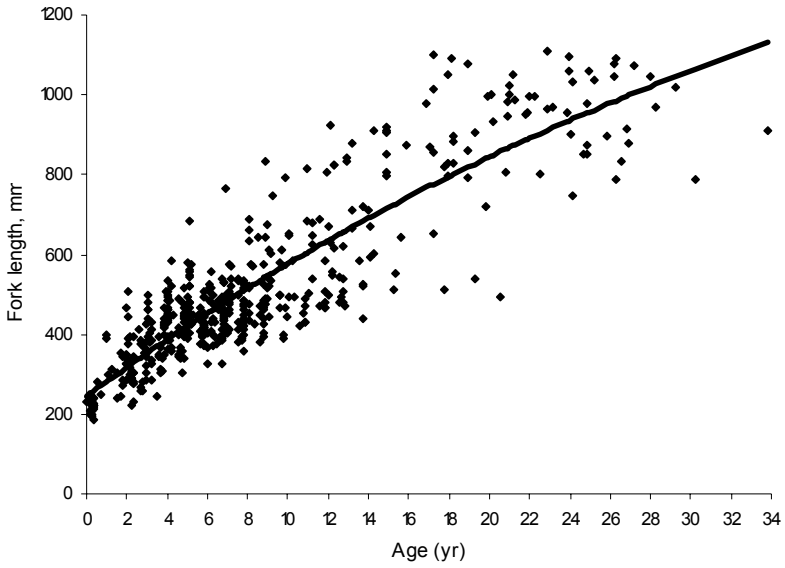


Figure 4.4: Length at age estimated from *H. portusjacksoni* sectioned dorsal spine analysis for both sexes. L_∞ = 1962.8 mm F_L, k = 0.022 year^{-1} and t_0 = -6.22 years.

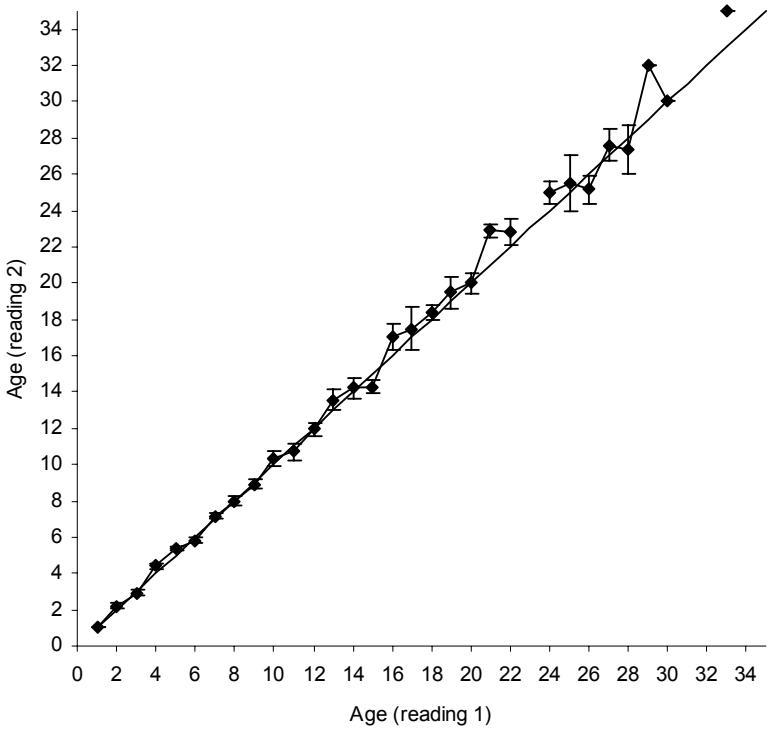


Figure 4.5: An age-bias plot with standard error and a 1:1 relationship line *H. portusjacksoni* (sexes combined) comparing the ages estimated using sectioned dorsal spines in the first and second reading (n = 511).

4.3.4 Comparison Between Methods

The age of 374 dorsal spines determined from whole and sectioned dorsal spines were compared. For whole dorsal spines age ranged from 0+ to 32 years (10.2 ±0.28 years) and for sectioned dorsal spines age ranged from 0+ to 34 years (9.1 ±0.36 years). There was a significant difference between the two methods (ANCOVA; $F_{1,951} = 4.34$, $p < 0.05$). The average bias between methods was -0.22 ±0.026 which was significantly different from 0

(T = -8.47, p <0.001). This indicated that whole dorsal spines returned lower ages than sectioned dorsal spines. This was supported by the age-bias plot indicating whole dorsal spines ageing individuals younger than 13 years higher than sectioned dorsal spines. However, sectioned dorsal spines aged individuals older than 13 years higher than whole dorsal spines (Figure 4.4). It also indicates that as individuals get older the age difference between the two methods increases. The average precision (CV) between methods was 73.09%.

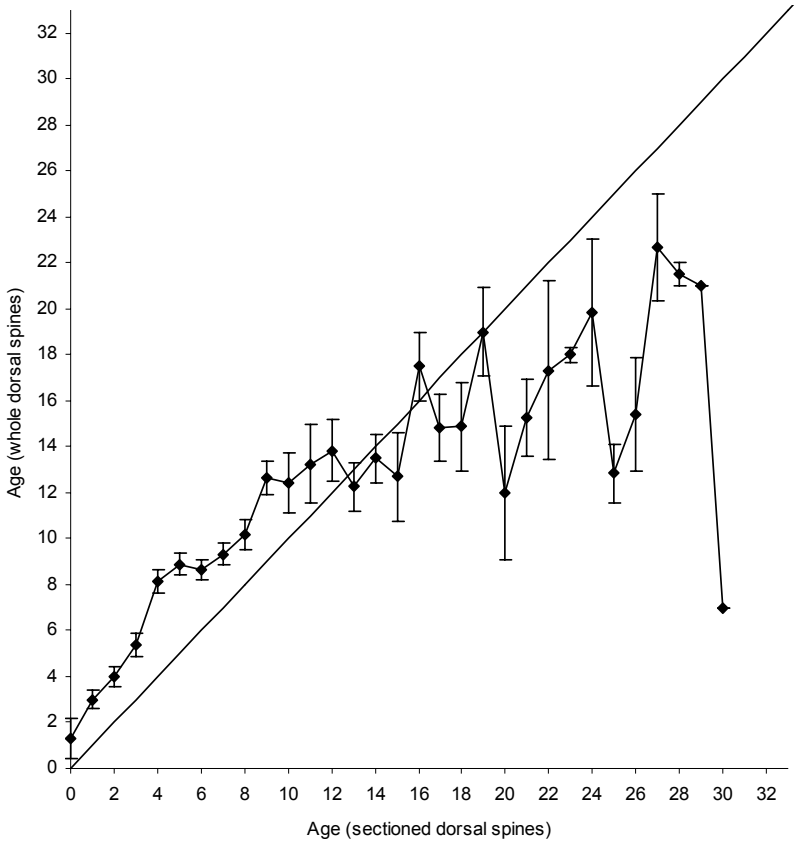
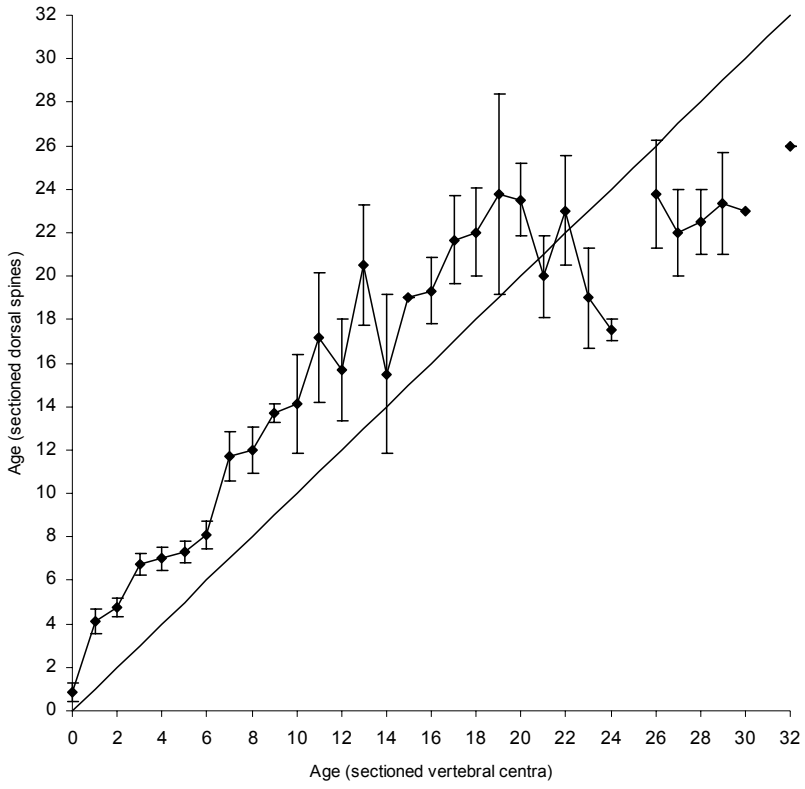


Figure 4.4: An age-bias plot with standard error and a 1:1 relationship line for *H. portusjacksoni* comparing the ages estimated using sectioned and whole dorsal spines (n = 374).

4.3.5 Comparison Between Vertebral Centra and Dorsal Spines

The age estimated from 249 sectioned dorsal spines and sectioned vertebral centra from the same sharks were compared. For sectioned dorsal spines age ranged from 0+ to 34 years (10.9 ±0.47 years) and for sectioned vertebral centra age ranged from 0+ to 32 years (8.3 ±0.48 years). There was a significant difference between the two structures (ANCOVA; $F_{1,485} = 75.05$, $p < 0.001$). The average bias between the two structures was -0.4 ±0.03 which was significantly different from 0 ($T = -13.06$, $p < 0.001$). This indicated that sectioned vertebral returned lower ages than sectioned dorsal spines. This was supported by the age-bias plot indicating sectioned dorsal spines ageing individuals younger than 21 years higher than sectioned vertebral centra. However, sectioned vertebral centra aged individuals older than 21 years higher than sectioned dorsal spines (Figure 4.5). It also indicates that as individuals get older the age difference between the two structures increases. The average precision (CV) between methods was 74.7%.



F**igure 4.5:** An age-bias plot with standard error and a 1:1 relationship line for *H. portusjacksoni* comparing estimated using sectioned vertebral centra and sectioned dorsal spines (n = 249).

4.4 Discussion

4.4.1 Validation

Both OTC and calcein were used to validate the annual formation of dorsal spine band pairs and showed a clear florescent mark in sectioned dorsal spines. Date of injections and the position of the fluorescent marks incorporated in the dorsal spines, together with the number of post-injected band pairs, all suggest an annual pattern of band pair formation.

Translucent bands form during the slower growth period of the winter months (August to September), while the opaque band pairs are formed during the faster period of growth during the summer months (November to March). This annual pattern of band pair formation in the dorsal spines was identical to the results reported when using vertebral centra. OTC was incorporated in the two dentine layers between 49 and 84 days post-injection. OTC vanishes on exposure to light and calcein is visible under normal light. Calcein has been used successfully in several elasmobranch species (Walker *et al.*, 1995; Gelsleichter *et al.*, 1997; McAuley *et al.*, 2006). It is therefore recommended that calcein be used for age validating elasmobranchs.

OTC has also been successfully used to validate the existence of annual band pairs in sectioned dorsal spines of *S. acanthias* (Beamish and McFarlane, 1985). These authors found one distinct OTC mark in the inner dentine layer however reported two different dentine layers of growth. Similar results were found by Tucker (1985), reporting annual band pairs in the enamel of *S. acanthias* with formation of dark opaque bands in summer. Tovar-Avila (2006) validated the age estimation technique of *H. portusjacksoni* in the southern part of Australia using individuals ranging from 377 to 970 mm T_L and therefore missing the neonates and the formation of the birth mark. And although his results, using both captive and wild animals, correspond to this study, the low number (n = 12) and size range limited the conclusion of the validation (Campana, 2001).

Although validation is becoming more commonly used as a technique for vertebral centra (Davenport and Stevens, 1998; Goldman *et al.*, 2006; Natanson *et al.*, 2006), it is still excluded from age estimation using dorsal spines (Clarke *et al.*, 2002b; Braccini *et al.*, 2007). As analyse such as MIR can be more or less excluded as a validation technique for

this structure, the use of chemicals such as OTC and calcein as a validation method should be a vital part of any age estimation using dorsal spines.

4.4.2 Age Estimation

Age estimation using whole dorsal spines was successful in all age classes ranging from 0+ to 30 years. Band pairs on whole dorsal spines were visible in the external cap from the base to the apex. The initial reading showed a relatively high readability score of 72.2%, suggesting that this method is suited for age estimation. Bias analyses showed some difference between the two readings. However, the differences between the two readings were not systematic and the variation was minor. A high consistency of interpretation was found between the two readings. Again this indicates that the method is suitable for ageing *H. portusjacksoni*. Although, there was a low band pair count bias and a high precision, the high percentage of worn and broken spines in sharks over 600 mm F_L and the lack of pigmentation on dorsal spines from sharks less than 400 mm F_L, makes this technique highly vulnerable to underestimation of the true age in this species.

Holden and Meadows (1962) and Ketchen (1975) were the pioneers of using dorsal spines to estimate age in elasmobranchs. Although both only used whole dorsal spines for their age estimation they established the first techniques for using this structure in age estimation. Beamish and McFarlane (1985) found annual band pairs on the external surface of the second dorsal spines of *S. acanthias* and successfully estimated the age of the species. Braccini *et al.* (2007) reported from their age estimation of whole dorsal spines of the *S. megalops* that the first dorsal spine gave better readability scores and a more precise reading than that of the second.

Age estimation using sectioned dorsal spines was successful in all age classes ranging from 0+ to 34 years. Band pairs in sectioned dorsal spines were visible in both the inner and outer dentine layer. The initial reading showed a relatively high readability score of 82.6%, indicating that this method is suitable for age estimation. Bias estimates showed little difference between two readings and a high consistency of the interpretation (CV), again indicating that the method is suited for ageing *H. portusjacksoni*. Tanaka (1990) found that the inner dentine layer gave the most precise age estimation in C. *acus* and established the technique for ageing sectioned dorsal spines. Both Clarke *et al.* (2002a) and Clarke *et al.* (2002b) continued to use sectioned dorsal spines to estimate the age of *C. squamosus* and *D. calceus*. Braccini *et al.* (2007) concluded that when using sectioned dorsal spines, the first dorsal spine was most suitable of age estimation.

The external and internal features of the dorsal spines and the techniques used to prepare them for age estimation is a source of bias that studies have to include and minimise. There were a high percentage of worn dorsal spines within the sample however, none were worn all the way down to the pulp cavity. Therefore worn spines can be used for age estimation in this species. However, the majority of the broken dorsal spines were damaged below the pulp cavity. Fortunately band pairs are visible in most part of the upper stem, so sections with band pair formation could be sampled from these specimens. It was considered easy to find the start and end of the two different dentine layers and the precision of band pair counts were high. However, great care should be taken when sectioning the dorsal spines. The optimal section is just below the pulp cavity, which is hard to find. A section above the pulp cavity in the lumen will exclude the younger band pairs, and a section too far below the pulp cavity will compress the band pairs. However the effects on spine growth of broken dorsal spines is unknown and might bias the ageing,

suggesting that great care should be applied when using broken spines to avoid underestimating the age.

Although the external features of dorsal spines of Squaliformes and Heterodontiformes appear very similar, the number of dentine layers is different. Several authors have reported three dentine layers in dorsal spines from *S. acanthias, C. acus* and *E. baxteri* (Holden and Meadows, 1962; Beamish and McFarlane, 1985; McFarlane and Beamish, 1987b; Tanaka, 1990; Irvine *et al.*, 2006a). However the results given here show that there are two distinct dentine layers in the dorsal spines of *H. portusjacksoni* as found in *C. squamosus* and *S. megalops* (Clarke *et al.*, 2002a; Braccini, 2006).

4.4.3 Comparison of Methods

Comparing the ages estimated by the two methods (whole dorsal spines versus sectioned dorsal spines) showed a significant difference between the slopes of whole and sectioned dorsal spines ageing. It also showed that whole dorsal spines on average estimated higher band pair counts than did sectioned dorsal spines, however the maximum age was higher for sectioned dorsal spines. There was also a significant bias between the two methods and coefficient of variance was high. The age-bias plot showed that whole dorsal spines overestimated the age of younger *H. portusjacksoni* and as individuals got older the age difference reversed. Together with the age-bias plot, all analyse agreed that sectioned dorsal spines on average age higher than whole dorsal spines and should be used for future age estimation on *H. portusjacksoni*.

Irvine *et al.*(2006a) compared whole and sectioned dorsal spines of *E. baxteri* and found that whole dorsal spines had a higher precision and gave higher band pair counts than those that had been sectioned. They used the second dorsal spine for examination for its

bigger size and less wear. Their result is not reflected by this research and might be related to wear of the dorsal spine. Their results showed that only 11% of *E. baxteri* had worn or broken spines compared to 27% in *H. portusjacksoni* (this study). This might be explained by the different habitat preference of the two species and therefore different ageing techniques gave the better result in each study.

4.4.4 Comparison of Structures

The comparison between the two different structures (sectioned second dorsal spines and sectioned vertebral centra) showed a significant difference between the structures. It also showed that sectioned dorsal spines on average estimated higher band pair counts than did sectioned vertebral centra. There was also a significant bias between the two structures and coefficient of variance was high. The age-bias plot showed that sectioned dorsal spines overestimated the age of younger *H. portusjacksoni* and as individuals got older the age difference reversed. When comparing the r^2 values for the age at length regression between the two structures, the dorsal spine regression line was 15.2% lower for females and 11.3% lower for males compared to the regression line derived using vertebral centra. Together with the age-bias plot, all analyse agree that sectioned vertebral centra provided greater estimates of age than sectioned dorsal spines and should be used for future age estimation on *H. portusjacksoni*.

Unfortunately all other elasmobranch species studied so far that possess dorsal spines have weakly calcified vertebra centra and has therefore not been able to compare the age estimates between the two structures.

4.5 Conclusion

It was considered easy to identify the individual band pairs in both whole and sectioned dorsal spines. Both OTC and calcein gave visible marks in the dorsal spines of *H. portusjacksoni*. They indicated that wide opaque bands were incorporated in the dorsal spines in summer and that the narrow translucent bands were incorporated in winter. Fluorescent marks also showed that *H. portusjacksoni* has two dentine layers in its dorsal spines compared to some squalid species that have three.

Both whole and sectioned dorsal spines were used successfully to estimate age. While both methods were accurate, sectioned dorsal spines are recommended since they do not underestimate the true age of older individuals as occurs with whole dorsal spines. Analyse of the two structures (sectioned dorsal spines and sectioned vertebral centra) used to age this species indicated that vertebral centra are a more reliable structure than dorsal spines for age estimation, and it is recommended that sectioned vertebral centra are used for future age estimation of *H. portusjacksoni*.

Chapter 5

A 6 hours old juvenile Port Jackson shark resting in the hand of the author (photo by the author)

Chapter 5

A Comparison of Age-Growth Models for the Port Jackson Shark (*Heterodontus portusjacksoni*).

5.1 Introduction

The understanding of age structure and growth rates of any population is vital to the aim of management and conservation. It gives an idea of size and age at maturity, maximum size and age, and the potential production. Fisheries around the world rely on precise age and growth determinations for their stock assessment and management of fishing practise (Musick and Bonfil, 2004).

Growth curves describe the mathematical relationship between the size of an animal and time. Several growth models and variations of growth models exist for determining growth parameters in fishes, where the Gompertz (1825) and von Bertalanffy (1938) are the most commonly applied (Summerfeldt and Hall, 1987). Although often criticised, the determination of a proper model to accurately describe the growth dynamics of a wide variety of species was achieved by von Bertalanffy (1938). The original von Bertalanffy Growth Equation (VBGE) has been the most widely applied growth equation to fisheries age and growth studies since it was introduced to the industry by Beverton and Holt (1957):

$$L_t = L_\infty (1 - e^{-k(t-to)}), \quad (1)$$

where L_t is the length at time t, L_∞ is the theoretical asymptotic size, t_0 is the theoretical age at zero length, and k is the rate at with L_∞ is achieved.

This model has been popular with former and present fisheries scientists because it is not just a mathematical model however it describes the biological growth as the physical balance between the two forces of anabolism and catabolism. A modified two-parameter version of the original VBGE was introduced by Fabens (1965). He removed the parameter t_0 in VBGE and replaced it with L_0 because of two reasons. The parameter t_0 is an artificial factor defining the age at which the animal would be of zero length if it had the same growth factor as in the post-larval phase and because L_0, length-at-age-at-birth, is generally more easier to obtain (Stevens, 1975). This model is popular with species that have small individuals which are hard to sample.

Other growth models have been applied to fish populations in the following years such as the Gompertz Growth Function (GGF) (Gompertz, 1825). The GGF was initially used to describe larval and early life history on fishes (Ricker, 1979) and in elasmobranchs has mostly been applied on rays and skates (Mollet *et al.*, 2002). The GGF is known to describe juvenile's S-shape growth better than any other model (Lucifora *et al.*, 2004). Goldman (2004) suggested that this model might better describe the growth of elasmobranchs which hatches from egg capsules, while others have suggested that this model might be the most appropriate model for elasmobranchs in which body mass is the main contributor to growth instead of length (Wintner *et al.*, 2002). The same arguments that were used for von Bertalanffy on the parameter t_0, exist for Gompertz. And therefore a more popular two-parameter version of the GGF using L_0 is normally applied to fisheries (Mollet *et al.*, 2002).

The growth coefficients k and L_∞ derived from VBGE have a reverse relationship (Beverton and Holt, 1959), where k describes the average growth rate at which one individual in the population will achieve its maximum size and L_∞ is the maximum size. k is an important factor and has even been used to evaluate the potential vulnerability of a species (Musick, 1999; Araya and Cubillos, 2006). Slow growth in elasmobranchs is associated with a late maturity and long life span. k values in elasmobranchs vary from 0.016 $year^{-1}$ in the female Rhinobatidae, *Rhinobatus productos*, to 1.34 $year^{-1}$ in male *R. taylori* (Cailliet and Goldman, 2004). Species with k-value $\leq$ 0.1 $year^{-1}$ are considered to be vulnerable having generally slow growth, high longevity and reduced litter size (Wintner *et al.*, 2002).

Sharks from the order Squaliformes, which together with Heterodontiformes are all oviparous, has a k-value from 0.039 $year^{-1}$ for female *Squalus mitsukurii* of the coast of Japan to 0.2 $year^{-1}$ for male *S. acanthias* in the Black Sea, with a mean over the order of less than 0.1 $year^{-1}$ (Cailliet and Goldman, 2004), indicating that this order of elasmobranchs are vulnerable. There is no published research on growth models for any of the other Heterodontiformes, and none from the New South Wales population of *Heterodontus portusjacksoni*. Growth parameters are used by fishery scientists to establish catch quotas for the world's fishery and are therefore vital biological information that needs to be estimated. Without the knowledge of growth rates or longevity the fishery is working blind.

The aim of the present study was to (1) examine the growth dynamics using vertebral centra and dorsal spines and (2) compare the fit of different growth models to *H. portusjacksoni* inhabiting the waters of central New South Wales, Australia.

5.2 Materials and Methods

5.2.1 Sampling and Measurements

H. portusjacksoni were sampled from commercial prawn and fish trawlers operating from Newcastle, New South Wales, Australia (32° 55′05″*S*, 151°45′37″*E*). Vertebral centra and second dorsal spines were then cleaned of excess tissue and cut in ~ 400 µm longitudinal sections and aged under a dissection microscope with transmitted light.

Age data from *H. portusjacksoni* were derived from sectioned vertebral centra 20 and from second sectioned dorsal spines. Shark ages were determined from the vertebrae centra of 300 sharks consisting of 133 females (210 to 1115 mm F_L) and 167 males (197 to 1003 mm F_L). Ages were also determined from the second dorsal spines of 527 sharks, consisting of 262 females (185 to 1197 mm F_L) and 265 males (196 to 1003 mm F_L). Complete details of the age data are described in Chapter 3 and 4 of this thesis.

5.2.2 Growth Models

Several growth models were fitted to the length-at-age data for the separate sexes to compare the fit of each model. To determine the model parameters by least-squares non-linear regression, the software SOLVER in Microsoft Excel 2002 (Microsoft, Redmond, Washington, USA) was used (White *et al.*, 2002; Skomal and Natanson, 2003; Siegfried and Sanso, 2006). The traditional von Bertalanffy Growth Equation (VBGE) (1) was fitted to collected data on length-at-age. The modified two-parameter VBGE (hereafter called 2VBGE), was used for comparison (Fabens, 1965; Cailliet *et al.*, 1992; Araya and Cubillos, 2006):

(2) $$L_t = L_\infty - (L_\infty - L_0)\, e^{-kt},$$

where L_0 is the length at birth. L_0 was calculated for each sex and structure as the mean F_L of all year 0+ (n = 43).

Since *H. portusjacksoni* both hatch from egg capsules and growth more in weight than length (see Figure 5.1), the alternative Gompertz Growth Function (GGF) as described by Ricker (1975) was used to compare the growth curves:

(3) $$L_t = L_\infty\, e^{-e(-k(t-to))},$$

where the parameters are the same as for VBGE.

A more popular two-parameter form of the GGF (hereafter called 2GGF) described by Mollet *et al.* (2002) was also used for comparison:

(4) $$L_t = L_0\, (e^{G\,(1-e\,(-kt))}),$$

where $G = \ln (L_\infty/L_0)$.

5.2.3 Statistical Analyses

Length-weight regressions were constructed for each sex and Analyses of Covariance (ANCOVA) was used to test the null hypothesis that the two regression relationships were not significantly different.

To determine which model gave the best fit of the length-at-age data Akaike's Information Criterion (AIC) was used (Soriano *et al.*, 1992; Quinn and Keough, 2002):

$$AIC = n \ln(\sigma^2) + 2p,$$

where n is the sample size, σ is the residual sum of squares between the real data and the estimated data from each model and p is the number of parameters.

The model with the lowest AIC value and highest coefficients of determination (r^2) was selected to have the best fit of the data. To compare the difference in fit between models, the ΔAIC and Akaike weights (w_i) were calculated. ΔAIC was the differences between the AIC for a model and the AIC of the best described model. Akaike weight was calculated to determine the probability of selecting the best model out of the candidates (Quinn and Keough, 2002):

$$w_i = (e^{(-\Delta AIC/2)})/\sum(e^{(-\Delta AIC/2)}),$$

where ΔAIC is the difference between the AIC for a model and the AIC for the best describing model.

The higher the ΔAIC, the better the probability that the right model was chosen. A One-way analyses of variance (ANOVA) was used to test if there were a significantly different between the r^2 values.

5.2.4 Annual Growth

Annual growth was calculated from sectioned vertebra centra and sectioned dorsal spines for both sexes and divided in juvenile and adult stages. For sectioned vertebra centra, female juvenile size ranged from 210 to 935 mm F_L (0 to 18 years) when the species is thought to mature (Powter, 2007), while female adult size ranged from 935 to 1090 mm F_L (18 to 33 years). Male juvenile size ranged from 197 to 790 mm F_L (0 to 12 years) when the species is thought to mature (Powter, 2007), while male adult size ranged from 790 to 904 mm F_L (12 to 24 years). For sectioned dorsal spines, female juvenile size ranged from 185 to 935 mm F_L (0 to 18 years) when the species is thought to mature (Powter, 2007), while female adult size ranged from 935 to 1020 mm F_L (18 to 29 years). Male juvenile size ranged from 196 to 790 mm F_L (0 to 14 years) when the species is thought to mature (Powter, 2007), while male adult size ranged from 790 to 910 mm F_L (14 to 34 years).

5.2.5 Longevity

Band pair counts provided an estimate of initial longevity, and since there was no commercial fishing on this species, there was no concern of underestimation (Walker *et al.*, 1998). As a comparison for the real age-data, theoretical longevity was estimated at which 95% of L_∞ was reached from the equation: $5\ln 2/k$ (Fabens, 1965; Piercy *et al.*, 2007).

5.2.6 Length-Frequency Distribution

H. portusjacksoni length-frequency distributions were obtained from extensive fish trawls surveys conducted with the commercial fishing boat *Avalon Star* off the Newcastle region (32° 55′05″*S*, 151°45′37″*E*) using a 90-150 mm mesh fish trawl. The surveys were carried out from September 2003 to June 2006. Length-data was allocated to 5 cm bins between 150-200 and 1150-1200 mm F_L. Fork length was used instead of total length for analysis, since wear occurred on the caudal fin. Sexes were combined for modal analysis. The modes identified were assumed to be age classes.

Monthly length-frequency histograms were developed for all months throughout the year. ELEFAN I (Simpfendorfer, 1993; Jackson *et al.*, 2000) was used to estimate the VBGE parameters from the population (Natanson *et al.*, 2002). Data for females and males were analysed separately. Specimens were pooled in months between years to provide sufficient sample size for each month.

5.3 Results

H. portusjacksoni length (F_L) and weight (T_W) were significantly related in both sexes and grew more in weight than in length (Figure 5.1). The slopes relationships between F_L and T_W for females (maximum 1197 mm F_L and 19500 g T_W) and males (maximum 1003 mm F_L and 8400 g T_W) showed a significant difference between the sexes (ANCOVA; $F_{1,695}$ = 234.3 $p < 0.001$), so sexes were separated for further analyses.

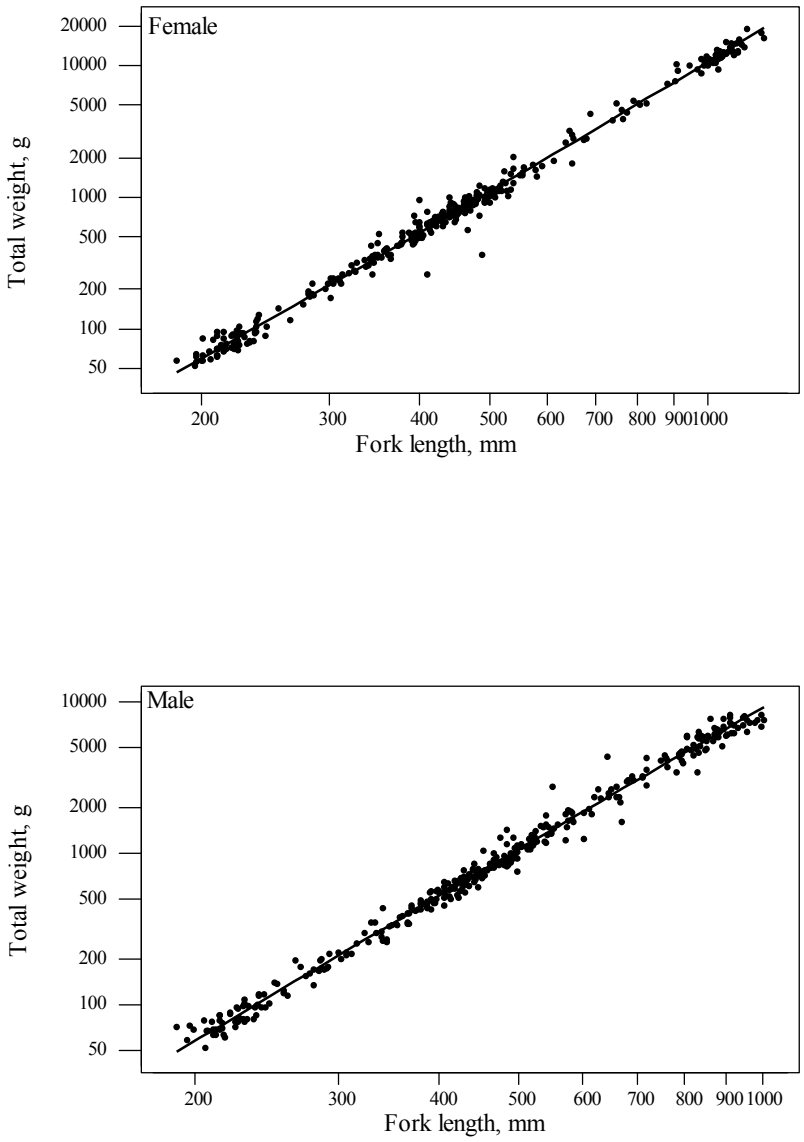


Figure 5.1: The relationship between fork length and total weight for females *H. portusjacksoni* ($\log T_W = -4.74 + 2.53(\log F_L) + 0.13(\log F_L)^2$, $r^2 = 99.2\%$, $F_{1,347} = 42659.44$, $p < 0.001$) and for males ($\log T_W = -6.18 + 3.68(\log F_L) - 0.09(\log F_L)^2$, $r^2 = 99.1\%$, $F_{1,348} = 38595.79$, $p < 0.001$).

5.3.1 Vertebral Centra

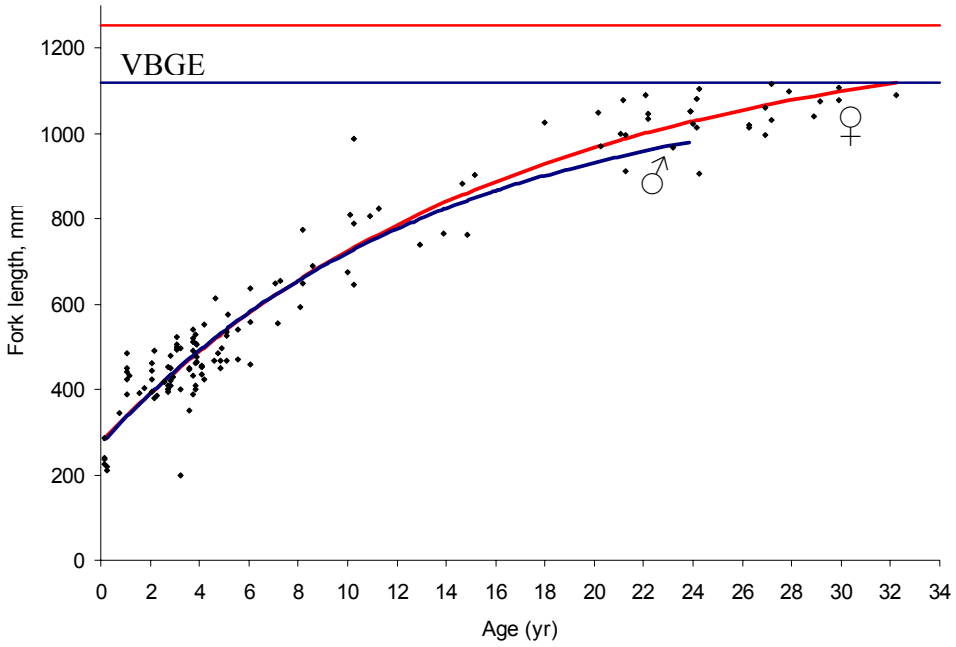
5.3.1.1 Growth Curves

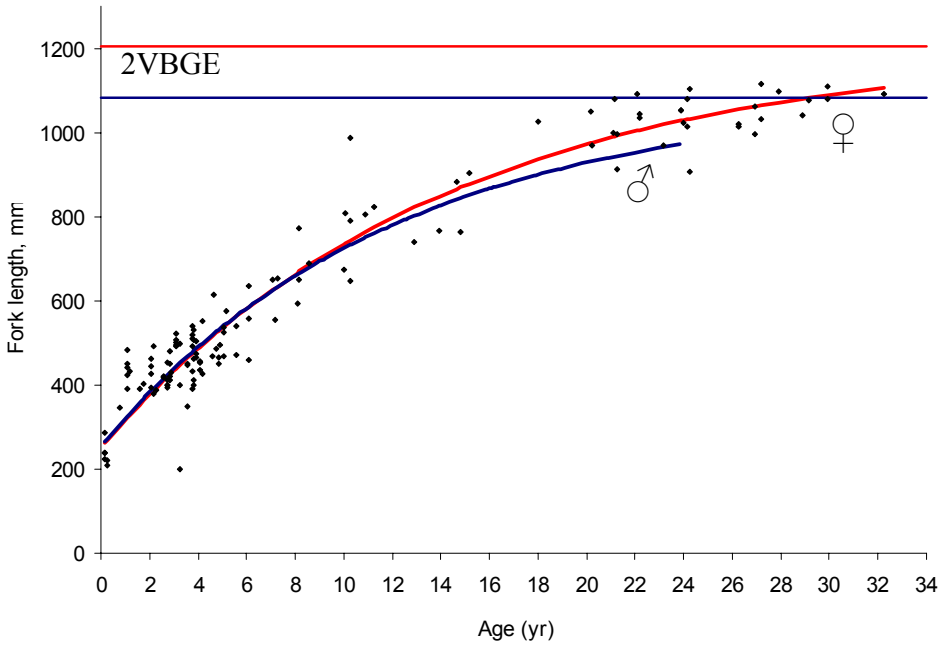
All growth models fitted the age-at-length data well for both sexes (Table 5.1). All models showed high coefficients of determination ($r^2 \geq 0.90$) and no significant differences between residuals (ANOVA; $p > 0.9$). The GGF described the growth of both sexes of *H. portusjacksoni* better than any of the other models with w_i values of 96.61% and 84.82% for females and males, respectively, indicating high probability that the best fitted model was chosen. However, ΔAIC was generally lower for males, indicating that male growth on average was better described by all growth models. Also there was little difference between VBGE and 2VBGE models, indicating that both described male *H. portusjacksoni* similarly (Table 5.1).

Growth parameters derived from the growth model with lowest AIC and highest r^2 (GGF) differed between the sexes, with females attaining a larger size and slower growth than males. Therefore, females had a lower growth coefficient ($k = 0.11$ year^{-1}) and higher asymptotic size ($L_\infty = 1134.14$ mm F_L) than males ($k = 0.13$ year^{-1}and 1012.95 mm F_L, respectively). However, in younger *H. portusjacksoni* up to 9.25 years of age, their growth was similar (Figure 5.2).

Table 5.1: Growth estimates and model selection criterion for male and female *H. portusjacksoni* derived from sectioned vertebral centra. Also shown are Akaike's information criterion (AIC); AIC differences between models (ΔAIC); Akaike weights (w_i); and sample size (*n*). L_0 = 252 and 254 mm F_L for female (*n* = 7) and male (*n* = 6), respectively.

Model	Parameter	Females (*n* = 129)					Males (*n* = 166)				
		Estimate	r^2	AIC	ΔAIC	w_i	Estimate	r^2	AIC	ΔAIC	w_i
VBGE	L_∞ (mm F_L)	1252.57	0.94	3408.37	6.79	3.39%	1118.51	0.90	4458.85	4.74	7.93%
	k (year^{-1})	0.061					0.076				
	t_0 (years)	-4.067					-3.675				
2VBGE	L_∞ (mm F_L)	1204.06	0.94	3411.64	10.06	0.63%	1082.99	0.90	4459.04	4.93	7.21%
	k (year^{-1})	0.071					0.084				
GGF	L_∞ (mm F_L)	1134.14	0.94	3401.58	0	96.61%	1012.95	0.90	4454.11	0	84.82%
	k (year^{-1})	0.111					0.131				
	t_0 (years)	2.583					1.629				
2GGF	L_∞ (mm F_L)	1083.26	0.94	3428.26	26.68	1.55×10^{-4} %	963.02	0.90	4469.37	15.26	0.04%
	k (year^{-1})	0.141					0.161				


VBGE
Fork length, mm
Age (yr)
♀
♂


2VBGE
Fork length, mm
Age (yr)
♀
♂

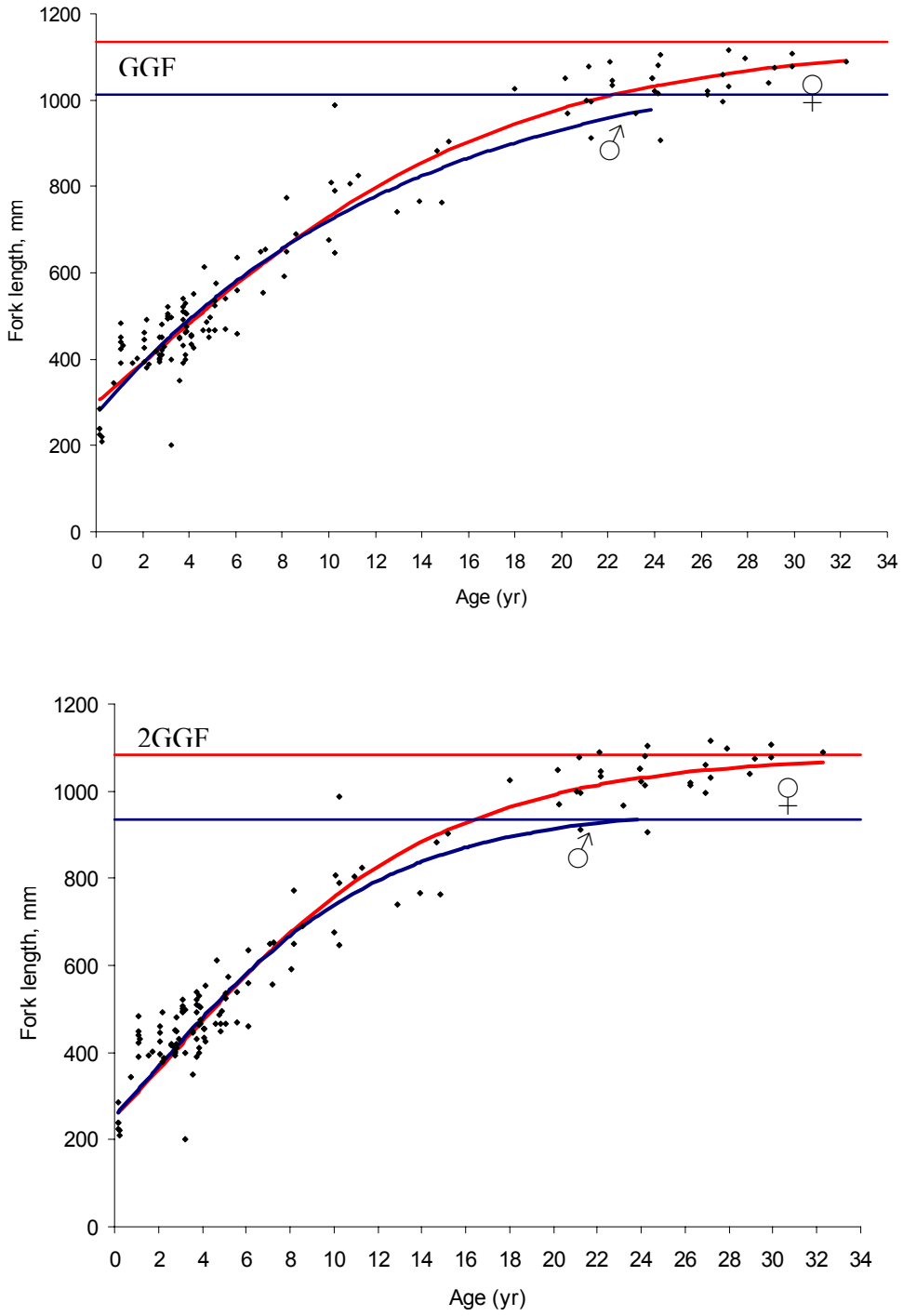


Figure 5.2: The predicted growth and asymptote of female (♀/ red) and male (♂/ blue) *H. portusjacksoni* using the Von Bertalanffy Growth Equation (VBGE), two-factor von Bertalanffy Growth Equation (2VBGE), Gompertz Growth Function (GGF) and two-factor Gompertz Growth Function (2GGF) model based on length-at-age data from sectioned vertebral centra.

5.3.1.2 Annual Growth

The annual growth for female juveniles was calculated to 40.28 mm year^{-1} and 10.33 mm year^{-1} for adults. Annual growth for male juveniles was calculated to 49.4 mm year^{-1} and 9.5 mm year^{-1} for adults.

5.3.1.3 Longevity

Based on the direct age estimates from Chapter 3, the oldest female was 32.3 years and the oldest male 23.8 years. Using the growth coefficients from VBGE (k) parameter, the theoretical longevity (Ricker, 1975) was estimated to be 31.2 years and 26.5 years for females and males respectively.

5.3.2 Dorsal Spines

5.3.2.1 Growth Curves

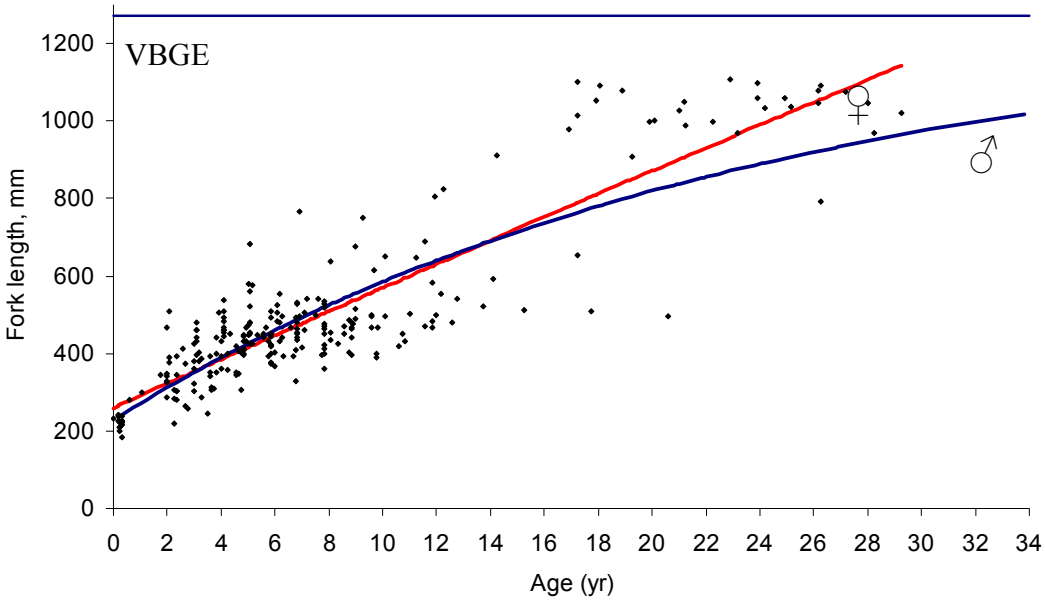
Each growth model fitted the age-at-length data well (Table 5.2). All models showed high coefficient of determination ($r^2 \geq 0.79$) and no significant differences between residuals (ANOVA; $p > 0.9$). The GGF described the growth of both sexes of *H. portusjacksoni* better than any of the other models with w_i values of 79% and 89.11% for females and males, respectively, indicating a high probability that the best fitted model was chosen. However, ΔAIC was generally lower for males in all models except VBGE, indicating that male growth on an average was better described by all growth models (Table 5.2). The only exception for this was for females.

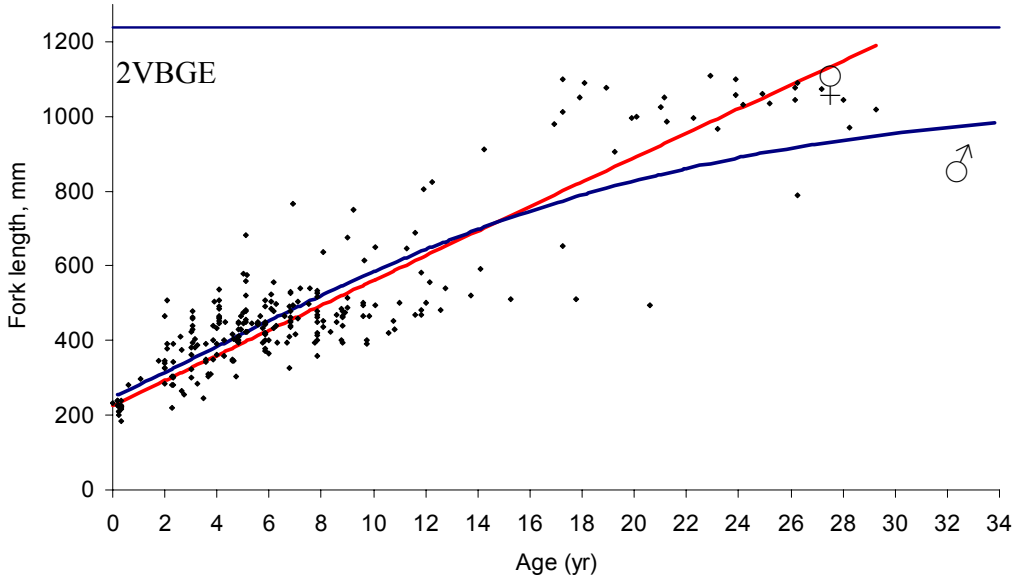
Growth parameters derived from the growth model with lowest AIC and highest r^2 (GGF) differed between the sexes. Females had a lower growth coefficient (k = 0.05 year^{-1})

and higher asymptotic size (L_∞ = 1731.23 mm F_L) than males (k = 0.089 $year^{-1}$and 1051.91 mm F_L, respectively). However, up to 15.3 years of age males grew faster than females. In older animals females exceeded the growth of males (Figure 5.3).

Table 5.2: Growth estimates and model selection criterion for males and females *H. portusjacksoni* derived from sectioned dorsal spines. Also shown are Akaike's information criterion (AIC); AIC differences between models (ΔAIC); Akaike weights (w_i); and sample size (*n*). L_0 = 225 and 221 mm F_L for female (*n* = 18) and male (*n* = 12), respectively.

Model	Parameter	Females (*n* = 253)					Males (*n* =258)				
		Estimate	r^2	AIC	ΔAIC	w_i	Estimate	r^2	AIC	ΔAIC	w_i
VBGE	L_∞ (mm F_L)	10646.17	0.8	7433.5	2.65	21%	1271.15	0.79	7519.78	7.11	2.55%
	k (year^{-1})	0.003					0.042				
	t_0 (years)	-8.102					-4.734				
2VBGE	L_∞ (mm F_L)	16543.68	0.79	7457.43	26.58	$1.34x10^{-4}$ %	1238.54	0.79	7518.18	5.51	5.67%
	k (year^{-1})	0.002					0.044				
GGF	L_∞ (mm F_L)	1731.23	0.80	7430.85	0	79%	1051.95	0.80	7512.67	0	89.11%
	k (year^{-1})	0.05					0.09				
	t_0 (years)	12.206					4.082				
2GGF	L_∞ (mm F_L)	1302.28	0.79	7459.3	28.45	$5.25x10^{-4}$ %	994.95	0.79	7519.68	7.01	2.68%
	k (year^{-1})	0.078					0.106				


VBGE
Fork length, mm
Age (yr)
♀
♂


2VBGE
Fork length, mm
Age (yr)
♀
♂

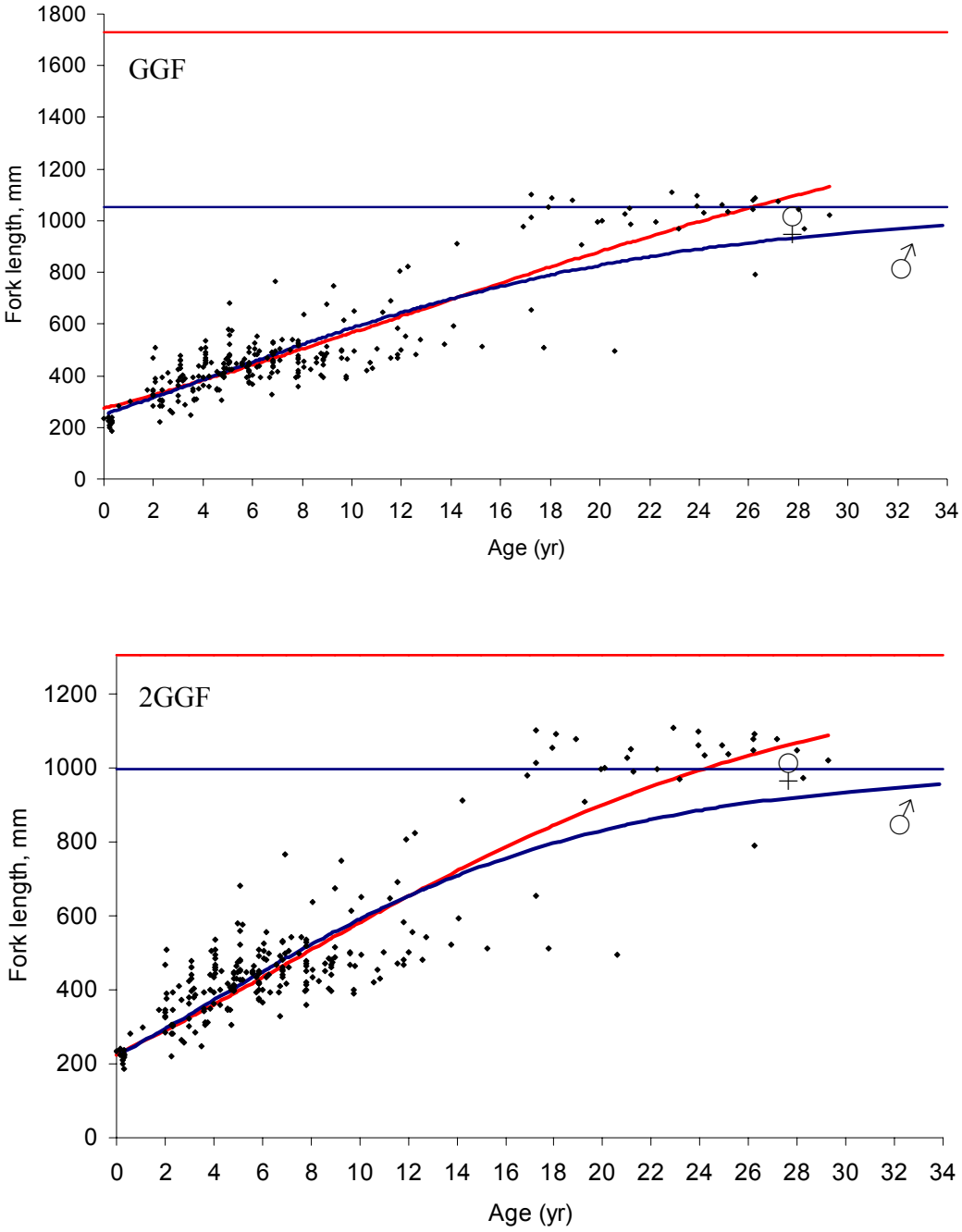


Figure 5.3: The predicted growth and asymptote of female (♀/ red) and male (♂/ blue) *H. portusjacksoni* using the Von Bertalanffy Growth Equation (VBGE), two-factor von Bertalanffy Growth Equation (2VBGE), Gompertz Growth Function (GGF) and two-factor Gompertz Growth Function (2GGF) model based on length-at-age data from sectioned dorsal spines.

5.3.2.2 Annual Growth

The annual growth for female juveniles was calculated to 41.67 mm year^{-1} and 7.73 mm year^{-1} for adults. Annual growth for male juveniles was calculated to 42.43 mm year^{-1} and 6.0 mm year^{-1} for adults.

5.3.2.3 Longevity

Based on the direct age estimates from Chapter 4, the oldest female was 29.3 years and the oldest male 33.8 years old. Using the growth coefficients from VBGE (k) parameter, the theoretical longevity (Ricker, 1975) was estimated to be 69.31 years and 38.59 years for females and males respectively.

5.3.3 Length-Frequency Distributions

Monthly length-frequency histograms showed a clear progression of juvenile's size-classes for *H. portusjacksoni* (Figure 5.4). Early year-class modes can be tracked through the monthly length-frequency samples. Juveniles are born at about 200-250 mm F_L, mainly in August-September and following the year-class modes to June-July, where they exit the class at 300-325 mm F_L. However, modes were less apparent in later age groups.

Using ELEFAN I gave the VBGE parameter L_∞ = 1207.5 mm F_L and k = 0.42 year^{-1} and L_∞ = 1050 mm F_L and k = 0.17 year^{-1} for females and males respectively.

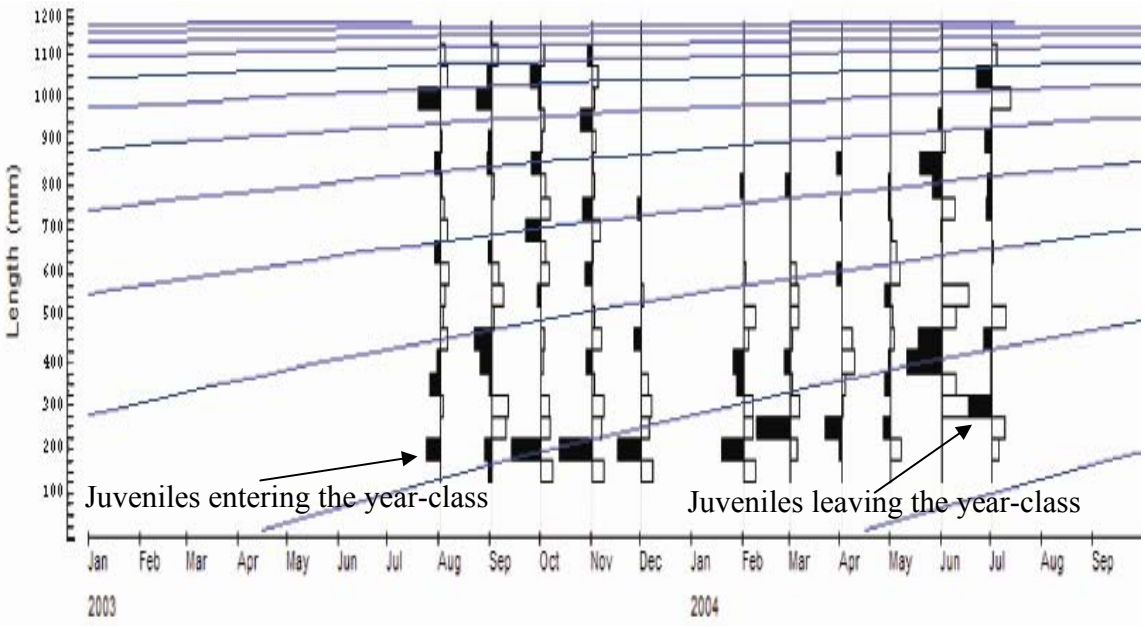


Figure 5.4: Length-frequency histograms with 5 mm bin sizes derived from ELEFAN I for sexes combined. Blue lines are VBGF curves (n = 1569).

5.4 Discussion

Length-weight relationship indicated that although sexes growth slopes were different they both grew more in weight than in length.

5.4.1 Vertebral Centra

When using vertebral centra to describe the growth of *H. portusjacksoni*, the Gompertz growth model best described the growth for both females and males. The main difference between the two Gompertz growth models and the von Bertalanffy growth models for both sexes, were that the Gompertz models gave a lower asymptotic size (L_∞) than both the von Bertalanffy models. The opposite was evident looking at k, which were calculated lower using the von Bertalanffy models than the Gompertz models. The one parameter that is

better biologically described with the VBGE than GGF is the theoretical age at zero length (t_0). The GGF gave a positive t_0 value, indicating that the age at length zero is after birth. The VBGE calculated a negative value of t_0, indicating that the age at length zero is before birth, giving more biological sense. However, with an incubation period of between 10 and 11 months (Rodda, 2000), this would indicate a t_0 value of around -1. Although the VBGE model gives a negative value for t_0, this value is too large. As discussed by Fabens (1965) and Stevens (1975) t_0 is an artificial factor and therefore not much weight was given to it when choosing the best growth model. Therefore the GGF was chosen to best describe the growth of *H. portusjacksoni*. The longevity calculated using L_∞ was not much different from the direct age estimated from sectioned vertebral centra and could therefore be used as a reliable estimate. Minor differences was determined in both AIC and r^2. For females there was a low difference in AIC between GGF and VBGE, while in males there were low differences in AIC between all models except 2GGF. Both sexes indicated no difference between r^2 for any of the other models. Although the smaller differences in AIC and r^2, the growth parameters GGF and the second best fitted growth model differed by 118.4 and 105.6 mm F_L, and 0.05 and 0.06 in growth, indicating that minor differences between model fit could give greater differences in growth parameters.

The growth parameters derived from the GGF model using sectioned vertebral centra were similar to *Mustelus manazo* of the coast of Japan with growth parameters L_∞ = 1341 T_L and k = 0.113 year^{-1}, and L_∞ = 1137 T_L and k = 0.124 year^{-1} for females and males, respectively (Yamaguchi *et al.*, 1996).

5.4.2 Dorsal Spines

The Gompertz growth function was also the best model to describe growth based on estimates of age from dorsal spines. This was especially evident for males. The *k*-value was higher and the L_∞ was lower for both the Gompertz models. Although female L_∞ was not just lower as for vertebral centra, L_∞ calculated with both the von Bertalanffy models gave an unrealistic high value, overestimating the asymptotic length. Even the GGF gave a relatively high L_∞. As for t_0, although the VBGE was negative it was as GGF, too high. The longevity calculated using L_∞ was clearly different from the direct age estimated from sectioned dorsal spines and could therefore not be used as a good estimate. However it is again worth mentioning that there was minor differences in both AIC and r^2 when choosing the model. For females there was a low difference between GGF and VBGE, while in males there were hardly and difference between any of the models. Both sexes indicated low differences between r^2 for any of the other models. Although the smaller differences in AIC and r^2, the growth parameters GGF and the second best fitted growth model differed by 8914.4 and 219.2 mm F_L, and 0.001 and 0.05 in growth, again indicating that minor differences between model fit could give greater differences in growth parameters.

The growth parameters for females derived from the GGF model using sectioned dorsal spines were similar to *M. manazo* of the coast of Japan with growth parameters L_∞ = 1765 T_L and k = 0.07 $year^{-1}$ (Caillet *et al.*, 1990) and the growth parameters for males where similar to those from *S. mitsukurii* of the coast of Japan with growth parameters L_∞ = 1093 T_L and k = 0.066 $year^{-1}$ (Taniuchi and Tachikawa, 1999).

5.4.3 Growth Models

Both vertebral centra and dorsal spines clearly indicated that the GGF described the growth of *H. portusjacksoni* better than any of the other growth models. The GGF indicated that growth was similar for both sexes up to 9.25 years old. Thereafter, females grew faster and to a larger size. Growth parameters derived from the GGF model suggested that female *H. portusjacksoni* attained a larger size (1134.14 mm F_L) and age (32.25 years) than did males (1012.95 mm F_L, 23.83 years) as reported in other elasmobranchs such as *Carcharhinus taurus, C. plumbeus, C. obscurus, C. carcharias* (Wintner and Cliff, 1999; Simpfendorfer *et al.*, 2002b; Goldman *et al.*, 2006; Licandoe *et al.*, 2006; Romine *et al.*, 2006). Cailliet and Goldman (2004) reported a range in *k* between 0.016 $year^{-1}$ to 1.34 $year^{-1}$ and a longevity between 5+ years for male *Spyrna tiburi* to 70 years for female *Centrophorus squamosus*. Which indicate that *H. portusjacksoni* has an intermediate growth rate compared to other elasmobranchs and reach a relatively old age. The popularity of VBGE was illustrated by Cailliet *et al.* (2006). They reviewed 28 of the most recent chondrichthyan growth studies and found that only four had compared and used more than the VBGE, the GGF was evaluated in only three of those studies. Mollet *et al.* (2002) estimated age and growth for captive *Dasyatis violacea* and compared VBGE to GGF. They concluded that GGF gave more reasonable parameters and was therefore better overall compared to VBGE. The opposite was reported by Braccini *et al.* (2007) investigating growth of *Squalus megalops.* They concluded that out of five growth models compared, GGF was considered the third best fit for both sexes after a two-phase VBGE and the original VBGE. Because of the wide range of elasmobranchs, no one growth model can best describe the growth of all elasmobranchs. This study, together with the ones

discussed above, all indicate that several models should be compared when estimating growth of elasmobranchs.

The sensitivity to the number of larger age groups is an important aspect and was tested by successive removing out the older individuals. However, none of the models indicated any sensitivity as the fit was more or less unchanged after the removal of individuals older than 20 years.

McLaughlin and O'Gower (1971) reported with caution that the annual growth rate of adults derived from recaptured wild *H. portusjacksoni* was 20 to 40 mm for adults and 50 to 60 mm for juveniles, which is higher than this study regarding both juveniles and adults for both structures. This might have occurred because of the different techniques and difficulties in measuring growth in long lived species such as elasmobranchs. Powter (2007) again estimated growth from *H. portusjacksoni* recaptured during field surveys and found juvenile annual growth to be 28.2 and 36.8 mm year^{-1} for females and males, respectively, and adult annual growth to be 34.2 and 36.4 mm year^{-1} for adult females and males, respectively. These estimates are again different to this study's estimate for both juveniles and adults. Juvenile annual growth was higher in this study while adult annual growth was lower. Since both studies used the same length of maturation, only the techniques and methods can explain the different results. It is important to mention that few growth studies calculate annual growth since this measurement is highly variable within the species. It is therefore more reliable to use the *k*-value when comparing between populations and species. The *k*-value reported in this study is in the lower to middle range of *k*-values reported for other elasmobranchs (Cailliet and Goldman, 2004). Tovar-Avila (2006) use dorsal spines to establish growth curves for *H. portusjacksoni* from Victoria and reported *k*-values of 0.07 and 0.084 for females and males, respectively. These values are

similar to the values found in this study using dorsal spines. Tovar-Avila (2006) also reported an L_∞ that was within range of the calculated value for males found in this study. However, the L_∞ for females was well below that calculated in this study using dorsal spines (1242 mm T_L). Although the difference could be due to reading techniques and experience in ageing, it also suggests the possibility of geographical differences in growth rates, and therefore the existence of different populations.

The large differences in results between the estimated values from sectioned dorsal spines and the observed asymptotic length (especially for female in this study) and based upon the results in Chapter 3 and 4, indicate the use of vertebral centra when estimating growth curves. When vertebral centra were used in this study, there was a large difference between this study and the results of Tovar-Avila (2006). This might be explained by the lack of individuals less than 3 years old for females and 2 years old for males in Tovar-Avila's (2006) study, giving his lowered *k*-value and higher L_∞.

Length-frequency modes were clearly identified in juveniles however, they were less discrete in adults. This is normally found in elasmobranchs because of the slow adult growth (Simpfendorfer, 1993). Simpfendorfer (1993) used length-frequency analysis to estimate growth for *R. taylori* and concluded that this method of estimating growth parameters was only effective in 0+ individuals because of the rapid decline in annual growth after 0+-age class. Francis and Mulligan (1998) indicated that using length-frequency analysis on *Galeorhinus galeus* over an age rage of 0 to 9 years gave similar growth curves and growth parameters as estimated when using length-at-age data from vertebral centra band pair counts. The VBGE parameters estimated from ELEFAN I gave L_∞ values that did not differ much from the calculated values using GGF. Although there was not much different in *k*-values for males, the *k*-value was almost four times higher for

females. So although using length-frequency modes for adults *H. portusjacksoni* was difficult, length-frequency analysis could be effective for estimating juvenile growth parameters however. Growth model to compare results and great caution should be applied when choosing the parameters.

5.5 Conclusion

Growth models were successfully applied to age estimates derived from sectioned vertebral centra and dorsal spines of *H. portusjacksoni*. All growth models gave a relatively good fit, however the Gompertz growth function gave the best biological and mathematical fit for both sexes and structures indicating that this species attain a maximum 1134 and 1013mm F_L and have a growth of 0.11 and 0.13 mm $year^{-1}$ for females and males, respectively. As concluded in previous chapters, the use of sectioned vertebral centra is preferred when estimating the growth of this species.

This research indicates that even small differences in fit between different models can result in large differences in growth parameters which are used for fisheries purposes. Great care should be taken when choosing a growth model and both mathematical and biological considerations should be applied.

Chapter 6

Port Jackson sharks resting under a ledge at Broughton Island (photo by the author)

Chapter 6

Validation of the Reading Techniques for Ageing the Port Jackson Shark (*Heterodontus portusjacksoni*).

6.1 Introduction

The importance of validating age estimation techniques was stressed by Beamish and McFarlane (Beamish and McFarlane, 1983) and since their report, many authors have included validation in their research. However, validation of the different steps involved in age estimation (i.e. periodicity of band pair formation, bias between estimations and readers) is usually not often found in the literature. Verification of the ageing structures and validation of their band pair formation is performed more often than validation of the reading techniques. Exceptions to this include studies comparing two or more readers ageing similar or different structures (Moulton *et al.*, 1992; Francis and Maolagain, 2000; Conrath *et al.*, 2002; MacNeil and Campana, 2002; Braccini, 2006; Carlson *et al.*, 2006; Tovar-Avila, 2006; Piercy *et al.*, 2007).

Inter-observer differences in age estimations could arise from several sources. The reading technique is one such source. The uncertainty about the types of bands to be counted can lead to over- or under-estimation. Sulikowski *et al* (2007) found no difference in readings between two readers using vertebral centra ageing *Raja texana*. The low bias between readers indicated an acceptable level of precision. Simpfendorfer *et al.*(2000) on the other hand reported difference in reading results between four different readers with up to 1.5 higher band pair counts for one of the readers ageing *Furgaleus macki* using vertebral centra. Another potential source of variation is reader experience. Few researchers

have tested the importance of the experience level of the reader. Without any prior knowledge or a lack of experience in reading growth structures, inexperience may lead to over- or under-estimating the age of the sample, which could give rise to a poor if not false age-structured population model. Officer *et al.* (1996) reported significant differences in increment counts in vertebrae from *Mustelus antarcticus* and *Galeorhinus galeus* between experienced and inexperienced readers. They found that counts of experienced readers were more precise and less biased. Although, it is generally thought that a high level of experience is needed to estimate the age of any hard structure without high bias and with great precision (Officer *et al.*, 1996; Campana, 2001), the amount of training needed to eliminate bias and strengthen precision to an acceptable degree is unknown.

Bias is defined as the difference between the mean of the age estimations and an accepted reference value and is often reported using an age-bias plot (Campana *et al.*, 1995). Precision is the lack of random error or the reproducibility of a repeated count on a given structure and can be a measure of the statistical variance of the age estimation, and is often calculated as the Coefficient of Variation (CV) (Chang, 1982). When bias and precision are combined to define the performance of an age estimate, accuracy is the value between the estimated and true age (Campana, 2001; Walther and Moore, 2005; Cailliet *et al.*, 2006).

Sulikowski *et al.* (2007) reported no bias and 4.8% CV between two experienced readers. Natanson *et al.* (2006) found no bias and 10.8% CV between two ageing laboratories, and no bias and 2.8% CV between two counts estimating the age of *Isurus oxyrinchus*. Campana (2001) reviewed 117 fish ageing studies and reported a mean CV of 7.6%. Mean CV for shark ageing studies using vertebral centra mostly exceeded 10%, which were different than average CV between different structures (Campana, 2001).

Campana (2001) reported that many ageing studies could be carried out with a CV value less than 7.6% or ass low as 5% as recommended by many ageing laboratories.

Both vertebral centra and dorsal spines have been shown (Chapter 3 and 4) to be useful structures for age estimation on *Heterodontus portusjacksoni*. However the potential bias and precision of other readers of these structures needs to be tested if these structures are to be adopted more widely. Because of the reported results that sectioned vertebral centra are recommended for age estimation (Chapter 4), this structure was used to test for bias and precision in this chapter.

The aims of this study were to determine the variation throughout time and the effects of experience in age estimation counts of *H. portusjacksoni* vertebral centra. This was done by estimating the difference in bias and precision (1) within repeated band pair counts over time (2) and between readers with different experience in ageing.

6.2 Methods

6.2.1 Sampling

H. portusjacksoni were sampled from commercial fishing boats operating from Newcastle, Australia (32° 55′05″*S*, 151°45′37″*E*). Vertebral centra were removed, cleaned and sectioned before being fixed on microscope slides. Three hundred centra from vertebrae 20 from 133 females (210 to 1115 mm F_L) and 167 males (197 to 1003 mm F_L) were aged. Further detailed information regarding the sampling and preparation of vertebrae are provided in Chapter 2 and 3.

6.2.2 Readers and Training

There were six readers in total. Two readers's had extensive experience in ageing (experience level 1). Reader 1 (the author) had experience in reading both elasmobranch vertebral centra and dorsal spines, and fish otoliths. Therefore he was considered to estimate the most accurate age. Reader 2 had experience in reading fish otoliths but was assigned to experience level 1 because it was assumed that this experience was transferable to ageing elasmobranch vertebral centra. These two readers collaborated by ageing 100 vertebral centra over a period of two days until they agreed upon the age of each sample.

Readers 3 and 4 were both marine biologists but had no prior experience in ageing elasmobranch vertebral centra. These two readers collaborated with reader 1 by ageing 50 vertebral centra over one day until they agreed upon the age of each sample and were therefore assign to experience level 2.

Readers 5 and 6 were both marine biologists but had no prior experience in ageing. No ageing collaboration with any of the other readers was undertaken and therefore they were assigned to experience level 3. Readers 5 and 6 were given a brief instruction (1 hour) about counting sectioned vertebral band pairs on the first day of the experiment.

6.2.3 Experimental Design

Prior to the experiment the author selected 300 vertebral centra of them 131 females ranging from 210 to 1115 mm F_L and 167 males ranging from 197 to 1003 mm F_L. These vertebral centra were randomly selected based on their readability score (Table 3.2) for the trials. The 300 vertebral centra were numbered randomly so that the sex, length and time of collection were unknown to the readers. All vertebral centra were randomly ordered between each trial so readers did not read the vertebral centra in the same order.

Reading of sectioned vertebrae centra was performed under a dissecting microscope (Nikon SMZ645) with reflected light. An annulus contained one opaque and one translucent band pair. The opaque band was defined as "dark" and wider while the translucent band was defined as "light" and narrow. Only translucent band pairs that were visible and whole through the corpus calcareum were counted. The angle change was determined to be the birth mark, and the first translucent band formed during the first winter. Complete details are described in Chapter 3.

6.2.4 Statistical Analysis

6.2.4.1 Bias

Within-Reader

Within-reader bias was calculated for each reader from the age estimated of each shark for each trials, using the following formula (Officer *et al.*, 1997):

$$(Rd_1 - Rd_2) / ((Rd_1 + Rd_2) / 2),$$

where Rd_1 and Rd_2 are the band pair counts recorded in reading one and two, respectively.

A one-sample *t*-test was used to test the null hypothesis that the average within-reader bias between the two trials was zero (Campana *et al.*, 1995). Age-bias plots were used as the second analysis to determine the existence of any systematic bias within-readers (Campana *et al.*, 1995).

Between-Reader

Two analyses were calculated to test for bias between-readers of the estimated age. Between-reader bias was calculated with the formula (Officer *et al.*, 1997):

$$(Rd_1 - Rd_2) / ((Rd_1 + Rd_2) / 2),$$

where Rd_1 Rd_2 and are the band pair counts recorded by two different readers. Bias was calculated between reader 1 and all other readers at each trial.

A one-sample *t*-test was used to test the null hypothesis that the average between-reader bias was zero at each trials (Campana *et al.*, 1995). Age-bias plots were used as a second analysis to determine the existence of any systematic bias between-readers (Campana *et al.*, 1995).

In addition, a one-factor analysis of variance (ANOVA) was used to test the null hypothesis that average age of the sample of 300 vertebral centra did not differ between readers within each trial. Prior to analysis the homogeneity of variances was tested with Cochran's test. Significant difference between mean values were investigated post-hoc with Student-Newman-Keuls test (Underwood, 1981).

6.2.4.2 Precision – Within and Between Readers

Within- and between-reader precision (i.e. the consistency of interpretation of band pairs) was calculated from the Coefficient of Variance (CV) (Chang, 1982) using the formula:

$$CV_j = 100 \text{ x } (\sqrt{\sum} [(x_{ij} - x_j)^2/(R(R - 1)]/ x_j),$$

where x_{ij} is the *i*th age estimation of the *j*th shark, x_j is the mean age of the *j*th shark and *R* is the number of times each shark is aged.

Within-reader precision was calculated from the age of each shark estimated in each trial. Between-reader precision was calculated for the comparison between reader 1 and all the other readers in each trial.

An upper CV value of 5% was used for the experiments based upon the established value from most ageing laboratories (Campana, 2001).

6.3 Results

6.3.1 Within Readers

Reader 1 and 3 estimated higher in the first trial than in the second, while reader 2, 4, 5 and 6 estimated higher in the second trial than the first (Table 6.1). However the *t*-test indicated that the bias was not significantly different from 0 between the two trials for readers 1, 3, 4 and 5, while reader 2 and 6 showed a significant difference between the two trials.

Reader 1 was the only reader that showed a high precision while all the other readers had CV values >8%. Reader 4 had the lowest precision (CV = 19.5%)

Overall there was no association between ageing experience in other structures or the level of training given and bias or precision. Reader 2 (experienced in otolith ageing) had the greatest bias in readings of all readers. There was little difference in bias between readers 3 (trained) and reader 5 (untrained) and between readers 4 (trained) and 6

(untrained). There was also little difference in precision between readers 3 (trained) and reader 6 (untrained) and readers 4 (trained) and 5 (untrained).

Table 6.1: Assessment of band pair counts for *H. portusjacksoni* by individual readers. Values shown for each variable are mean ± standard error. *T*-test shows results of a one-sample *t*-test that mean bias in not significantly different from 0.

Reader	Age 1	Age 2	Bias	*T*-test	CV
1	9.35 ±0.44	9.34 ±0.44	0.006 ±0.004	1.44, $p = 0.15$	1.1% ±0.18
2	9.82 ±0.5	10.56 ±0.5	-0.13 ±0.014	-9.01, $p < 0.001$	8.2% ±0.64
3	10.44 ±0.44	9.67 ±0.4	0.04 ±0.02	1.94, $p = 0.06$	11.0% ±0.83
4	7.78 ±0.47	7.3 ±0.44	-0.076 ±0.04	-1.91, $p = 0.06$	19.5% ±1.65
5	7.65 ±0.39	7.93 ±0.4	-0.033 ±0.035	-0.94, $p = 0.35$	17.0% ±1.43
6	8.06 ±0.35	8.71 ±0.39	-0.068 ±0.02	-3.02, $p = 0.003$	14.5% ±0.76

Age-bias plots indicate that only reader 1 had no systematic bias between the two trials with the mean estimated age lying along the 1:1 relationship line throughout the age range of the sample (Figure 6.1). However, an increase in variation (S.E.) after age 27 indicates that sharks older than 27 years are more difficult to age estimate.

Reader 2 systematically estimated higher in the second trial than the first trial and had a high variation between the two trials after age 26 where the variation increased, indicating that reader 2 also found older individuals harder to age estimate.

Reader 3 and 4 aged older for sharks greater than 10 years in the first trial than in the second. The variation in reader 3 became greater after 21 years.

Reader 5 and 6 aged individuals older than 20 years, younger in the second trial. The variation increased for reader 5 for sharks greater than 18 years old, while for reader 6 variations increased for sharks greater than 14 years old individuals.

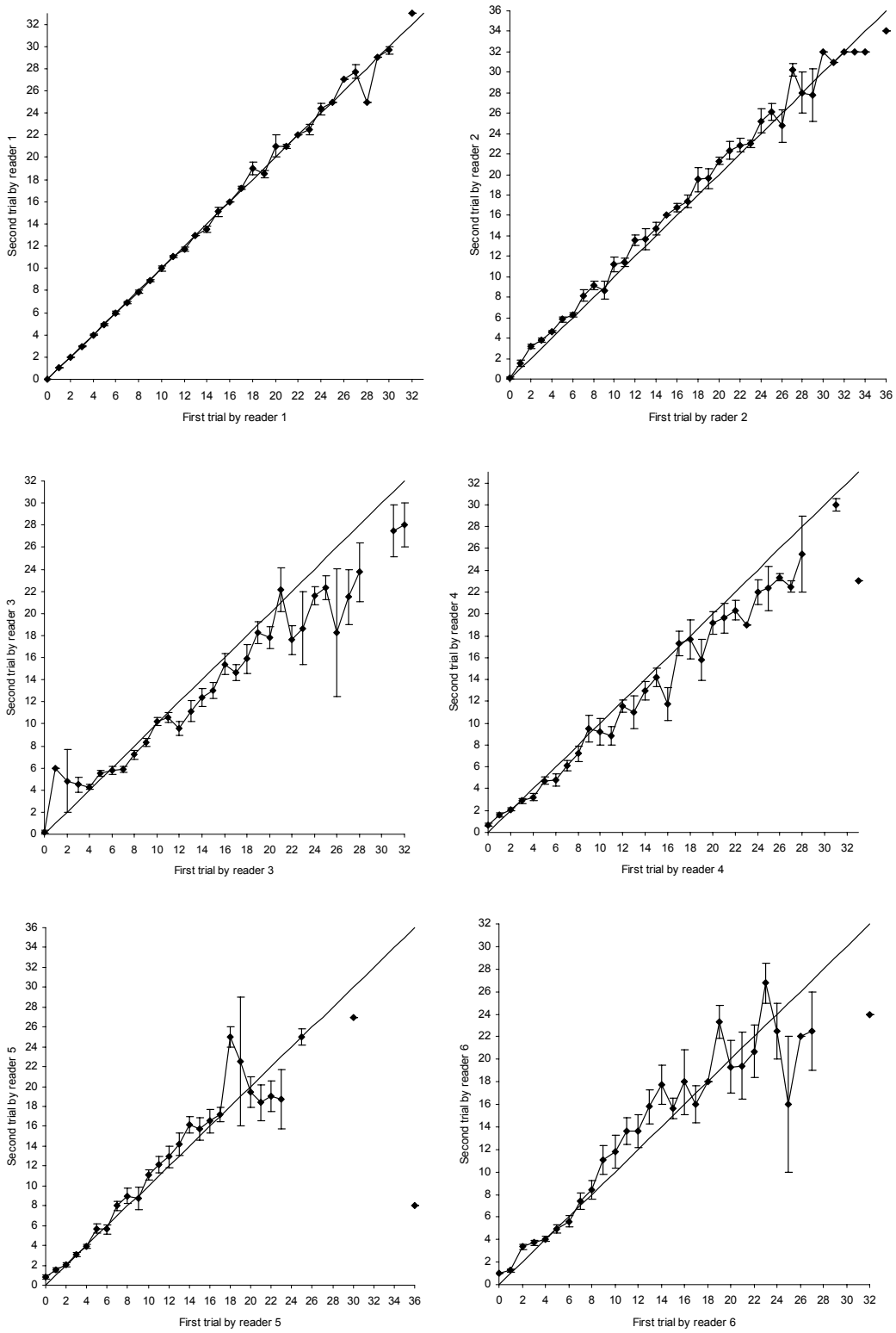


Figure 6.1: Comparing the ages estimated using sectioned vertebral centra in the first and second trial for each reader 1 to 6. Age-bias plots shows standard error bars and a 1:1 relationship line for *H. portusjacksoni* (n = 300).

6.3.2 Between Readers

Reader 1 estimated higher than reader 2 in the first trial while in the second trial, reader 2 estimated higher than reader 1 (Table 6.2). The *t*-test indicated that the bias between the two readers was not significantly different from 0 in the first trial, however the bias between them was significantly different from 0 in the second trial. The value for CV (2.5%) indicated a high precision between the two readers.

Reader 1 estimated lower than reader 3 in both trials and the *t*-test indicated that the bias between the two readers was significantly different from 0 in both trials. However the CV value was higher than the set value of 5%.

Reader 1 estimated higher than reader 4 in both trials and the *t*-test indicated that the bias between the two readers was significantly different from 0 in both trials. However the CV value was again higher than the set value of 5% between reader 1 and 4.

Reader 1 estimated higher than both reader 5 and 6 in both trials and the *t*-test indicated that the bias between the two readers was significantly different from 0 in both trials. The CV values was higher between reader 1 and both readers 5 and 6 than the set value of 5%.

Although reader 3 and 4 both showed acceptable level of precision when compared to reader 1 neither indicated that their age estimation training lowered their bias compared to reader 5 and 6.

Table 6.2: Assessment of band pair counts for *H. portusjacksoni* by individual readers. Values shown for each variable are mean ± standard error. *T*-test shows results of a one-sample *t*-test that mean bias was not significantly different from 0.

Reader	Bias 1	*T*-test 1	Bias 2	*T*-test 2	CV
2	0.024 ±0.02	1.46 $p = 0.15$	-0.11 ±0.01	-7.6 $p < 0.001$	2.5% ±0.2
3	-0.148 ±0.02	-7.43 $p < 0.001$	-0.113 ±0.02	-5.19 $p < 0.001$	4.7% ±0.8
4	0.525 ±0.04	13.7 $p < 0.001$	0.5 ±0.03	15.62 $p < 0.001$	4.5% ±0.3
5	0.339 ±0.03	10.85 $p < 0.001$	0.306 ±0.03	10.07 $p < 0.001$	5.2% ±0.7
6	0.058 ±0.02	2.14 $p = 0.033$	-0.017 ±0.02	-0.74 $p = 0.46$	5.4% ±0.5

Age-bias plots indicate that compared to reader 1, reader 2 systematically aged older for all sharks greater than 6 years of age. There was an increase in variation (S.E.) after age 14 indicating that sharks older than 14 years were aged more differently by the two readers (Figure 6.2).

Reader 3 aged all individuals younger than 21 years to be older and individuals older than 21 years to be younger, compared to reader 1. An increase in variation after age 12 indicated that sharks older than 12 years were aged more differently by the two readers.

Both readers 4 and 5 systematically aged sharks younger than reader 1 across all ages. An increase in variation after age 8 indicated that sharks older than 8 years were aged more differently by the two readers.

Reader 6 aged all individuals younger than 4 years to be older, and individuals older than 4 years to be younger than the ages estimated by reader 1. Bias increased with the age of the individuals. An increase in variation after age 10 indicated that sharks older than 10 years were aged more differently by the two readers.

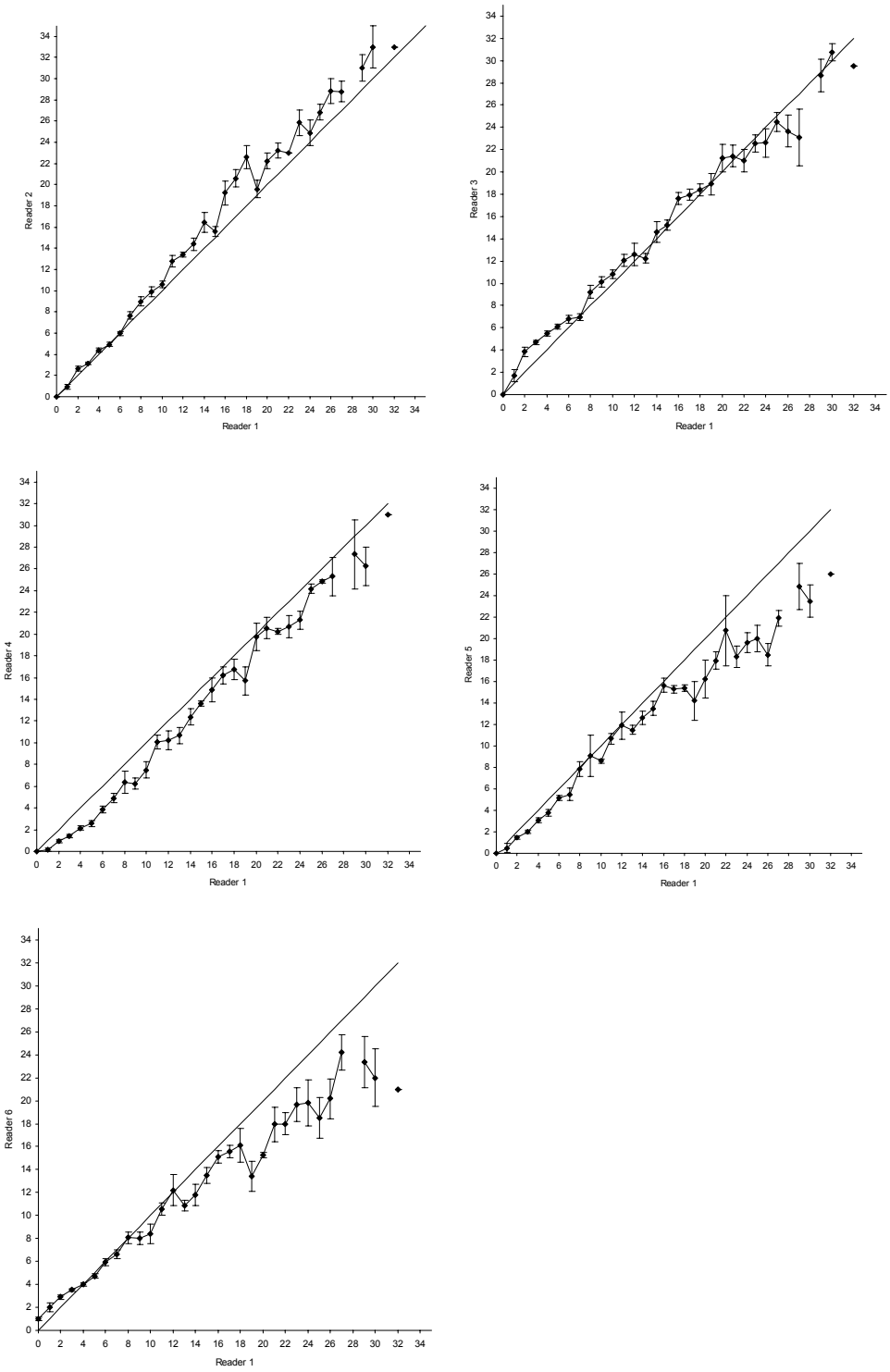


Figure 6.2: Age bias plots with standard error and a 1:1 relationship line for *H. portusjacksoni* comparing the ages estimated using sectioned vertebral centra between reader 1 and readers 2 to 6 (n = 300).

One-way ANOVA indicated that there was a significant difference between readers in estimated mean age in each trial (Table 6.3). In the first trial reader 3 had the highest age estimate and reader 5 had the lowest, while in the second trial reader 2 had the highest age estimate and reader 4 had the lowest (Table 6.1). Post-hoc comparison of mean ages in the first trial found that: (I) reader 4 was significantly different to readers 1, 2 and 3; (II) reader 5 was significantly different to 2 and 3; and (III) and reader 6 was significantly different from reader 2. Post-hoc comparison of mean ages in the second trial found that: there was no significance between: (I) reader 2 and readers 1 and 3; (II) reader 4 and readers 5 and 6; and (III) reader 5 and readers 4 and 6.

Table 6.3: Summary of results of one-way analysis of variance testing for significant differences between readers in estimated mean age of a sample of *H. portusjacksoni* in two trials.

		1 (Cochrans $C = 0.22, p > 0.05$)			2 (Cochrans $C = 0.22, p > 0.05$)		
Source of variation	df	MS	*F*	*p*	MS	*F*	*p*
Readers	5	414.93	7.29	< 0.001	424.77	7.72	< 0.001
Residual	1794	56.88			55.03		

6.4 Discussion

6.4.1 Bias

The within-reader bias varied between the different readers. The within-reader analyses indicated that reader 1 had an average bias 5 times lower than the next lowest bias of reader 5 and almost 22 times lower than reader 2. Although only readers 2 and 6 showed a significant difference between the two trials, the age-bias plots indicated that all readers,

except reader 1, showed systematic differences between the two trials. Reported findings indicated that within- and between-reader bias is relatively low (Francis and Maolagain, 2000; Goldman *et al.*, 2006; McAuley *et al.*, 2006; Sulikowski *et al.*, 2007). Carlson *et al.* (2006) reported a 76.5% agreement between all band pair counts between the trials of two experienced elasmobranchs agers estimating the life history of *Carcharhinus limbatus*. Officer *et al.* (1996) reported a minimum within-reader bias from ageing *M. antarcticus* and *G. galeus* of 0.01 and a maximum bias of 0.15 between two trials and concluded that there was no significant different between the two trials. The maximum bias value (0.13) reported in this study is slightly less than reported by Officer *et al.* (1996) and might indicate that this value is within the limit of bias in age estimation of elasmobranchs. However, on the bases of the experience of reader 1 in this study and the most experienced reader in Officer *et al.* (1996) study, a lower limit of within-reader bias of 0.01 should be set for ageing elasmobranchs.

Between-reader analyses showed that only reader 6 had no significant bias in both trials. Although, age-bias plots indicated a systematic difference between reader 1 and all the other readers. Officer *et al.* (1996) also compared between-reader bias and reported a minimum bias between the most experienced reader and the other less experienced readers of 0.03 and a maximum of 0.1 between the readers, and concluded that there was no significant difference between the two readers. The maximum between-reader bias (0.5) reported in this study is higher than that reported by Officer *et al.* (1996) and clearly indicates that this value is too high. On the basis of the two most experience of readers 1 and 2 in this study and the two most experienced readers in Officer *et al.* (1996), a lower limit of 0.05 for between-reader bias should be set for ageing elasmobranchs. Although this

bias limit might seem high compared to the bias limit set for within-reader, it is important to indicate that bias is normally greater between readers than within one reader.

6.4.2 Precision

Only reader 1 had an acceptable precision under 5% when comparing the two trials. However readers 2, 3 and 4 all had precision values under the 5% when compared to reader 1. Carlson *et al.* (2006) reported a 3.9% within-reader precision between the two trials of two experienced elasmobranchs agers determining the life history of *C. limbatus*. Sulikowski *et al.*(2007) reported a higher within-reader precision of 7.2 and 7.7% for two different reader estimates of the age of *R. texana*, which falls outside the acceptable limit for precision. Although not reported, one has to assume that both readers had experience in ageing elasmobranchs. However, between-reader precision was 4.8% in the same study, indicating (as found in this study) that within-reader precision is normally higher than between-reader precision. Sulikowski *et al.* (2007) concluded that since there was no indication of bias, that the precision was acceptable. Carlson *et al.* (2006) reported an acceptable precision of 3.9% between two experienced readers estimating the age of *C. limbatus*. Francis and Maolagain (2000) reported a much lower precision of 10-15% for between-reader bias.

The importance of accurate age estimates is crucial and the most often underestimation of populations can contribute to overly optimistic estimates of growth and mortality which can result in overexploitations of a population or species (Campana, 2001). Campana (2001) indicated a mean precision value of 7.6 % for both otolith and vertebral centra ageing, and mention that many ageing laboratories used 5% as a reference point. However, it is important to mention that many elasmobranchs have greater longevity that

teleosts and therefore the level of precision can be raised above that of short lived teleosts without compromising the accuracy in ageing.

6.4.3 Level of Experience

The amount of training provided to readers 3 and 4 in this study appears to have been inadequate. Readers 3 and 4 were given training that consisted of an explanation of the ageing techniques and supervised ageing (with immediate correction) of 50 samples conducted over 1 day. However, both readers 3 and 4 had higher bias than the two inexperienced readers (reader 5 and 6) and showed high variation in bias over the age of the individuals. And when comparing reader 3 and 4 to reader 1, reader 4 had the highest bias of all readers in both trials, while readers 3 had higher bias than reader 6 in both trials. This clearly indicates that the amount of training needed to age vertebral centra needs to be greater than what was used in this study. Francis and Mulligan (1998) reported low precision in ageing *G. galeus* when both readers where inexperienced in ageing shark vertebrae and concluded that reading shark vertebrae is a learning process and a high level of experience is needed to accurately read band pairs. Simpfendorfer *et al.* (2000) did not report how much training was given to his two inexperienced readers when estimating the age of *F. macki*. However they did report that both the inexperienced readers had the lowest number of samples for which consensus counts could be reached and the lowest level of agreement between consensus and final counts. Officer *et al.* (1996) also reported that their reader with limited experience in ageing elasmobranchs had the highest bias of all readers when ageing *M. mustelus* and that no significant difference was found between the limited experienced elasmobranch reader and a reader with no experience in ageing elasmobranchs.

Officer *et al.* (1996) concluded that experienced elasmobranchs agers provided higher precision and less bias that inexperienced agers.

The result of the present study indicated that the performance of ageing is highly dependent on the experience level of readers. Pairwise comparisons of readings found high variance for the readers in experience level 2 and 3. Again, indicating that a high level of experience in needed to accurately age elasmobranchs.

Campana (2001) recommended using a minimum of 200 samples as a reference collection to monitor ageing consistency over both long and short term and suggested using 500 samples to avoid memorisation. Out of this reference collection a sub-sample off around 100 was suggested for training purposes. This study used 50 samples for unexperienced readers and 100 samples for a reader experienced in reading fish otoliths. The results indicate that there were no differences between the two ageing groups and therefore the number of samples used for training needs to be greater and that extensive training over a longer period of time is required to maintain the experience needed to limit bias and maintain a high precision.

6.4.4 Experience in Ageing Other Structures

Experience in ageing other structures cannot be assumed to provide sufficient experience compared to ageing elasmobranch vertebral centra. Reader 2, who had extensive experience in ageing fish otoliths, did not perform better than readers 3-6. Reader 2 was only one of two readers that showed a significant difference within-reader bias and had the highest bias of all the readers. When comparing reader 2 with reader 1, reader 2 had low bias and no significant difference was found in the first reading. However, in the second reading there was not much difference in bias between reader 2 and 3, and reader 2 had higher bias than

reader 6. Although reader 2 had extensive experience in reading fish otoliths, that experience was not transferable to reading sectioned vertebral centra. This was also reported by Officer *et al.* (1996) where a reader with extensive experience in ageing otoliths had the second largest bias, after a reader with limited experience in ageing shark vertebrae, and the lowest precision when ageing *M. antarcticus*. The same otolith reader again had the second largest bias, after an ager with no experience in ageing, and the lowest precision when ageing *G. galeus*. Similar results was reported by McAuley *et al.* (2006) where an experienced otolith ager showed lower individual consensus counts and had a lower agreement with final band pair count than any of the two other readers experienced in ageing elasmobranchs. Again, an experienced otolith reader was reported to have the lowest precision compared to both experienced and inexperienced elasmobranch agers when ageing *F. macki* (Simpfendorfer *et al.*, 2000).

All these results indicate that a high level of experience and training is needed to accurately age estimate elasmobranchs and that experience of reading other structures such as teleost otoliths is not transferable to reading elasmobranch vertebral centra. The high level of bias and low level of precision between readers together with the high bias and low precision between the author and the other readers reported in this study indicate and strengthen this conclusion. The failure to extensively increase the experience in ageing elasmobranch vertebral centra could lead to over- or underestimated age which again could lead to overexploitation of a species population.

6.5 Conclusion

Validation of the all the procedures in ageing is crucial when estimating age and growth studies in all animals. The effects of bias and precision may over- or under-estimate age which again can result to over exploitation of resources. This result shows that using the right statistics both bias and precision can be calculated and assessed. Experience is one such bias that can alter the age estimation and it is here recommended that extensive experience in ageing the structure of the species under question is not underestimated. However, the level of training to gain extensive experience has not been fully investigated and as indicated in this study needs to be elevated above 100 samples, if not within family level, at least to class.

Chapter 7

A Port Jackson shark resting at Seal Rocks (photo by the author)

Chapter 7

General Discussion and Conclusion

7.1 Introduction

Ensuring sustainable fisheries is becoming more essential as we see our resources diminish year by year. The increasing demand for shark fins and meat, and the large amounts of sharks caught as by-catch (Walker *et al.*, 2005). Therefore demersal shark species, such as *Heterodontus portusjacksoni*, might have to contribute to the increasing world shark market. With the limited important life history information that exists for this species along the New South Wales coast, this thesis has assessed the life history characters for this species before harvesting has started.

The present chapter provides a summary of findings for this thesis. For further detailed discussion of the findings presented here, please consult to the relevant text in Chapter 2 through 6.

7.2 Anatomy of the Ageing Structures

General knowledge of the hard structures used to estimate age an essential first step in assessing the usefulness of potential ageing structures. Information about how these structures grow and their appearance gives insight to their importance and application to age estimation. The anatomy of the two ageing structures, vertebral centra and dorsal spines, was described for *H. portusjacksoni* from New South Wales, Australia. Vertebral centra from *H. portusjacksoni* were similar to other shark vertebrae previously described. Dorsal spines were also found to be of similar appearance and structure to other sharks.

Both vertebral centra and dorsal spines grew proportionally throughout the life of the animal and were therefore cleared suitable for further investigation of their potential for ageing. Only minor differences were found in shape between described species. Therefore, the vertebral centra and dorsal spines of *H. portusjacksoni* have the general appearance of most described sharks, however further research on the anatomy of hard parts in other species hard parts is needed to indicate if there are more differences than so far reported in the literature.

7.3 Vertebral Centra and Dorsal Spines for Age Estimation

Accurate age estimates are important for fisheries science. Only age estimations can give insight into longevity and growth rates making age data very important in stock assessment and resource management. Since there is still evidence of species without the annual pattern of band pairs, the periodicity of the band pair formation was validated throughout all age groups for *H. portusjacksoni*. Validation is an important part of age and growth data and the need to validate the formation of band pairs is essential. Fluorescent injections, marginal increment analysis and centrum edge analysis all concluded that the band pairs in *H. portusjacksoni* were annual and that the translucent band pairs were deposited in the winter months. This is typical for elasmobranchs with the slower growth in the winter months giving the hypo-mineralised (translucent) bands.

Age data was derived from vertebral centra and dorsal spines by reading band pairs on both whole and sectioned structures. Both vertebral centra and dorsal spines have been frequently used for age estimation, however few studies have compared the two structures or whole and sectioned samples. Assessment of the different hard parts and methods of preparation is crucial in the decision to adopt the right technique for a species.

The use of sectioned hard structures has been recommended by several authors and seems to be more frequently used on elasmobranchs in recent years. This study agrees with the hypothesis that whole vertebral centra and whole dorsal spines underestimate *H. portusjacksoni* age. Whole structures have the tendency to underestimate older individuals as the band pairs grow more densely and are therefore harder to interpret.

Precision and accuracy should be an important part of all age estimations and although the within-reader bias and precision is low between experienced readers bias concerning experience level has not been assessed by many authors. This study demonstrated that extensive experience is needed to limit bias and increase precision, and that experience is not transferred from ageing teleost to elasmobranchs. This conclusion is important as the lack of precision and high bias in age estimation will have a strong influence on growth curves which identify the important fishery parameters of longevity (L_∞) and growth (k).

7.4 Growth Models and Parameters

The von Bertalanffy growth equation (VBGE) is still popular with fisheries scientists although it has been criticised by several authors for the artificial parameter t_0. Therefore several variations of the VBGE have been produced and an alternative to the VBGE the Gompertz growth function (GGF) has been applied to several elasmobranchs.

The need to use several growth models to characterise the growth of a given species is crucial as one model might be limited in reflecting early and even asymptotic growth (Cailliet *et al.*, 2006). The VBGE might give a suitable description of the species growth, however the use of other models might describe different life stages better than the other.

Selection of the appropriate model should be on the base of biological relevance, statistical fit and convenience.

This research indicated that *H. portusjacksoni* has a typical elasmobranch life history, with slow growth rate and long life span. As with many other elasmobranchs this life history trait makes this species vulnerable to any changes in their population and it is therefore recommended that any opening of fishery on this species is regulated to size and that the species is monitored across the whole population. As *H. portusjacksoni* has a defined population structure and unknown size, the effect of fishery on this species might be damaging.

7.5 Future Research

It is hard to analyse the importance of future research needs since most are closely linked and give way to the other. However, it one should do so this would be the order of importance:

1. As all hard calcium structures might deposit band pairs during growth, future research in non-lethal age estimation techniques would benefit the ageing research and limit the so far necessary death of research animals.
2. The anatomy of ageing structures in elasmobranchs is limited. Therefore more research and knowledge needs to be gained in the anatomy of all ageing structures.
3. The knowledge of ageing structures individual growth and across all life stages is important to be able to successfully use these structures as ageing tools.
4. Future research into training required by elasmobranch age readers to limit bias that occurs in precision and accuracy is needed. So far there is a general knowledge that extensive experience in ageing elasmobranch vertebral centra is needed to

successfully age estimate any fish species. However, little or no research has been devoted to how to train researchers in this technique.

5. It is also worth clarifying that this study suggests the existence of different populations of *H. portusjacksoni* and it would therefore be useful to test this by a genetic study across the species.

6. Age and growth parameters are important data for population management. Although individuals from all size classes were sampled, there is still a chance for sampling bias due to gear selectivity, or that the sampling area not representative for the whole population and therefore does not represent natural growth. A more representative sample from fishery-dependent and –independent sampling along the whole population area would allow age and growth determination to be representative for the whole population and therefore for the species.

7. With the increasing development of growth equations and variations of growth models, the need to compare these models and identify the best fit for each species will be required to match the increasing demand for better sustainability and stock assessment.

Appendix A

Species of elasmobranchs for which age estimation have been reported.

Order	Family	Scientific name	Common name	Reference
Carcharhiniformes	Carcharhinidae	*Carcharhinus brevipinna*	Spinner	(Allen and Wintner, 2002)
Carcharhiniformes	Carcharhinidae	*Carcharhinus limbatus*	Blacktip	(Branstetter, 1987a; Wintner and Cliff, 1996)
Carcharhiniformes	Carcharhinidae	*Carcharhinus leucas*	Bull	(Thorson and Lacy Jr, 1982; Branstetter and Stiles, 1987; Wintner *et al.*, 2002; Neer *et al.*, 2005)
Carcharhiniformes	Carcharhinidae	*Carcharhinus isodon*	Finetooth	(Carlson *et al.*, 2003)
Carcharhiniformes	Carcharhinidae	*Carcharhinus acronotus*	Blacknose	(Carlson *et al.*, 1999; Driggers *et al.*, 2004)
Carcharhiniformes	Carcharhinidae	*Carcharhinus plumbeus*	Sandbar	(Casey *et al.*, 1985; Casey, 1992; Joung *et al.*, 2004)
Carcharhiniformes	Carcharhinidae	*Carcharhinus porosus*	Smalltail	(Lessa and Santana, 1998)
Carcharhiniformes	Carcharhinidae	*Carcharhinus longimanus*	Oceanic whitetip	(Seki *et al.*, 1998; Lessa *et al.*, 1999)

Order	Family	Scientific name	Common name	Reference
Carcharhiniformes	Carcharhinidae	*Carcharhinus obscurus*	Dusky	(Schwartz, 1983; Natanson *et al.*, 1995; Simpfendorfer, 2000; Simpfendorfer *et al.*, 2002b)
Carcharhiniformes	Carcharhinidae	*Carcharhinus falciformis*	Silky	(Oshitani *et al.*, 2003)
Carcharhiniformes	Carcharhinidae	*Carcharhinus signatus*	Night	(Santana and Lessa, 2004)
Carcharhiniformes	Carcharhinidae	*Carcharhinus brachyurus*	Bronze whaler	(Walter and Ebert, 1991)
Carcharhiniformes	Carcharhinidae	*Carcharhinus cautus*	Nervous	(White *et al.*, 2002)
Carcharhiniformes	Carcharhinidae	*Carcharhinus sorrah*	Spottail	(Davenport and Stevens, 1998)
Carcharhiniformes	Carcharhinidae	*Carcharhinus tilsoni*	Australian blacktip	(Davenport and Stevens, 1998)
Carcharhiniformes	Carcharhinidae	*Galeocerdo cuvier*	Tiger	(Branstetter *et al.*, 1987; Natanson *et al.*, 1999)
Carcharhiniformes	Carcharhinidae	*Negaprion brevirostris*	Lemon	(Gruber and Stout, 1983; Brown and Gruber, 1988)

Order	Family	Scientific name	Common name	Reference
Carcharhiniformes	Carcharhinidae	*Prionace glauca*	Blue	(Stevens, 1975; Cailliet *et al.*, 1983b; MacNeil and Campana, 2002; Skomal and Natanson, 2003; Lessa *et al.*, 2004)
Carcharhiniformes	Carcharhinidae	*Rhizoprionodon terraenovae*	Atlantic sharpnose	(Carlson and Baremore, 2003; Loefer and Sedberry, 2003)
Carcharhiniformes	Carcharhinidae	*Rhizoprionodon taylori*	Australian sharpnose	(Simpfendorfer, 1993)
Carcharhiniformes	Carcharhinidae	*Isogomphodon oxyrhynchus*	Daggernose	(Lessa *et al.*, 2000)
Carcharhiniformes	Sphyrnidae	*Sphyrna lewini*	Scalloped hammerhead	(Schwartz, 1983; Piercy *et al.*, 2007)
Carcharhiniformes	Sphyrnidae	*Sphyrna tiburo*	Boonthhead	(Carlson and Parson, 1997)
Carcharhiniformes	Scyliorhinidae	*Galeus melastomus*	Blackmouth catshark	(Correia and Figueiredo, 1997)
Carcharhiniformes	Triakidae	*Furgaleus macki*	Whiskery	(Simpfendorfer *et al.*, 2000)

Order	Family	Scientific name	Common name	Reference
Carcharhiniformes	Triakidae	*Galeorhinus galeus*	School	(Ferreira and Vooren, 1991; Francis and Mulligan, 1998; Walker *et al.*, 2001)
Carcharhiniformes	Triakidae	*Mustelus canis*	Smooth dogfish	(Conrath *et al.*, 2002)
Carcharhiniformes	Triakidae	*Mustelus antarcticus*	Gummy	(Moulton *et al.*, 1992; Walker *et al.*, 2001)
Carcharhiniformes	Triakidae	*Mustelus henlei*	Brown smooth-hound	(Yudin and Cailliet, 1990)
Carcharhiniformes	Triakidae	*Mustelus manazo*	Spotted dogfish	(Yamaguchi *et al.*, 1996)
Carcharhiniformes	Triakidae	*Mustelus californicus*	Grey smoothhound	(Yudin and Cailliet, 1990)
Carcharhiniformes	Triakidae	*Mustelus henlei*	Brown smoothhound	(Yudin and Cailliet, 1990)
Carcharhiniformes	Triakidae	*Mustelus lenticulatus*	Rig	(Francis and Francis, 1992; Francis and Maolagain, 2000)
Carcharhiniformes	Triakidae	*Triakis semifasciata*	Leopard	(Kusher *et al.*, 1992; Smith *et al.*, 2003)
Lamniformes	Alopiidae	*Alopias vulpinus*	Common thresher	(Cailliet and Bedford, 1983)

Order	Family	Scientific name	Common name	Reference
Lamniformes	Alopiidae	*Alopias pelagicus*	Pelagic thresher	(Liu *et al.*, 1999)
Lamniformes	Alopiidae	*Alopias superculiosus*	Bigeye thresher	(Liu *et al.*, 1998)
Lamniformes	Lamnidae	*Carcharodon carcharias*	White	(Cailliet, 1985; Wintner and Cliff, 1999)
Lamniformes	Lamnidae	*Carcharodon taurus*	Grey nurse	(Branstetter and Musick, 1994)
Lamniformes	Lamnidae	*Isurus oxyrinchus*	Shortfin mako	(Pratt and Casey, 1983; Campana *et al.*, 2002b)
Lamniformes	Lamnidae	*Lamna nasus*	Porbeagle	(Francis and Stevens, 2000; Campana *et al.*, 2001; Campana *et al.*, 2002b; Natanson *et al.*, 2002)
Lamniformes	Lamnidae	*Lamna ditropis*	Salmon	(Tanaka, 1980)
Heterodontiformes	Heterodontidae	*Heterodontus portusjacksoni*	Port Jackson	(McLaughlin and O'Gower, 1971; Izzo, 2005; Tovar-Avila, 2006)
Rajiformes	Rhinobatidae	*Rhinobatos annulatus*	Sand	(Rossouw, 1984)
Squaliformes	Centrophoridae	*Centrophorus squamosus*	Deepwater	(Clarke *et al.*, 2002a)

Order	Family	Scientific name	Common name	Reference
Squaliformes	Centrophoridae	*Centrophorus acus*	Needle dogfish	(Tanaka, 1990)
Squaliformes	Centrophoridae	*Deania calceus*	Squalid	(Clarke *et al.*, 2002b)
Squaliformes	Centrophoridae	*Deania calcea*	Birdbeak	(Machado and Figueiredo, 2000)
Squaliformes	Squalidae	*Squalus acanthias*	Spiny dogfish	(Holden and Meadows, 1962; Ketchen, 1975; Hall, 1976; Soldat, 1982; Polat and Gumus, 1995; Avsar, 2001; Jones and Ugland, 2001; Henderson *et al.*, 2002)
Squaliformes	Squalidae	*Squalus megalops*	Piked spurdog	(Watson and Smale, 1999; Braccini *et al.*, 2007)
Squaliformes	Squalidae	*Squalus mitsukurii*	Shortspine spurdog	(Wilson and Seki, 1994)
Squaliformes	Squalidae	*Squalus blainvillei*	Longnose spurdag	(Cannizzaro *et al.*, 1995)
Squaliformes	Squatinidae	*Squatina californica*	Pacific angel	(Cailliet *et al.*, 1992)
Squaliformes	Dalatiidae	*Centroscymnus crepidater*	Longnose velvet dogfish	(Irvine *et al.*, 2006b)

Order	Family	Scientific name	Common name	Reference
Squaliformes	Dalatiidae	*Etmopterus baxteri*	New Zealand lantern	(Irvine *et al.*, 2006a)
Hexanchiformes	Hexanchidae	*Hexanchus griseus*	Blutnose sixgill	(McFarlane *et al.*, 2002)
Hexanchiformes	Hexanchidae	*Notorynchus cepedianus*	Sevengill	(Dykhuizen and Moller, 1992)
Orectolobiformes	Ginglymostomatidae	*Ginglymostoma cirratum*	Nurse	(Carrier and Luer, 1990)
Orectolobiformes	Rhincodontidae	*Rhincodon typus*	Whale	(Wintner, 2000)
Orectolobiformes	Orectolobidae	*Orectolobus ornatus*	Ornate wobbegong	(Huveneers, 2007)
Orectolobiformes	Orectolobidae	*Orectolobus halei*	Banded wobbegong	(Huveneers, 2007)
Orectolobiformes	Orectolobidae	*Orectolobus maculatus*	Spotted wobbegong	(Huveneers, 2007)

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