Assessing the population dynamics and stock viability of striped trumpeter (*Latris lineata*) in a data limited situation

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For my family...Anj and Kails

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Statement of co-authorship

Chapters 2 – 5 of this thesis have been prepared as scientific manuscripts. In all cases experimental design and implementation of the research program, data analysis, interpretation of results and manuscript preparation were the primary responsibility of the candidate, but were carried out in consultation with supervisors, and with the assistance of co-workers. Contributions of co-authors are outlined below:

Chapter 2 (Paper 1): Age validation, growth modeling and mortality estimates for striped trumpeter (*Latris lineata*) from southeastern Australia: making the most of patchy data. *Sean Tracey (80%), Dr Jeremy Lyle (20%)*

Chapter 3 (Paper 2): Reproductive biology and per-recruit analyses of striped trumpeter (*Latris lineata*) from Tasmania, Australia: implications for management. *Sean Tracey (80%), Dr Jeremy Lyle (15%), Ass Prof Malcolm Haddon (5%)*

Chapter 4 (Paper 3): Application of Elliptical Fourier analysis of otolith form as a tool for stock identification. *Sean Tracey (85%), Dr Jeremy Lyle (13%), Dr Guy Duhamel (2%)*

Chapter 5 (Paper 4): Investigating genetic structuring of *Latris lineata* (Forster in Bloch and Schneider 1801) (Percoidei: Latridiidae) at localised and trans-oceanic scales. *Sean Tracey (80%), Adam Smolenski (15%), Dr Jeremy Lyle (5%)*

Dr Jeremy Lyle (Tasmanian Aquaculture and Fisheries Institute) and Dr Alistair Hobday (School of Zoology) were the primary supervisor for this Ph.D. study and as such both contributed to the development of ideas as well as providing analytical advice and assistance.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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Abstract

Research into small-scale fisheries is often insufficient, resulting in limited data, because this type of fishery is inevitably constrained by financial considerations. This creates a challenge to provide adequate information to support sustainable management, particularly given the shift from single species management to more integrated spatial and multi-species management and, ultimately, to ecosystem based fisheries management (EBFM).

Striped trumpeter (*Latris lineata*) is widely distributed around the temperate latitudes of the southern hemisphere. The species is iconic to Tasmania where it supports a small commercial fishery, and it is increasingly targeted by recreational fishers. This fish is common on most rocky reefs between 50 – 250 m around Tasmania. However, the historical data for striped trumpeter from Tasmania is patchy in time and space, reflecting opportunistic sampling over many years. Using striped trumpeter as an example of a small-scale data-limited fishery, this study applies a variety of techniques to describe key biological and ecological processes required for sustainable fisheries management.

The study was divided into three themes. First, standard and novel analytical techniques were applied to evaluate data to provide key biological parameters required for single-species assessment. Second, stock structure was investigated on both local and global scales using molecular techniques and otolith morphometrics. Finally, recruitment processes were investigated based on otolith microchemistry and modelling of larval dispersal.

Seasonal growth variability was observed over the first five years, with growth rates peaking approximately one month after the observed peak in sea surface temperature. The oldest fish in this study was 43 years. Lifetime growth was modelled using a modified two-phase von Bertalanffy growth function, with the transition between growth phases linked to changes in physiological and life history traits, including offshore movement as fish approach maturity.

Total mortality was estimated using catch curve analysis based on the standard and two-phase von Bertalanffy growth functions, and estimates of natural mortality were calculated using two empirical models, one based on longevity and the other based on the parameters L_∞ and k from both growth functions.

The spawning season around Tasmania occurs in the austral spring, with peak spawning activity in September and October. Size at 50% maturity was estimated at 543 mm fork length (FL) for females (estimated age = 6.8 years) and 529 mm FL for males (estimated age = 6.2 years). Striped trumpeter is a multiple spawner with batch fecundity estimates ranging from 205,054 for a 2 kg fish (540 mm FL) to 2,351,029 for a 9.5 kg fish (800 mm FL). At the current minimum legal size limit of 450 mm total length (equivalent to approximately 425 mm FL), yield-per-recruit was estimated to be close to maximum, and spawning biomass-per-recruit (SPR) ranged from 35 – 52% of virgin stock, depending on the mortality estimates used.

Otolith morphometrics, in particular elliptical Fourier analysis of otolith shape, indicated little to no connectivity between the striped trumpeter population of Tasmania and the St. Paul/ Amsterdam Island populations. A molecular assessment of mtDNA confirmed this finding. In addition, the DNA sequence analysis indicated that the New Zealand striped trumpeter population was genetically distinct from the Tasmanian and St. Paul/ Amsterdam Island populations. DNA sequence analysis also indicated that the population around Tasmania is a single population.

The affinity of juvenile striped trumpeter to inshore reefs has been suggested from anecdotal fishing observations. Using otolith microchemistry the comparative contribution

of juvenile striped trumpeter from shallow inshore habitats to the adult population was estimated. Juvenile striped trumpeter from a strong recruitment pulse (1993 cohort) were collected at age two from inshore reefs and as adults at age six from deeper offshore reefs around the coast of Tasmania. Natural variations were identified in the concentrations of lithium and strontium within the incremental structure of the observed otoliths. Discriminant analysis suggested that 70% of adults sampled originated from an inshore juvenile habitat, 13% were from deeper reefs and 17% could not be statistically allocated with confidence.

An integrated bio-physical larval dispersal model was developed in an attempt to explain the high degree of inter-annual recruitment variability displayed by this species. The model utilised information developed through the course of this study on reproductive biology, ontogenic habitat preferences and stock structuring as well as additional information on striped trumpeter larval biology from aquaculture trials to generate realistic scenarios. While the model was unable to accurately predict observed interannual recruitment variability, it did provide insights to important source and settlement regions as well as the importance of the addition of biological components, such as: timing of spawning, growth and mortality.

Through efficient data-mining, novel methods and technological advancements this study has provided robust scientific advice to support the management of the striped trumpeter fishery. Information has been collated to support traditional single-species management and also for developing spatial fisheries measures, leading to a more ecosystem based approach to fisheries management. Otoliths proved to be valuable in several areas, and small-scale fisheries would be advised to initiate otolith collections even though analysis may not be planned for some time. This study demonstrates how targeted research could be used in other small-scale data limited fisheries in a cost effective manner to provide information for sustainable management.

Acknowledgements

Firsty I extend thanks to my supervisors, Dr Alistair Hobday and Dr Jeremy Lyle for providing much support and inspiration through the course of this journey. Alistair for helping me see the ‘big picture’ in each step of this journey and for being patient while I fumbled through a relatively steep learning curve with MatLab. Jeremy I thank for providing me with the majority of the data set used within this study as well as hours of discussion on the striped trumpeter and meticulous editing of each manuscript as they evolved. I also thank Jeremy and Dr Gretta Pecl, in their role as my supervisors in my capacity as an employee at TAFI, for agreeing to allow me to undertake this study whilst in full-time employment.

I would like to express my gratitude to many staff and students at TAFI and the ‘Team Hobday’, who assisted, discussed and supported me throughout this study. The finfish section Graeme Ewing, Alistair Morton, Simon Willcox and Ed Forbes. Finfish alumni Dirk Welsford and Alan Jordan. Resource modellers Malcolm Haddon and Phil Ziegler. Habitat mappers Vanessa Lucieer and Miles Lawler. The striped trumpeter aquaculture team for providing discussion, samples, advice and data; Steve Battagalene, Dave Morehead, Jenny Cobcroft, Ross Goldsmid.

Many people have provided samples for this study, firstly I would like to thank the crew of the FRV Challenger Matt Francis and Jac Gibson for your tireless efforts collecting stripeys when ever possible and all the crew from the other sections that have participated

in stripey sampling. Ray Murphy collected the majority of the juvenile stripeys over 1995 – 1998 from inshore reefs around the southeast of Tasmania. Guy Duhamel, Peter Smith, Clive Roberts and Margaret McVeigh provided samples from overseas.

Luke Finley for embarking on geometric morphometrics in his PhD thesis, and the hours of discussion on the in's and out's of this technique. Adam Smolenski for many hours in the Central Science Molecular Biology Lab showing me everything from DNA extraction to sequence analysis. Leonid Danyushevsky and Sarah Gilbert from the Centre of Ore Deposit and Environmental Sciences (CODES) for providing access to the LA-ICPMS and providing advice on the analysis of the data.

I am immensely thankful for the generosity of the Fisheries Society of the British Isles. They provided not only a research grant to fund the otolith microchemistry component of this study, but also a travel grant which assisted in me attending the ASLO Ocean Sciences conference held in Honolulu 2006 where I presented the larval dispersal model to an international audience.

Sincere thanks to my parents for providing me support over the years and believing in me even when it looked like the Kombi may not have returned, thank you.

Finally but definately not last sincere gratitude to my beautiful girls, my wife Angela Williamson and our daughter Kailea, without you two this achievement would not have been possible. You have provided inspiration, support and love which has kept me going even when it looked like the mountain was unsurmountable, thank you, I love you both tremendously.

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Chapter 1

General Introduction

There is a global shift from single species management to integrated spatial and multi-species management leading to ecosystem based fisheries management (Sainsbury et al., 2000). This trend is placing additional pressure on researchers to provide more information relevant to target species and the environments they inhabit. Tackling these information requirements will require a focused strategy that invests in research that provide the best return on investment while generating robust and timely information to support sustainable management.

Fisheries are an economic-driven natural commodity. Hence, resources for research and management are typically allocated in proportion to the fisheries of greatest value. This fund allocation requirement creates a situation whereby there are few species that are well understood and the fisheries can be managed accordingly. However, it leaves many species for which there is limited knowledge.

In Tasmania for example, there are four single species fisheries that earn over a million dollars annually (relatively small scale in terms of global fisheries) (Fig. 1.1). These are all invertebrate species for which there has been significant research investment into their biology and population dynamics. The scalefish fishery on the other hand earned just over a million dollars in 2004/2005, but is comprised of over 10 key species along with a large number of minor species (Fig. 1.1) (ABARE, 2006). Although these species are fished under a multi-species scalefish licence, the species are assessed individually. This means that the resource allocation for research into individual species is extremely limited. With few exceptions, insufficient information is available to support assessments that facilitate maximal economic gain in an ecologically sustainable manner. The socio-economic value of these 'data limited' fisheries is however, also an important consideration. Based on the number of commercial fishing licences registered in Tasmania these low value, small scale fisheries (not considering post-fishing processes) employ a greater number of fishers than the more valuable fisheries.

As economics is a strong, and justified driver of research funding data limited fisheries will always exist. So the challenge for researchers and managers is to judiciously determine

the key information required to maximize yield in a sustainable manner.

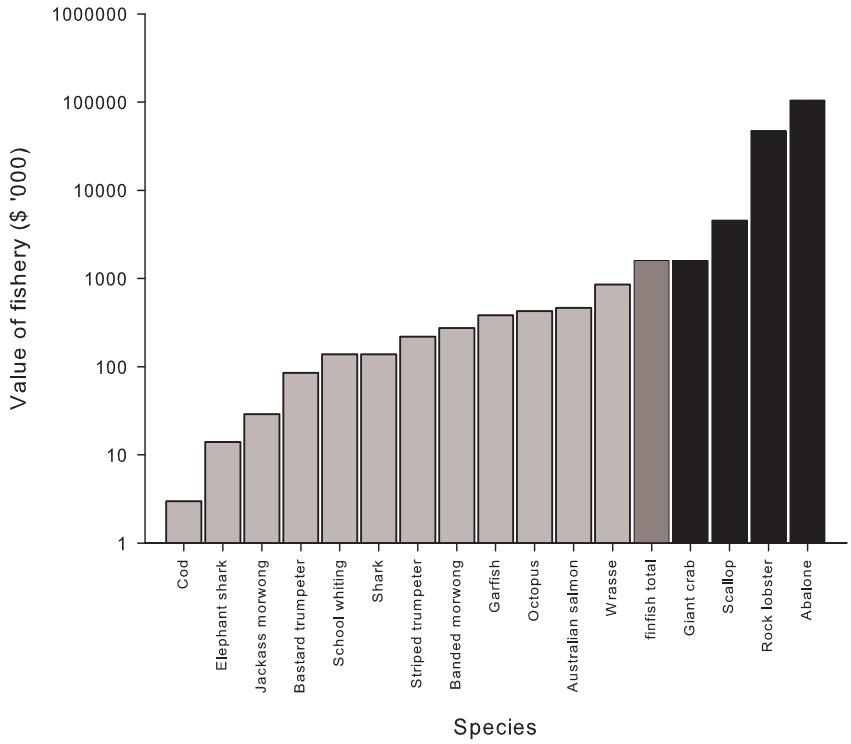

Figure 1.1: The 15 most valuable fishery species from Tasmania based on data from 2004/05. Grey shading signifies fisheries worth less than one million dollars annually. The dark grey column is the combined value of the scalefish fisheries, and the black columns are valued annually at greater than one million dollars (ABARE, 2006). Note $\log_{10}$ scale on y-axis.

1.1 Problem statement

In Tasmania the only information generally available to underpin the assessment and management of scalefish fisheries are the compulsory commercial catch and effort data and in some instances, limited and occasional biological information collections may also be

available. This situation has resulted in 'data poor' fisheries from which it is difficult to create defensible stock assessments. In this study the striped trumpeter *Latris lineata* is used as a case in point.

1.2 The case of the striped trumpeter

Trumpeters (family Latridae) are a southern hemisphere family with a centre of diversity in Australasian waters where four species are present: the species of interest to this study - striped trumpeter *Latris lineata* (Table. 1.1 & Fig. 1.2), bastard trumpeter *Latridopsis forsteri* (also known as copper moki), blue moki *Latridopsis ciliata* and telescope fish *Mendosoma lineatum* (also known as real bastard trumpeter). Recent exploration around central South Pacific seamounts has discovered a fifth latrid species, the silver trumpeter *Latris pacifica* (Roberts, 2003). In terms of genetic relatedness, striped and silver trumpeter are sister species, bastard trumpeter and blue moki are sister species, while the telescope fish is the most divergent latrid (Smith et al, 2003).

Striped trumpeter has a broad geographic distribution, occurring around the temperate latitudes of southern Australia, New Zealand (Last et al., 1983), including the sub-Antarctic Auckland Island (Kingsford et al., 1989), the Gough and Tristan Da Cunha Island groups in the southern Atlantic Ocean (Andrew et al., 1995), the Amsterdam and St. Paul Island groups in the southern Indian Ocean (Duhamel, 1989) and the Foundation seamount in the southern Pacific Ocean (Roberts, 2003) (Fig. 1.3).

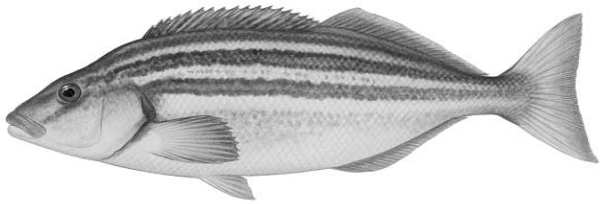

Figure 1.2: An adult striped trumpeter *Latris lineata*

The catch and effort data for striped trumpeter has been supplemented by limited biological information collected over the last 15-years, the latter being spatially and temporally 'patchy'. In this study we define 'patchy' as the irregular sampling of fish in space and time as well as the irregularity of the types of biological information collected. An example of this 'patchiness' is shown in Figure. 1.4. One of the main sources of biological information over the last 6 years has been opportunistic sampling by 'vessels of opportunity'; that is where informal arrangements had been made with fishing or research vessels to provide samples for research. The majority of this 'opportunistic' sampling occurred from the Tasmanian Aquaculture and Fisheries Institute (TAFI) research vessel 'Challenger' whilst involved in research targetting other issues/species.

These limitations in the data create a challenge for fisheries researchers: the data must be 'mined' efficiently using both standard and non-standard approaches to provide robust information on stock status.

1.3 The Tasmanian fishery

Striped trumpeter has a long history of exploitation in Australia. In 1882, the striped trumpeter was described as the 'most excellent of all Tasmanian fishes' (Tasmania, 1882). These days the species contributes as a target species or byproduct to multi-species fisheries caught using a variety of fishing methods, with hooks and gillnets being the primary

Table 1.1: Taxonomic description of striped trumpeter

Category	Taxon
Class	Osteichthyes
Order	Perciformes
Family	Latrididae
Genus	*Latris*
Species	*lineata*
Common name	striped trumpeter

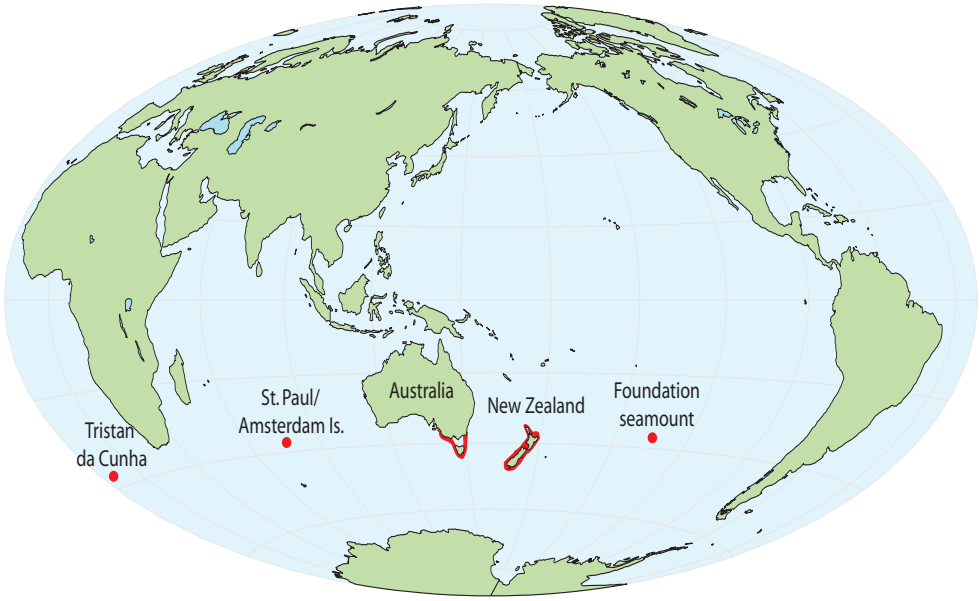

Figure 1.3: Current known distribution of striped trumpeter *Latris lineata* (red shading).

methods. Juvenile striped trumpeter are taken predominantly by gillnet in inshore waters (typically within 3 nautical miles of the coast) and usually in depths <50 m whereas adult fish are targeted in deeper offshore waters by hook methods (dropline, handline, bottom longline, trotline) and taken as a byproduct in large mesh gillnets (shark nets).

Responsibility for the management of striped trumpeter was passed to Tasmania in 1996 through an Offshore Constitutional Settlement (OCS) arrangement with the Commonwealth. A memorandum of understanding accompanied the OCS, specifying trip limits for Commonwealth only fishers of 100 kg for South East Non-Trawl (SENT) permit holders and 20 kg for all other permit holders.

When the Tasmanian scalefish fishery management plan was implemented in 1998, gear restrictions were introduced for all commercial scalefish fishers operating in State waters. However, after the introduction of the management plan, those fishers who held a Tasmanian licence and a Commonwealth permit to fish in the southern shark or SENT

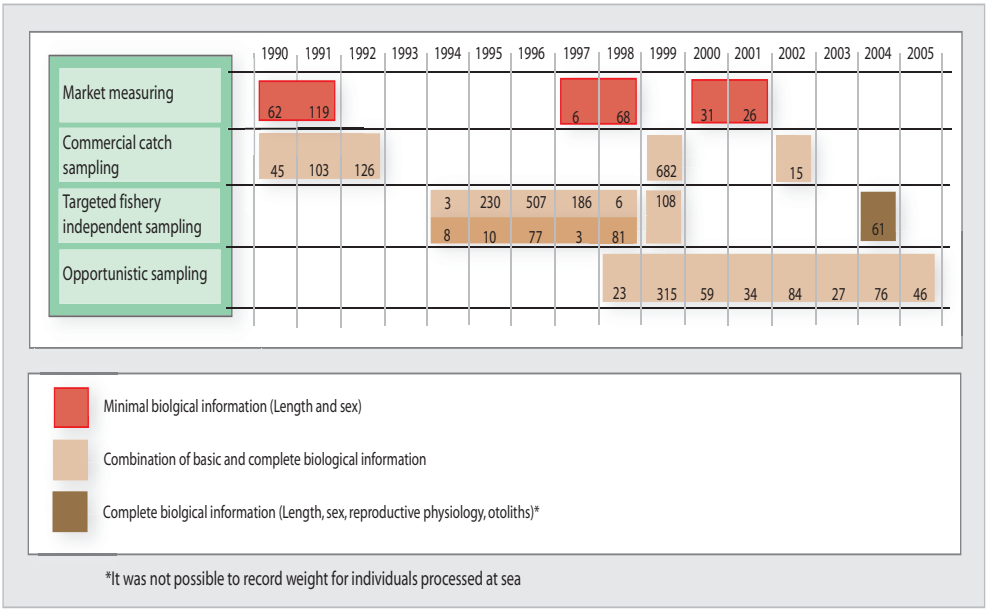

Figure 1.4: Timeline showing the sources of biological information collected over the last 15 years for the striped trumpeter fishery adjacent to Tasmania. Numbers in the top of the boxes are the sample sizes from inshore gillnetting and the numbers in the bottom of the boxes are the sample sizes from offshore fishing methods.

fisheries were effectively allowed to target unrestricted quantities of striped trumpeter in offshore waters using their Commonwealth gear allocations (this was a significant change to their original 20 kg or 100 kg restrictions). In addition, Tasmanian rock lobster fishers were also allowed to take unrestricted quantities of striped trumpeter in offshore waters using their State scalefish gear allocations.

In August 2000, the State Government introduced a combined 250 kg trip limit for striped trumpeter, yellowtail kingfish and red snapper for all fishers (Commonwealth and State) in inshore and offshore waters relevant to Tasmania. This measure was introduced to limit the potential for expansion of effort directed at these species. A daily bag limit of five and possession limit of eight striped trumpeter was also introduced for recreational fishers. The legal minimum size limit for striped trumpeter was raised from 35 to 45 cm total length (TL) in November 2004 in recognition that the smaller size limit was substantially below

the size at maturity. The recreational bag limit was also replaced with a possession limit of eight fish (Ziegler et al., 2006).

Annual production was high, at over 110 tonnes in the early 1990s (with Victorian vessels taking between 17–39% of the reported catch), but then fluctuated generally between 70-80 tonnes through the early to mid 1990s before increasing again to over 100 tonnes by the late 1990s. Catches almost halved in 2000/01, to less than 50 tonnes, and have remained low since that time (Fig 1.5). The reported combined Commonwealth and state catches of 23 tonnes for 2005/06 was about 14% lower that in the previous year and represented the lowest catch reported since the mid 1980s. However, Commonwealth catches are believed to be substantially under-reported and together with an unknown level of recreational catch, represent a major source of uncertainty in estimating the total mortality(Ziegler et al., 2006).

Striped trumpeter catches have been reported from all areas around Tasmania apart from the north coast, with the fishery focussed off the north-east, east, south-east, south and north-west coasts (Fig 1.6). The decline in catches over recent years has also seen the area of the fishery contract and catches are now concentrated off the south east coast(Ziegler et al., 2006).

The most conspicuous trend in catch history was the initial increase in production for all methods up until 1999/2000, followed by general declines in catches for gillnet and handline methods. By contrast, dropline catches rose slightly between 2002/03 and 2003/04 but declined again in 2005/06. Regionally, expansion of the fishery during the late 1990s was the result of increased catches from all areas. Subsequent declines also occurred in all regions, with the most recent drop in catches influenced particularly by further falls in landings from north-east and east coasts. South-east coast catches have remained relatively stable in recent years (Ziegler et al., 2006).

Strong 1993 and 1994 year-classes recruited to the fishery between 1995/96 and 1997/98 and influenced subsequent catches (and catch rates). A slight increase in inshore gillnet catches in 1998/99 followed by a decline suggest that the 1996 year-class, which would

have recruited to the inshore gillnet fishery in 1998/99, was also relatively strong. The subsequent decline in graball catches presumably reflects the movement of the relatively strong year-classes offshore but also suggests that there has been limited recruitment in recent years.

Industry representatives suggest that the 250 kg trip limit has represented a strong disincentive for some operators to fish for the species and may have contributed to the fall in dropline and handline catches since 2000/01.

An estimate of the recreational take of striped trumpeter (38 tonnes in 2000/01) indicates that the recreational catch may well be equivalent to the commercial catch and, therefore, a significant component of the overall fishery. While more recent estimates of recreational catches are not available, recreational fishing activity has probably increased in recent years(Ziegler et al., 2006).

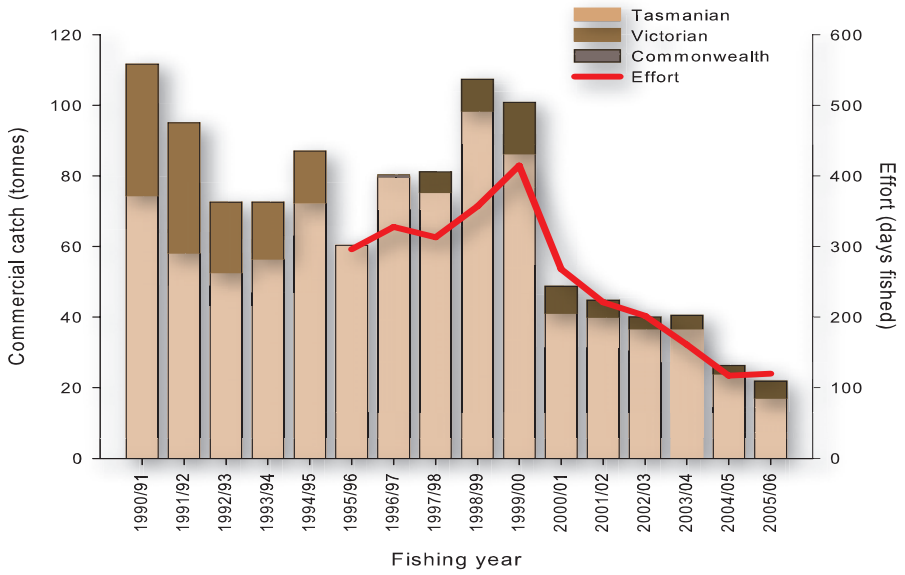

Figure 1.5: Commercial catch (tonnes) and effort (days fished) for striped trumpeter fishery based on commercial catch returns. (Ziegler et al., 2006).

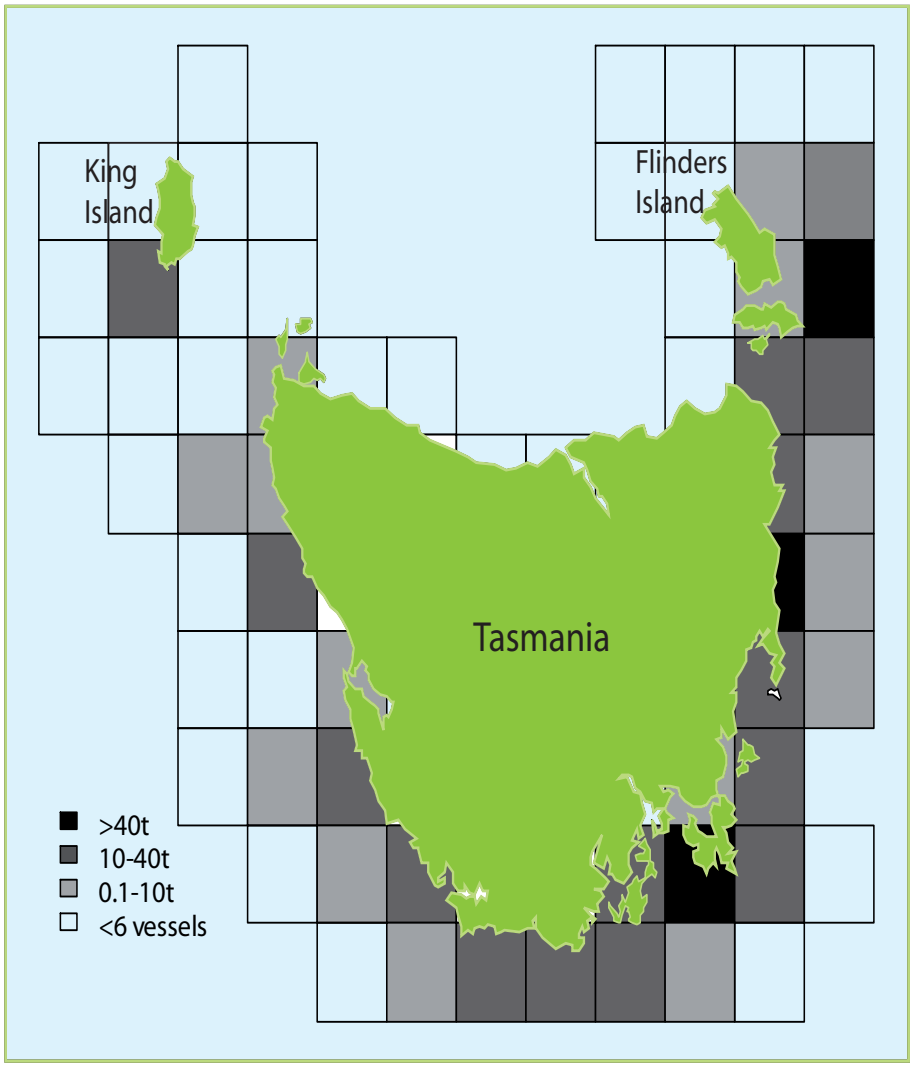

Figure 1.6: Striped trumpeter catches (tonnes by fishing block pooled from 1995 to 2006. Blocks with less than 6 vessels reporting catch are shown as empty (Ziegler et al., 2006).

1.4 State of knowledge for striped trumpeter

At the commencement of this study there was limited knoweldge of the biological traits of striped trumpeter, with the exception of research focused on aquaculture of the species. A summary of the state of knowledge for striped trumpeter is as follows:

- Observed strong recruitment variability (Murphy & Lyle, 1999)
- Juveniles observed inshore, adults observed offshore (Murphy & Lyle, 1999)
- Approximate estimates of reproductive parameters: size/age at maturity, timing of spawning season (Hutchinson, 1993)
- Unvalidated age and longevity estimates (Murphy & Lyle, 1999)
- Basic information on larval life history (Furlani & Ruwald, 1999)

1.5 Thesis Aim

The aim of this study is to optimise the utility of existing but patchy datasets for striped trumpeter through efficient and innovative data-mining, and to undertake targeted and strategic biological sampling to describe key biological and ecological processes useful for modern fisheries management.

1.6 Specific objectives

The main data requirement to supplement the catch and effort data available for striped trumpeter is pertinent biological information, including: age, growth, mortality and reproduction. In addition, moving towards an ecosystem approach to fisheries will require an understanding of the spatial dynamics of the species, such as: stock structure and habitat

utilisation.

This study is divided into three areas. First, standard and novel analytical techniques are applied to available biological data to examine life history strategies and provide input parameters required for stock assessment. Second, stock structure is investigated at local and global scales, using molecular techniques as well as otolith morphometrics. Finally, ontogenic habitat preferences are identified using modelling of larval dispersal and analysis of otolith microchemistry signatures of juveniles and adults.

1.7 Research Structure

This thesis has been developed as a series of manuscripts written for publication in peer-reviewed literature. Each data chapter represents a separate manuscript, with slight alterations for inclusion within this thesis. Chapters can be read in isolation, however, they are written and arranged in a manner to complement the others. The thesis consists of six data chapters (chapters two – seven) with a general discussion (chapter eight). The principal objectives, rationale and published citation of each chapter are outlined below:

CHAPTER TWO:

Investigate and quantify the population parameters of age, growth and mortality.

Age, growth and mortality are fundamental parameters in assessments of exploited fish populations, considered the cornerstone of single-species assessment. Biases, inaccuracies or significant uncertainties in these parameters are at the root of many a fisheries collapse, with these effects exacerbated in data-limited situations. The aim of this chapter is to develop biologically robust parameters of lifetime growth, seasonal growth and mortality for striped trumpeter.

Tracey, S.R., Lyle, J.M. (2005). Age validation, growth modeling and mortality estimates of striped trumpeter (*Latris lineata*) from southeastern Australia: Making the most of patchy data. Fishery Bulletin 103:169–182.

CHAPTER THREE:

Assess the reproductive timing and maturation processes of striped trumpeter.

An understanding of reproductive biology and ecology is necessary to implement effective management based on size restrictions as well as being important for spatial management. Little is known on the reproductive cycle or outputs of striped trumpeter from the wild, though anecdotal evidence suggests that the reported age and size at maturity may be inaccurate. The current data available on the extent and timing of peak spawning period is also limited.

The aim of this chapter is to utilize existing data to define the spawning season and then build upon this by sampling during this period to assess size at maturity, gonad development and fecundity. These reproductive parameters are then combined with information from the previous chapter on age, growth and mortality to generate per-recruit analyses to assess the effectiveness of current management measures for striped trumpeter.

Tracey, S.R., Lyle, J.M., and Haddon. M. (2007) Reproductive biology and per-recruit analyses of striped trumpeter (*Latris lineata*) from Tasmania, Australia: implications for management. Fisheries Research 84:358–367.

CHAPTER FOUR:

Application of elliptical fourier analysis of otolith form as a tool for stock identification.

Understanding marine population structure is important from both conservation and scientific/ecological perspectives.

The aim of this chapter is to evaluate the utility of geometric morphometrics applied to striped trumpeter otoliths to distinguish stocks. This technique is relatively new to fisheries science, but is showing promise as a cheaper alternative to methods such as molecular techniques. To further test the hypothesis of discrete stocks a physiological examination of

growth will be facilitated by ageing individuals from two geographically separated populations and comparing the resultant growth curves for significant difference using likelihood ratio tests.

Tracey, S.R, Lyle, J.M., and Duhamel, G. (2006). Application of elliptical Fourier analysis of otolith form as a tool for stock identification. Fisheries Research 77:138–147

CHAPTER FIVE:

Population structuring of striped trumpeter at localised and trans-oceanic scales.

The aim of this chapter is to determine the genetic relatedness between populations from Tasmania, New Zealand and St. Paul/ Amsterdam Islands using genetic techniques. Further genetic analysis is also undertaken to address the hypothesis of a single genetically homogenous population around Tasmania.

Tracey, S.R., Smolenski, A.J., and Lyle, J.M. (2007) Genetic structuring of *Latris lineata* at localised and trans-oceanic scales. Marine Biology 152:119–128

CHAPTER SIX:

The role of inshore reef nursery habitats for temperate fish displaying a depth-stratified ontogenic migration.

Anecdotal evidence indicates that striped trumpeter undergo migration from inshore reefs to deeper offshore reefs at approximately five years of age. This 'vertical' migration crosses the thermocline, typically located between 50 and 200 m.

The aim of this chapter is to use otolith microchemistry to test this hypothesis and assess the relative importance of the inshore reefs as juvenile habitats to the striped trumpeter population. This information can then be used to assist in determining effective spatial harvest strategies for the striped trumpeter fishery.

CHAPTER SEVEN:

A bio-physical model of pelagic larval dispersal in the Tasman Sea

The high degree of interannual recruitment variability displayed by striped trumpeter is most likely linked to the extended larval/paperfish stage. The nine month duration of this stage allows for dispersal to other regions as well as extended exposure to variable environmental conditions.

The aim of this chapter is to explore the factors contributing to recruitment variability displayed by this species by developing an integrated bio-physical larval dispersal model. The model utilises information developed through the course of this study on reproductive biology, ontogenic habitat preferences and stock structuring, as well as information on striped trumpeter larval biology from aquaculture studies.

Chapter 2

Age, growth and mortality of striped trumpeter

2.1 Abstract

Abstract. Age estimates for striped trumpeter (*Latris lineata*) from Tasmanian waters were produced by counting annuli on the transverse section of sagittal otoliths and were validated by comparison of growth with known age individuals and modal progression of a strong recruitment pulse. Estimated ages ranged from one to 43 years; fast growth rates were observed for the first five years. Minimal sexual dimorphism was shown to exist between length, weight and growth characteristics of striped trumpeter. Seasonal growth variability was strong in individuals up to at least age four, and growth rates peaked approximately one month after the observed peak in sea surface temperature. A modified two-phase von Bertalanffy growth function was fitted to the length-at-age data, and the transition between growth phases linked to apparent changes in physiological and life history traits, including offshore movement as fish approach maturity. The two-phase curve was found to represent the mean length at age in the data better than the standard von Bertalanffy growth function. Total mortality was estimated using catch curve analysis based on the standard and two-phase von Bertalanffy growth functions, and estimates of

natural mortality were calculated using two empirical models, one based on longevity and the other based on the parameters L_∞ and k from both growth functions. The interactions between an inshore gillnet fishery targeting predominately juveniles and an offshore hook fishery targeting predominately adults highlights the need to use a precautionary approach when developing harvest strategies.

2.2 Introduction

Striped trumpeter *Latris lineata* are widely distributed around the temperate latitudes of southern Australia, New Zealand (Last et al., 1983), the Gough and Tristan Da Cunha Island groups in the southern Atlantic Ocean (Andrew et al., 1995) and the Amsterdam and St. Paul Island groups in the southern Indian Ocean (Duhamel, 1989). They are opportunistic carnivores associated with epibenthic communities over rocky reefs at moderate depths from 5 to 300 m along the continental shelf. The species can grow to a relatively large size, 1200 mm in total length and 25 kg in weight (Gomon et al., 1994). Spawning apparently occurs offshore, and females are highly fecund multiple-spawners (Furlani & Ruwald, 1999). Although there have been a number of ichthyoplankton surveys in Tasmanian waters, only a few striped trumpeter larvae have been collected, caught during the late austral winter through early spring months at near shore (30 – 50 m) and shelf edge sites ($\simeq$ 200 m) (Furlani & Ruwald, 1999). Larval rearing trials have shown that the presettlement phase is complex and extended; individuals can remain in this neritic-pelagic phase for up to 9 months after hatching before metamorphosis into the juvenile form. As juveniles they settle on shallow rocky reefs.

In Tasmania striped trumpeter are taken commercially over inshore reefs (5 – 50 m), generally as a bycatch of gillnetting, and are targeted using hook methods (handline, dropline, longline and trotline) on deeper reefs (80 – 300 m). Small, subadult individuals dominate inshore catches whereas larger individuals are taken from offshore reefs. In recent years the combined annual commercial catch has been typically less than 100 metric tons. Striped trumpeter also attract significant interest from recreational fishers, who use both hooks and gillnets. Furthermore, the aquaculture potential of this species is currently being assessed in Tasmania (Furlani & Ruwald, 1999; Cobcroft et al., 2001). Despite wide interest in this species, there is a general paucity of information on age, growth and stock structure of wild populations.

Assessing the growth of a species is a fundamental component of fisheries popula-

tion dynamics. Ever since Beverton & Holt (1957) introduced the von Bertalanffy growth model to fisheries research it has become ubiquitous to describe the increase in size of a species as a function of age. The parameters common to the von Bertalanffy growth function (VBGF) are used in stock assessment models such as empirical derivatives of natural mortality (Pauly, 1980) and assessments of yield per recruit and spawning stock biomass (Beverton & Holt, 1957). Despite the von Bertalanffy growth parameters being well established as cornerstones of many stock assessment models, several authors have highlighted limitations of the original derivation of the growth function to adequately represent growth of a population (Knight, 1968; Sainsbury, 1979; Roff, 1980; Schnute, 1981; Bayliff et al., 1991; Hearn & Polacheck, 2003). This limitation becomes especially evident with limited or patchy data. The limitations of the von Bertalanffy growth function have created three scenarios: 1) use the VBGF and retain the use of the parameters to derive per-recruit estimates at the possible expense of physiological integrity; 2) derive or employ a model that is not based on the von Bertalanffy parameters, such as a linear, logistic or another polynomial function, for instance, the Gompertz equation (Schnute, 1981) and in doing so the expediency of the von Bertalanffy parameters in stock assessment is compromised; 3), use or develop an extension of the von Bertalanffy equation with the caveat that, by introducing additional parameters, the problem of reduced parsimony by over parameterisation would need to be considered.

While investigating the life history characteristics of striped trumpeter, we became aware became aware that the original description of the VBGF would not adequately represent growth of this species, in part because of the patchy data available for analysis.

This study aims to describe the age and growth of striped trumpeter from Tasmania. Seasonal growth oscillations are considered for the first four years using actual length-at-age data of a strong cohort. We then employ and evaluate an extension of the VBGF that offers a better fit to the sample population of aged individuals and allows the flexibility to assign representative growth and mortality parameters to different life phases of the population. Growth parameters derived for both the standard von Bertalanffy and extended

von Bertalanffy models are used in catch curve analysis and the empirical models of Pauly (1980) and Hoenig (1983) to allow comparison of mortality estimates.

2.3 Materials and methods

Striped trumpeter were collected opportunistically from various sites off the east and south-east coasts of Tasmania from a variety of fisheries dependent and independent sources spanning the period 1990–2002 (Table 2.1). Inshore catches were predominately taken by gillnets ranging in mesh sizes from 64 to 150 mm. Offshore catches were taken by hook and line methods. Samples ranged from intact specimens, for which the full range of biological information was collected, to processed frames from which length and, depending on condition of the body, sex and gonad weight were recorded. All specimens were measured for fork length ($\pm$ 1 mm) and, where possible, total weight was recorded ($\pm$ 1 gram). Otoliths were collected when possible. This *ad-hoc* sampling approach created a temporally irregular data set.

Table 2.1: Composition of Tasmanian sampling data from 1990 through 2002 showing data from inshore gillnet and offshore hook fisheries. Numbers in parentheses represent individuals aged from each particular sampling regime.

Year	Gill net	Hook	Total
1990	–	45	45
1991	–	332	332
1992	–	126	126
1994	3	8	11
1995	228	12	240
1996	529	55	584
1997	193	2	195
1998	7	171	178
1999	205	902	1107
2000	–	91	91
2001	–	60	60
2002	–	97	97
Total	1165	1901	3069
	(268)	(508)	(776)

Kolmogorov-Smirnov tests (α=0.05) were used to determine whether significant differences existed between male and female length frequency distributions or between length frequency distributions by depth strata.

Analysis of residual sums of squares (Chen et al., 1992) was used to determine whether a significant difference existed between the sex specific length-weight relationships that were fitted by minimising the sum of square residuals and described by the power function

$$W = a.FL^b \tag{2.1}$$

where W = whole weight (g); FL = fork length (mm); and, a and b = constants. Sex ratios were compared for significant deviation from 1:1 by chi-square tests.

2.3.1 Ageing technique

Sagittal otoliths were removed from 873 individuals and a sub-sample of 295 otoliths were individually weighed to the nearest milligram. One randomly selected otolith from each fish was embedded in clear polyester casting resin. A transverse section was taken through the primordial region (width approximately 300 μm) and mounted on a microscope slide. A stereo dissector microscope at 25x magnification was used to aid the interpretation of increments in the mounted sections. Increment measurements were performed using Leica $IM^{©}$ image digitisation and analysis software (Leica Microsystems, Wetzlar, Germany). All counts and increment measurements were made without knowledge of fish size, sex or date at capture to avoid reader bias.

Position of the first annual increment was determined by the close correspondence of otolith microstructure and body size between known age individuals reared from eggs in aquaria and wild caught specimens. To ensure growth in cultured individuals was reflective of growth in wild specimens, a hypothesis of comparable growth was tested by fitting traditional VBGF's to the length at age data of 288 cultured individuals (maximum known age 4 years) and 268 wild specimens (maximum otolith derived age 4 years). A likelihood

ratio test (Kimura, 1980) was then used to test for significance. The VBGF model was in the form:

$$L = L_\infty(1 - e^{(-k(t-t_0)}) + \epsilon \tag{2.2}$$

where L_t = length at age t; L_∞ = average asymptotic length; k = a constant describing how rapidly L_∞ is achieved; t_o = the theoretical age where length equals zero; and ϵ = independent normally distributed error term.

Modal progression of length frequencies from a strong cohort of juvenile fish was sampled over a three-year period (1995 – 97). This cohort provided an opportunity to validate annual periodicity in increment deposition. By applying an ageing protocol based on position of the first increment and assuming each opaque+translucent zonal pair represented one years growth, this recruitment pulse could be tracked over seven years in age frequency progression.

A random subsample of 335 otoliths was read a second time by the primary reader, and a second subsample of 46 otoliths by a second reader, experienced in otolith interpretation. Precision was assessed by determining percentage agreement between repeated reads, age bias plots (Campana et al., 1995) and calculating the Average Percent Error (APE) (Beamish & Fournier, 1981).

2.3.2 Growth modelling

The length frequency progression of a strong and discrete cohort of fish indicated that striped trumpeter may be subject to seasonal growth variability. This variability was described by integrating a sinusoidal function (Pitcher & MacDonald, 1973; Haddon, 2001) into a standard VBGF and by applying this function to the actual weekly length-at-age data of individuals sampled from the strong 1993 cohort over the period 1995 through 1997, where the model was described as:

$$L_t = L_\infty \left(1 - e^{-\left[C.sin\left(\frac{2\pi(t-S)}{52}\right)+k(t-t_0)\right]}\right) + \epsilon \tag{2.3}$$

where C = the magnitude of the oscillations above and below the non-seasonal growth curve of the sinusoidal cycle; S = the starting point in weeks of the sinusoidal cycles; and 52 = the cycle period in weeks.

The timing of seasonal growth was compared with weekly average sea surface temperature (SST) on the southeast coast of Tasmania over the sampling period, calculated by using optimum interpolation (Reynolds et al., 2002) of raw remotely sensed data from the area[1]. A sine function was fitted to the weekly average SST by using least squares regression to compare the timing and phase of growth and temperature and test for a significant correlation.

All individuals aged were assigned a 'decimal' age, where the decimal portion represented the proportion of the year between a nominal average date of spawning (1st October) and the date of capture. We assume a nominal peak spawning date of October 1st based on an assessment of monthly averaged gonadosomatic index (Tracey et al., 2007), which is consistent with that observed for wild caught cultured broodstock held under ambient conditions[2] .

Growth of the sampled population was initially described using the standard von Bertalanffy growth function (Eq. 2.2). However, a preliminary visual assessment of the fit suggested it did not produce an adequate representation of the entire data set. In an attempt to find a model that better represented the data, the fit of the standard $VBGF_S$ was compared with an extension of the traditional von Bertalanffy growth model fitted by minimisation of the sum of negative log-likelihood assuming normal distribution of the error term. The model chosen was similar to that used by Hearn & Polacheck (2003) and involved fitting a VBGF function either side of an age at transference, described as:

[1]Data sourced from the NOAA-CIRES Climate Diagnostics Center, Boulder, CO 80305. http://www.cdc.noaa.gov/. [Accessed 15 Sep. 2002]

[2]Morehead, D. 2003. Personal Communication.

$$L_t = \begin{cases} (L_{\infty 1}(1 - e^{-k_1(t-t_{o1})}) + \epsilon), & \text{for } t < t^\delta \\ (L^\delta + (L_{\infty 2} - L^\delta)(1 - e^{-k_2(t-t_{o2})}) + \epsilon), & \text{for } t \geq t^\delta. \end{cases} \tag{2.4}$$

where $L_{\infty 1}, k_1, t_{o1}$ = VBGF parameters applied to the first growth phase; $L_{\infty 2}, k_2, t_{o2}$ = VBGF parameters applied to the second growth phase; L_δ = length of transference from one growth phase to the next; and, t_δ = age of transference from one growth phase to the next; calculated as:

$$t^\delta = t_{01} - \frac{1}{k_1} \ln\left(1 - \frac{L_t}{L_{\infty 1}}\right). \tag{2.5}$$

Having fitted Equation 2.4, the discontinuity from the first growth stanza to the second was smoothed assuming normal distribution around the age at transference by integrating a normal probability cumulative distribution function (PDF) where the mean is equal to the age of transference (4.4 years) and a standard deviation arbitrarily set at 1.0. This model is referred to as the two-phase von Bertalanffy growth function ($VBGF_{TP}$) and is now represented as:

$$L_t = \begin{cases} \left(1 - \int_{t=-t_0}^{+t^\delta} \frac{1}{\sigma\sqrt{2\pi}} e^{\left(\frac{-(t-t_{0_1})^2}{2\sigma^2}\right)}\right)(L_{\infty_1}(1 - e^{-k_1(t-t_{o_1})}) + \epsilon) +, & \text{for } t < t^\delta \\ \left(\int_{t=t^\delta}^{+t_{max}} \frac{1}{\sigma\sqrt{2\pi}} e^{\left(\frac{-(t-t^\delta)^2}{2\sigma^2}\right)}\right)(L^\delta + (L_{\infty_2} - L^\delta)(1 - e^{-k_2(t-t_{o_2})}) + \epsilon), & \text{for } t \geq t^\delta. \end{cases} \tag{2.6}$$

where t_{max} = maximum age present in the sample; and, σ^2 = standard deviation of cumulative density function with mean t_δ.

The model that best represented the data was judged on a combination of parsimony

as determined by the Akaike Information Criterion (AIC) (Akaike, 1974), quality of fit by minimisation of the negative log-likelihood value derived from each model, visual inspection of the residuals, and as an index of fit to the upper end of the size at age range the percent deviation of L_∞ for each model from maximum observed length (L_{max}).

The hypothesis of sexual dimorphism in growth was tested using likelihood ratio tests (Kimura, 1980) for both the $VBGF_S$ and $VBGF_{TP}$ models fitted to the length-at-age data of all individuals whose sex had been determined.

2.3.3 Mortality Estimation

Mortality estimates were calculated by using the parameters of both the $VBGF_S$ and $VBGF_{TP}$ functions. An estimate of instantaneous rate of total mortality (Z) for the offshore hook fishery was calculated for 1998 by applying a length converted catch curve analysis (LCCCA *sensu* Pauly, 1983) to the length frequency data.

Estimates of instantaneous rate of natural mortality (M) were calculated by using two empirical equations. The first derived by Pauly (1980), described as:

$$log_{10}M = -0.0066 - 0.279.log_{10}L_{\infty\gamma} + 0.6543.log_{10}k_\gamma + 0.4634.log_{10}T, \qquad (2.7)$$

where $L_{\infty\gamma}$ and k_γ = parameters derived from the $VBGF_S$ or from the second growth phase of the $VBGF_{TP}$; and T = average annual sea surface temperature (^{o}C) at the area of capture.

The mean annual sea-surface temperature on the east coast of Tasmania in 1998 was estimated as 14^{o}C .

The second equation used was the regression equation of Hoenig (1983):

$$lnZ = 1.46 - 1.01.lnt_{max}; M \simeq ZassumingF \simeq 0, \quad (2.8)$$

where t_{max} = the maximum age for the species in years.

Estimates of fishing mortality (F) were calculated by subtracting natural mortality from total mortality.

2.4 Results

Males ranged in length from 203 to 815 mm (n = 504) and females from 269 to 950 mm (n = 565). Length frequency distributions did not differ significantly between sexes (Kolmogorov-Smirnov; Z = 0.91, p = 0.38).

Pooling the length frequency data of all individuals produced a bimodal frequency distribution. However, when grouped by depth (Fig. 2.1), the data revealed a significant depth-based stratification between the shallow (<50 m stratum) and the deeper strata was evident (Kolmogorov-Smirnov; Z = 13.8, p <0.001) occurring at around 450 mm in length.

Analysis of residual sums of squares indicated no significant difference between the sex specific length-weight relationships (F = 0.02, df = 2, p = 0.10); consequently a power regression was applied to the length-weight data of all individuals combined (Table. 2.2).

The sex ratio of males to females (1.0:1.3) from the inshore net fishery showed a low level of significant difference from 1:1 (χ^2 = 3.88, p = 0.049, n = 232) whereas, the ratio of males to females (1.0:1.1) caught from the offshore hook fishery did not show significant difference from 1:1 (χ^2 = 0.933, p = 0.334, n = 840).

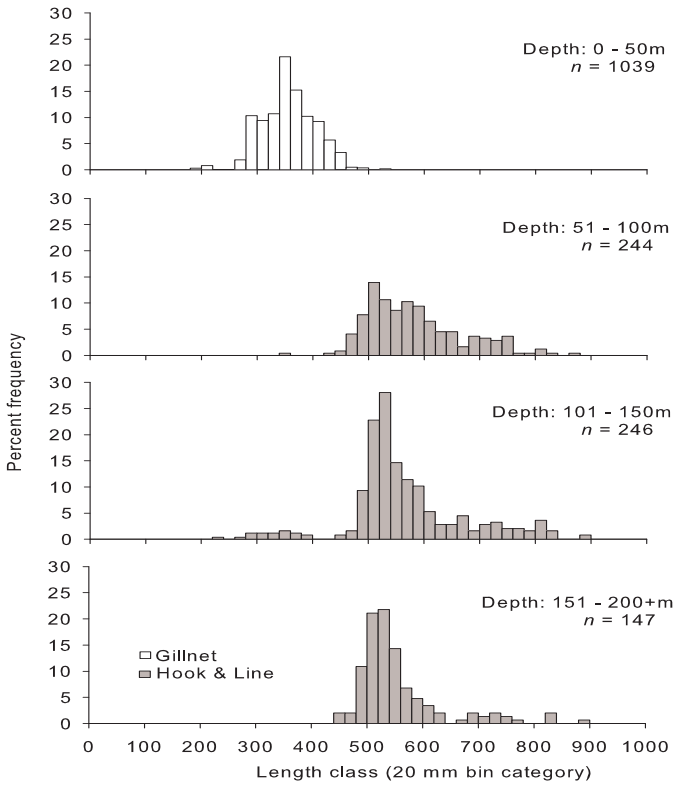

Figure 2.1: Length frequency distribution by 50 m depth strata for striped trumpeter (*Latris lineata*) samples collected from 1990 through 2002.

2.4.1 Age estimation

Age was successfully estimated for 776 (89%) individuals. Transverse otolith sections showed typical distinct alternate light and dark zone formations within the otolith matrix. Viewed under transmitted light the zones showed as dark (opaque) and light (translucent) (Fig. 2.2). A robust linear relationship existed between otolith mass and individual age (Table 2.2).

The core area of each section consisted of an opaque region. Immediately adjacent to

this was a faint thin translucent zone followed by the first broad opaque annual increment. In some cases the transition from core to the first expected increment could not be discerned, due to a continuation of the opaque region, caused by the expected thin translucent zone being to faint to see. In such cases, increment measurements were required to ensure that the annulus was not overlooked. Mean increment radius ($\pm$ sd) from the primordia to the first annulus was 491 $\pm$ 63 μm (n = 122), the deposition of the second annulus occurred at a mean radius of 733 $\pm$55 μm (n = 122). The next four opaque and translucent zone pairs were relatively broad compared with subsequent zones that consistently narrowed as they approached the growing edge (Fig. 2.2).

Table 2.2: Predictive equations used to compare weight and length, otolith weight and age, and reader variability across age classes, for striped trumpeter (*Latris lineata*). W = total weight (g), FL = Fork length (mm), OW = Otolith weight (mg), t = age (years), P_1 = primary reader (1st count), P_2 = primary reader (2nd count), S_1 = Secondary reader (1st count).

Dependent variable	Independent variable	n	Equation	r^2
(W)	(FL)	491	$W = 2 \times 10^{-5} \times L^{3.00}$	0.99
(OW)	(t)	295	$OW = 7.32 + (1.70 \times t)$	0.89
(P_2)	(P_1)	339	$P_2 = 0.05 + (0.99 \times P_1)$	0.99
(S_1)	(P_1)	46	$S_1 = 0.27 + (0.97 \times P_1)$	0.97

To validate the first increment we compared somatic and otolith growth of wild individuals with that of individuals cultured under ambient conditions. Larval rearing trials of striped trumpeter juveniles have produced mean lengths of 190 mm at 14 months and 261 mm at 24 months. The smallest individuals recorded from the wild in this study were 190 – 220 mm and were captured in January 1995. Based on the rearing trials it seemed reasonable to assume that the wild caught individuals of this size were between one and two years of age. If a birth date of 1st October is assumed, these individuals would have been about 16 months old and therefore spawned in 1993. Viewing the sectioned otoliths of these small wild caught individuals revealed only one increment within the margin, analogous to the increment composition of cultured individuals at a similar length.

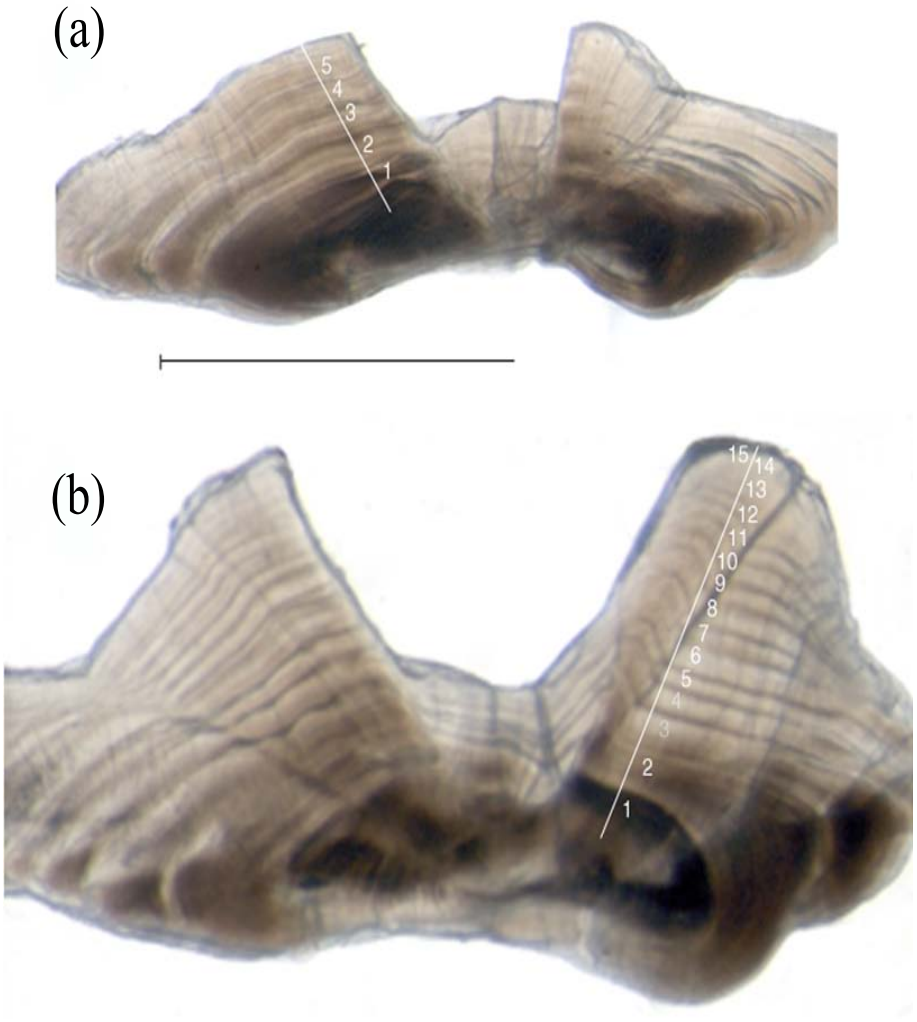

Figure 2.2: Photomicrograph of transverse otolith sections of striped trumpeter (*Latris lineata*) from (a) a 5-year old male (515 mm, FL), and (b) a 15-year old female (724 mm, FL), using transmitted light. Scale bar = 1 mm.

To test for comparable growth between wild and cultured individuals as a means to facilitate confident validation of the first increment deposition, von Bertalanffy growth curves were fitted to length at age data of both wild (based on otoliths) and cultured individuals (known age) to age four. A likelihood ratio test indicated that wild caught individuals increased in length faster than cultured individuals, (χ^2 = 5.3, df = 6, p = 0.51), however, the average lengths at approximately age one were similar (F = 4.4, df = 23, p = 0.04). Tracking length frequency distributions (Fig. 2.3) from 1995 – 1997 based on inshore gillnet samples revealed progression of a strong cohort. Based on size structure, this cohort was assumed to have been spawned in 1993. A second cohort was evident in the last quarter of 1996, assumed to have been spawned in 1994. Modal progression of this strong cohort proved useful in validation of annual periodicity of increment formation, as it was also clearly evident in the age structure of the samples over the period 1995 – 2001, present as one year olds in 1994 – 95 and seven years in 2000 – 01 (Fig. 2.4). However, inferences about population age structure cannot be drawn from the age frequency histograms since some sample sizes were low and there was discriminatory sampling (by gear type) over the period. For instance up to 1996 – 97 most of the aged samples were from inshore gillnet catches whereas subsequent samples were derived primarily from hook catches.

Precision of repeated age estimation was high. Second reads by the primary reader were 79% in agreement with first reads, yielding an APE of 0.93%. Eighteen percent of second reads gave rise to a one-year difference and 3% of second reads differed by two years, with no significant tendency to overestimate or underestimate age. An age bias plot did not differ significantly from 1:1 for the primary reader (Table 2.2). Precision of a second reader's age estimates when compared with those of the primary reader were also satisfactory, yielding an APE of 1.59% and no significant bias was revealed at any age class (Table 2.2).

The maximum observed ages for males and females were 29 and 43 years respectively. Based on available data, it is unclear whether apparent differences in longevity between the sexes are representative since very few individuals over the age of 25 were sampled. How-

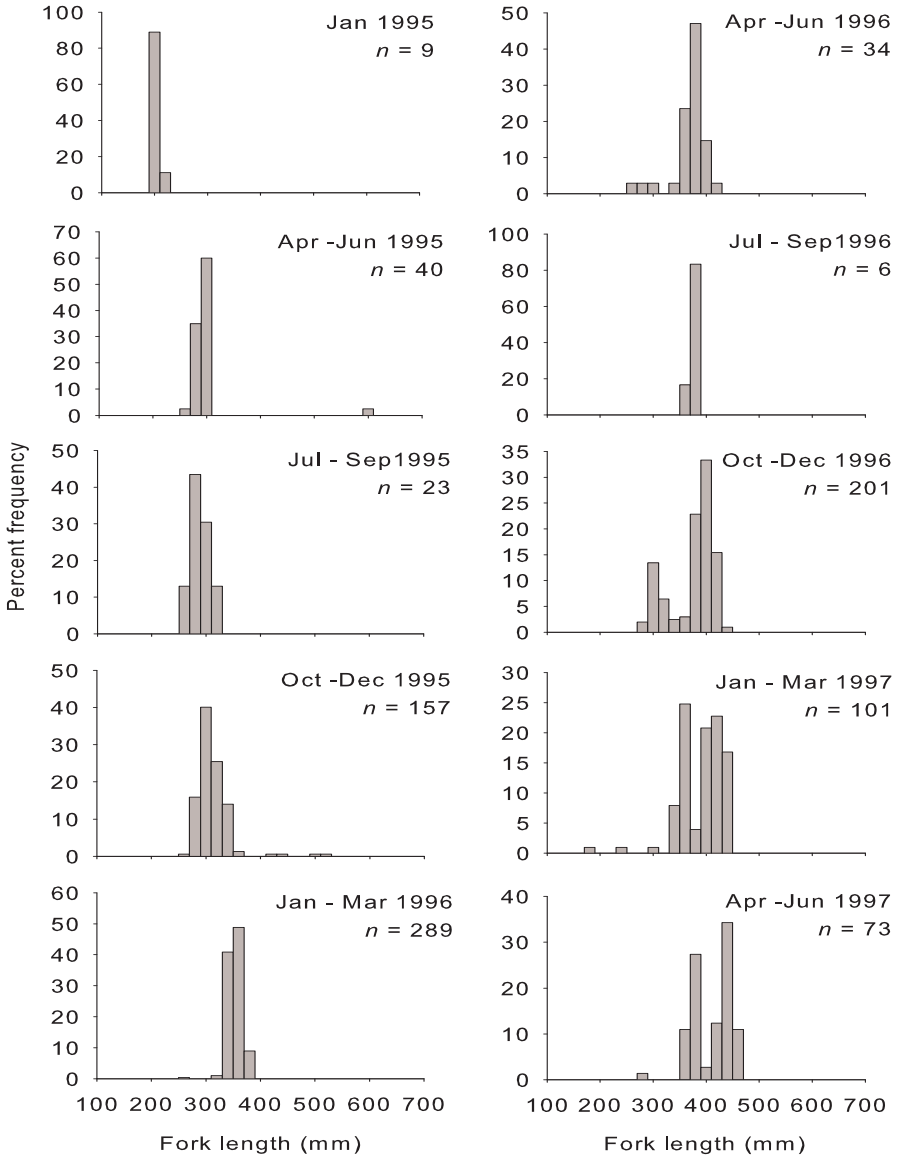

Figure 2.3: Quarterly length frequency distribution (by 20 mm size class) of striped trumpeter (*Latris lineata*) sampled from January 1995 to June 1997.

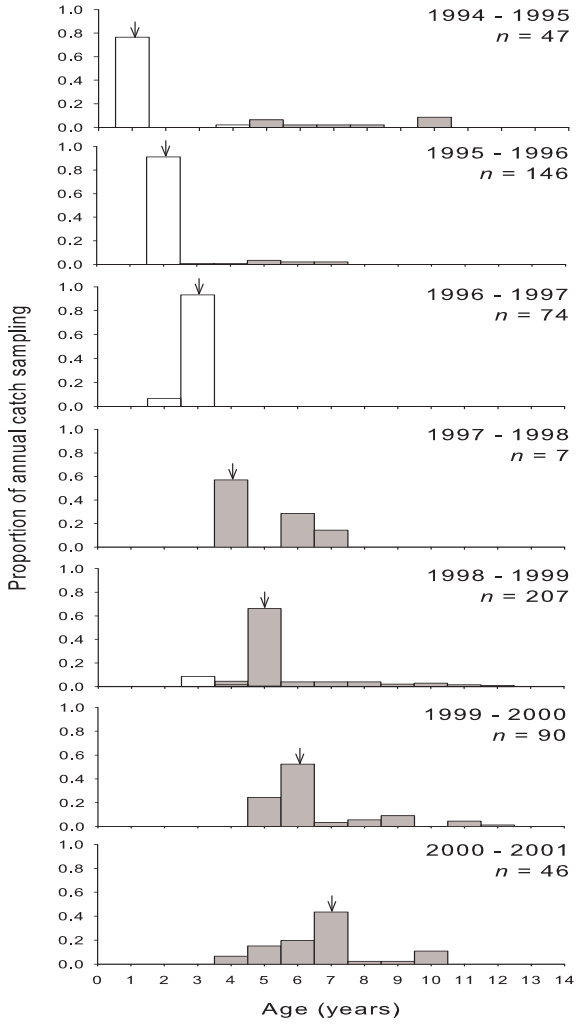

Figure 2.4: Age frequency distribution of striped trumpeter (*Latris lineata*) from 1994-2001. Gill net caught fish represented by shaded columns, hook caught fish represented by un-shaded columns. Arrows represent progression of cohort spawned in 1993.

ever, there was no significant difference in the age frequency composition of the pooled samples based on sex (Kolmogorov-Smirnov; Z = 1.05, p = 0.22).

2.4.2 Growth modelling

The strong 1993 cohort allowed close monitoring of the actual length at age of striped trumpeter, with average size increasing from 190 mm (1.3 years) in January 1995 to 300 mm (2.1 years) by November 1996 (Fig. 2.3) and 420 mm (4.0 years) by November 1997.

The seasonal VBGF model indicated that the majority of observed growth in this cohort occurred between January and May (late austral summer through autumn), with little growth apparent between June and December (Fig. 2.5). The sine wave representing seasonal fluctuations suggests that the peak growth rate occurred in May. Comparing this sine function with that derived for SST (Fig. 2.6) identified a first order serial correlation, the strongest correlation identified when a 34-day lag period was incorporated in the growth phase.

The parameters of the $VBGF_S$ and $VBGF_{TP}$ fitted to the aged individuals are presented in Table 2.3. The $VBGF_{TP}$ gave the more parsimonious fit to the pooled length-at-age data according to the deterministic AIC value and underestimated L_∞ in relation to L_{max} to a lesser extent than the $VBGF_S$ (Table 2.3), reflecting a better fit to the data in the older age classes. In conjunction with a visual assessment of residuals, it was apparent that the $VBGF_S$ underestimated length at age above 20 years (Fig. 2.7). The better fit by the $VBGF_{TP}$ supports the hypothesis that a more complex growth model was required for striped trumpeter.

The $VBGF_{TP}$ was sensitive to the value, age at transference. A profile of negative log likelihood for a range of age at transference values (Fig. 2.8) assisted in determining the correct absolute minima. The negative log likelihood profile revealed a low minima range across age at transference from 3.5 – 4.6 years, however converged to a lowest value at age 4.4 years. Fitting the PDF to the growth curve substantially smoothed the point of transition, producing a curve that represented the data well. Setting an arbitrary standard

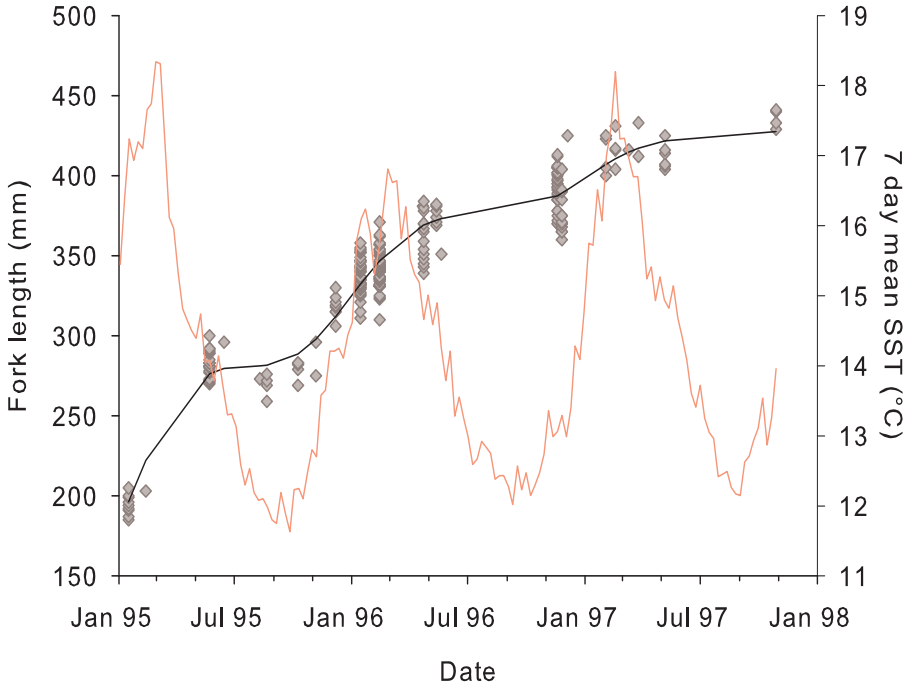

Figure 2.5: Length at age data (♢) of the 1993 striped trumpeter (*Latris lineata*) cohort fitted with a modified von Bertalanffy growth function to represent seasonal growth (black line), plotted against a 7 day average SST at the time of sampling (red line).

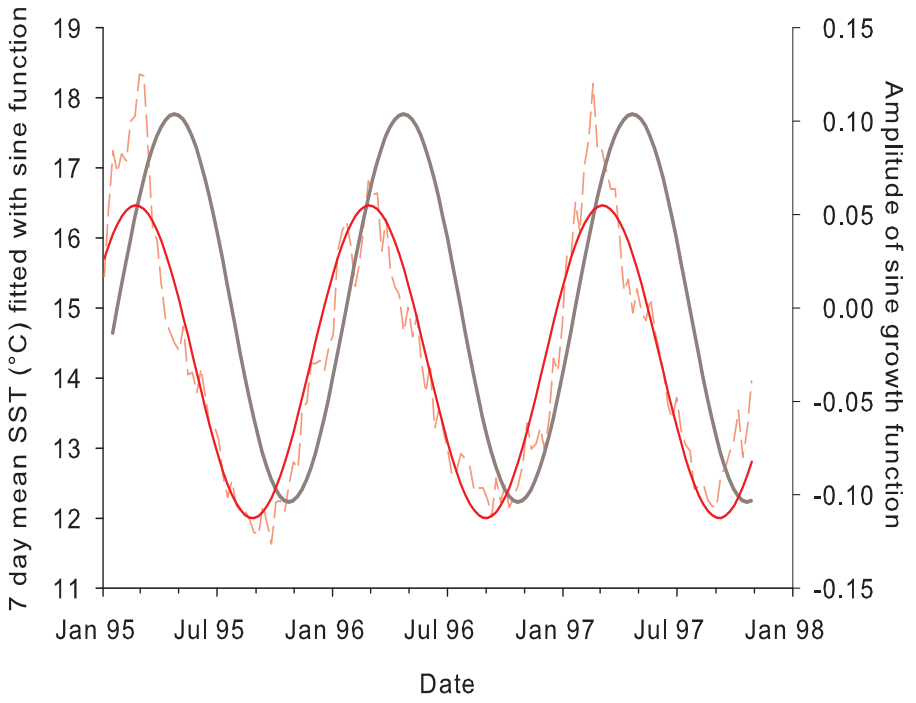

Figure 2.6: Seven day mean SST (dashed red line) fitted with a sine wave (thick red line) plotted against the sine function (thick black line) extracted from the seasonal von Bertalanffy growth function fitted in Fig. 2.5.

deviation of 1.0 around the age at transference provided a normally distributed two-tailed range at transference (90 percent confidence adjusted for bias) from 1.3 to 7.8 years.

A Likelihood Ratio Test (LRT) identified a slight significant difference between male and female $VBGF_S$ growth curves (χ^2 = 13.20, *df* = 3, p = 0.04) but there was no significant difference when the $VBGF_{TP}$ was tested (χ^2 = 10.83, *df* = 6, p = 0.09).

Table 2.3: Parameter estimates derived from the two growth functions applied to the length at age data of striped trumpeter (*Latris lineata*) in Tasmania. Growth parameters are defined in the text, NOP = number of parameters in the model, AIC = Akaike information criterion and L_{max} = the maximum length of all individuals included in the growth models, DOL_{max} = the percent deviation of the terminal L_∞ value from L_{max}.

		$VBGF_S$	$VBGF_{TP}$
Growth Parameters	$L_{\infty 1}$	773.27	532.77
	k_1	0.15	0.43
	t_{01}	-1.46	0.03
	L^d	–	450.11
	$L_{\infty 2}$	–	871.59
	k_2	–	0.08
	t_{02}	–	3.49
	t^d	–	4.40
	$\sigma^2 of t^d$	–	1.00
Diagnostics	NOP	3.00	9.00
	-log likelihood	3759.98	3700.13
	AIC	5335.12	5211.30
	% DOL_{max}	-13.70	-2.70

2.4.3 Mortality estimation

Ages 9 – 23 and 7 – 25 were included in the LCCCA regressions of the $VBGF_S$ and the $VBGF_{TP}$ respectively to estimate Z (Fig. 2.9). Individuals below these ranges were assumed, by their respective model, not to have fully recruited to the offshore fishery and

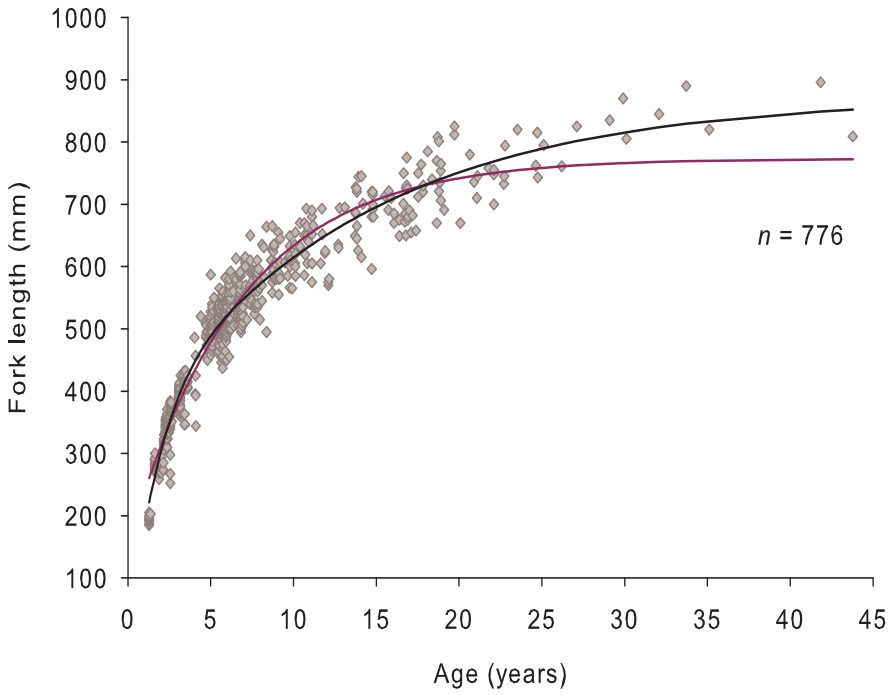

Figure 2.7: Pooled length at age data of striped trumpeter (*Latris lineata*). The black line represents the optimal two-phase von Bertalanffy growth function ($VBGF_{TP}$), with mean age at transference 4.4 years and standard deviation equal to 1; the purple line represents the optimal standard von Bertalanffy growth function ($VBGF_S$).

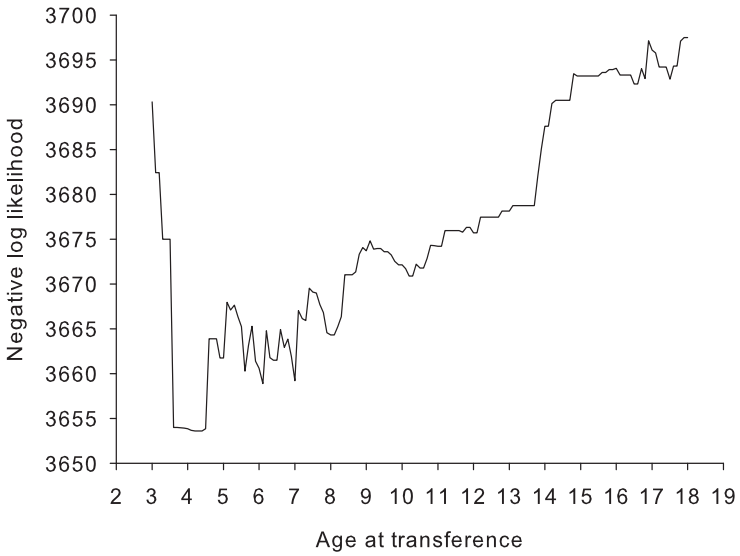

Figure 2.8: Negative log likelihood profile plot of increasing age at transference values for striped trumpeter (*Latris lineata*).

individuals over the age of 25 were excluded due to poor sample size. These age ranges effectively excluded the strong 1993 recruitment pulse from the regression, there by avoiding the complication of including a known strong year class in the analysis.

Application of the $VBGF_{TP}$ model resulted in lower estimates of Z and M (based on the Pauly equation), compared with those calculated using the $VBGF_S$ parameters (Table 2.4). The estimate of Z based on the Hoenig (1983) equation was assumed to be close to M as F is low for this species. The Hoenig M was very similar to the Pauly estimate when $VBGF_{TP}$ parameters were used. In this case M was just below 0.1, implying an annual natural mortality rate of about 9%. The $VBGF_{TP}$ estimates imply that F was slightly higher than M in the offshore fishery. By contrast, the standard $VBGF_S$ parameters produced a substantially higher estimate of M (0.15) based on the Pauly equation than predicted by the Hoenig approximation, implying an annual natural mortality rate of about 14%. Derived estimates of F using the $VBGF_{TP}$ were slightly higher than M, whereas F

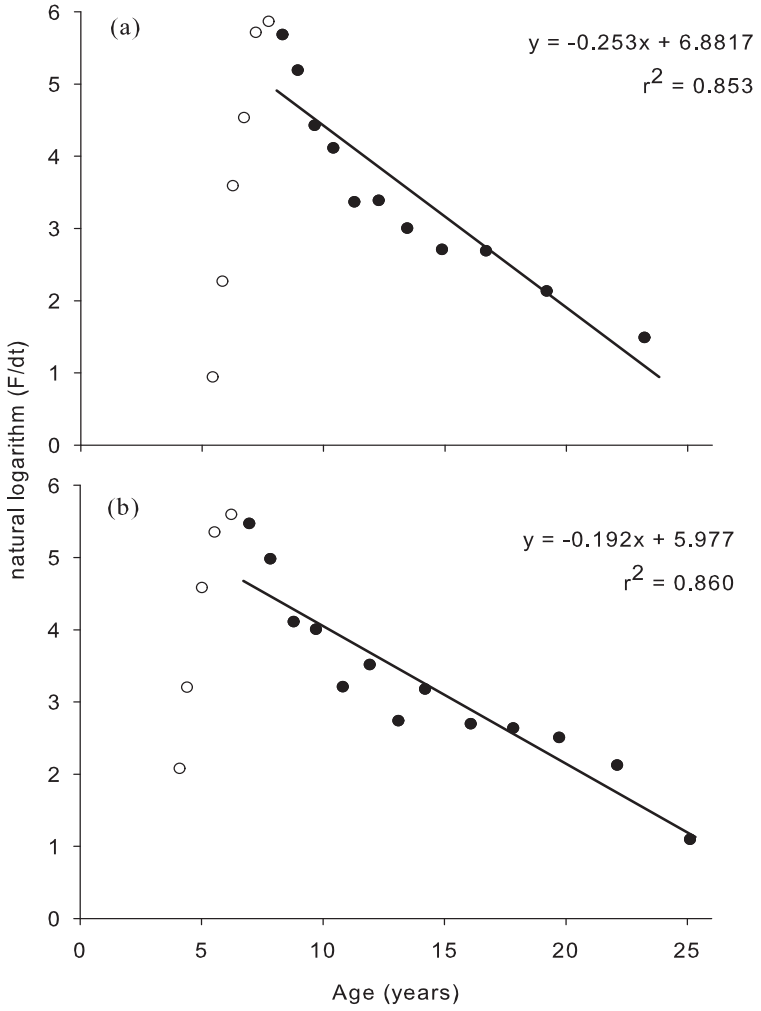

Figure 2.9: Length converted catch curve analysis for striped trumpeter (*Latris lineata*) length and age data from 1998. (a) Age composition based on the standard von Bertalanffy growth function ($VBGF_S$), (b) age composition based on the second stanza of the two-phase von Bertalanffy growth function ($VBGF_{TP}$). Solid points were included in the respective linear regressions.

relative to M was variable for the $VBGF_S$, depending on the equation used to derive M.

Table 2.4: Estimates of instantaneous rates of total (Z), natural (M) and fishing (F) mortality of striped trumpeter (*Latris lineata*) using age based catch curve analysis and the empirical equations of Hoenig (1983) and Pauly (1980). $VBGF_{TP}$ = estimates derived from the parameters of the two-phase von Bertalanffy growth function, $VBGF_S$ = estimates derived from the parameters of the standard von Bertalanffy growth function and LCCCA = Length converted catch curve analysis.

	Z		M		F	
Method	$VBGF_S$	$VBGF_{TP}$	$VBGF_S$	$VBGF_{TP}$	$VBGF_S$	$VBGF_{TP}$
LCCCA	0.253	0.192	–	–	–	–
Hoenig	–	–	0.096	0.096	0.157	0.096
Pauly	–	–	0.151	0.092	0.102	0.100

2.5 Discussion

This study represents the first report of age and growth of striped trumpeter. Despite having available a patchy data set we have been able to validate age and overcome the limitations of the von Bertalanffy equation to represent this data by the use of a robust growth model. Striped trumpeter are long-lived, with maximum age in excess of 40 years, and growth particularly rapid up to age five after which it slows dramatically.

The species has a complex early life history involving a long planktonic larval phase of around nine months; an inshore juvenile phase and then movement offshore into deepwater.

Gear selectivity (gillnets in the shallow and hook catches in the deeper waters) may have influenced the size structure of our samples, especially when grouped by depth, although it is highly unlikely that the size differences could be completely attributed to gear type alone. For instance, small individuals ($<$ 400 mm) were occasionally taken by hooks in the deeper strata and individuals over 500 mm were taken by gillnets in less than 50 m. The commercial hook fishery for striped trumpeter is largely restricted to depths of greater than 50 m, and despite considerable hook fishing effort at shallower depths, targeting other

demersal reef species, notably the wrasses, *Notolabrus fucicola* and *N. tetricus*, minimal catches of striped trumpeter are taken and those that are caught tend to be small in size . Rather, size structuring by depth is believed to reflect the movement of striped trumpeter offshore into deeper water as they grow and mature.

Seasonal growth was dramatic in young striped trumpeter (Fig. 2.5). This phenomenon is common in temperate species (Jordan, 2001; Haddon, 2001; McGarvey & Fowler, 2002), and has been linked to fluctuations in environmental factors, such as water temperature and oceanographic conditions, as well as biotic factors, such as seasonality in primary productivity (Harris et al., 1991; Jordan, 2001). This study supports a correlation between water temperature and seasonal growth (Fig. 2.6), with maximum growth observed consistently over a three-year period approximately one month after the peak sea-surface temperatures.

Knowledge of growth and growth variability is essential to the understanding of a stocks population dynamics. To achieve an accurate assessment of these characteristics, several issues need to be addressed. Foremost, is a rigorous approach to validation and precision tests of ageing (Campana, 2001). In this study, a combination of age validation protocols outlined by Fowler & Doherty (1992) and Campana (2001) were subscribed to; (1) otoliths must display an internal structure of increments (Fig. 2.3); (2) otoliths must grow throughout the lives of fish at a perceptible rate, which was confirmed via the otolith weight at age regression (Table 2.2); (3) the age of first increment formation must be determined, and (4) increment periodicity across the entire age range of interest must be verified. We used cultured individuals to determine the first increment, although the accuracy of validation using cultured individuals was questioned by Campana (2001). In this study, cultured individuals are able to adequately reflect growth from the wild, as the close correspondence between the growth of cultured and wild striped trumpeter over a period of several years supports the assertion that growth of cultured fish was generally representative of wild growth. The slightly slower growth rate observed in cultured striped trumpeter can be attributed to jaw malformation; a phenomenon that has been shown to affect feeding ability (Cobcroft et al., 2001).

Modal progression of the 1993 cohort through time provided indirect validation for annual periodicity in increment formation up until age seven. Validation across all age classes was not possible in this study, although validation after the age of five years was significant. That is, validation was achieved past the average age at which fish moved offshore into deeper water, and past the age at which there was a significant reduction in growth rate. These factors were assumed to be the point at which there was a possibility for some other deterministic increment being present in the otolith matrix or a change in otolith development.

The second consideration to address when studying animal growth is model selection. Akaike's information criterion is a standard method of model selection that provides an implementation of Occam's razor, in which parsimony or simplicity is balanced against goodness-of-fit (Forster, 2000). However, model selection should not rely on statistical fit alone, it should also reflect the theoretical viewpoint of growth to provide a biologically sensible interpretation across the entire range of ages in the sampled population (Haddon, 2001). In the case of striped trumpeter, the standard von Bertalanffy function provided a poor representation of growth in older individuals, resulting in an unrealistically low L_{∞}. This problem was largely overcome by the application of a two-phase growth function. Similar to that used on large pelagics, such as Thunnus maccoyii (Bayliff et al., 1991; Hearn & Polacheck, 2003). In their application of the model, Hearn & Polacheck (2003) considered biological traits when discussing the justification for age at transference, namely the reduction in growth rate, and inshore to offshore migration. Here we have considered analogous traits to seed the age of transference for striped trumpeter. In this species there is a marked transition in size structure between shallow and deeper reefs that occurs at around 450 mm or between 4 and 5 years (Fig. 2.8). In addition, a visual assessment of the length at age data highlighted a marked decrease in growth rate at a similar age.

Solving for the age at transference produced a point estimate that results in a sharp discontinuity in the growth curve; an observation that Hearn & Polacheck (2003) highlighted as biologically unrealistic. The range of low negative log likelihood values described by

the age at transference profile is due to the patchiness of data around these ages creating uncertainty in the model. We have assumed in this case that the converged value of 4.4 years is accurate and that the variability around this point is normally distributed with a standard deviation equal to one. By including the normal probability distribution function we have effectively created a smooth transition between growth phases. This function implies that age at transference has some level of inherent variability, which is likely to be more biologically plausible.

A further extension of the two phase model was tested by applying the seasonal growth version of the VBGF (described in Eq. 2.3) to the first phase and a standard VBGF to the second phase, but was disregarded due to the effect of over parameterisation on parsimony. However, this approach did highlight the flexibility of the two-phase model to allow for a more dynamic representation of population growth characteristics.

This study supports the assertion by Hearn & Polacheck (2003) that discontinuity in growth rate may be a more common phenomenon in fish than implied by growth models reported in the literature. The use of such a two-phase growth model, where age at transference coincides with the transition phase from one fishery to another, has proven informative. It allows separate growth parameters to be allocated to each fishery, and as such, provides a precursor to developing a more biologically robust production model with dynamic parameters at age and fishing method.

The predictive regression developed by Pauly (1980) that estimates natural mortality is based on the direct relationship between longevity (t_{max}) and the magnitude of the physiological growth parameters k and L_∞. As such, it would be reasonable to assume that if a good fit exists between length at age that the growth parameters, when employed in such an empirical model, would yield a natural mortality estimate approximately equal to that determined by a regression model that is based on t_{max} (Hoenig, 1983). The two-phase growth function also provided a more conservative estimate of M than the standard von Bertalanffy model. Overestimates of M can lead to unrealistically high estimates of productivity and potential yield that may in turn lead to overexploitation of a stock.

Protracted longevity, slow growth in later life, large body size, recruitment variability and relatively low natural mortality once individuals reach adulthood are all characteristics typical of a k-selected species. Such species are often regarded as being susceptible to growth over-fishing and stock depletion (Booth & Buxton, 1997). For instance, increased fishing effort on the inshore fishery, as has been observed with the recruitment of strong cohorts, will impact on subsequent recruitment to the offshore fishery and spawning stock. The current analysis suggests that fishing mortality is slightly higher than natural mortality and, in the absence of further strong recruitment, a decline in the stock size is likely if fishing pressure is not reduced.

Chapter 3

Reproductive biology and per-recruit analyses

Abstract. This paper profiles the reproductive biology of and investigates the resilience of Tasmanian striped trumpeter under different levels of fishing pressure and size at entry to the fishery using per recruit analysis. The spawning season around Tasmania occurs in the austral spring, with peak spawning activity in September and October. Size at 50% maturity was estimated at 543 mm fork length (FL) for females (estimated age = 6.8 years) and 529 mm FL for males (estimated age = 6.2 years). Striped trumpeter is a multiple spawner with batch fecundity estimates ranging from 205,054 for a 2 kg fish (540 mm FL) to 2,351,029 for a 9.5 kg fish (800 mm FL). At the current minimum legal size limit of 450 mm total length (equivalent to approximately 425 mm FL), yield-per-recruit was estimated to be close to maximum, and spawning biomass-per-recruit (SPR) ranged from 35 - 52% of virgin stock, depending on the mortality estimates used. Although these SPR are at a level considered sustainable, this methodology does not incorporate temporal variability, specifically recruitment variability. Therefore conservative management measures are recommended until a dynamic model is applied to the striped trumpeter population.

3.1 Introduction

Successful fisheries management relies on understanding the regenerative ability of fish populations and having an accurate assessment of biological parameters, including reproductive traits, such as; size and age at maturity, fecundity and duration of spawning season, along with growth and mortality (Quinn & Deriso, 1999). These factors are used in stock assessment models to ensure that yield is maintained near the maximum attainable level whilst ensuring that the stock remains above an undesirable threshold, such as 20% unfished spawning stock biomass (Mace, 1994). In the absence of a dynamic quantitative stock assessment, exploring yield and spawning biomass-per-recruit simulations at different levels of exploitation can provide a basis for management strategies (Sissenwine & Shepherd, 1987; Deriso, 1987; Mace, 1994).

Striped trumpeter (*Latris lineata*) is a large temperate reef species that is relatively long lived, attaining a maximum age of over 40 years (Tracey & Lyle, 2005) and growing to 25 kg in weight (Gomon et al., 1994). The species is widely distributed around the southern hemisphere, found in all the major oceanic basins (Tracey et al., 2006). The species has had a long history of commercial and recreational exploitation in Tasmania, targeted for its high quality large white fillets. Striped trumpeter display life-stage specific habitat preferences which have effectively created two fisheries for the species; juvenile fish are taken predominantly by gillnets (recreational and commercial) in coastal reefs, usually in depths $<$50 m, whereas sub-adult and adult fish are taken over deeper offshore reefs (up to 350 m) by hook methods (dropline, handline, longline, trotline) and as a by-catch in large mesh gillnets (shark nets) (Tracey & Lyle, 2005).

In Tasmania, management of the striped trumpeter fishery is based on minimum size and possession limits. Since 2001 commercial operators have been restricted to a 250 kg trip limit while recreational fishers have a possession limit of eight fish per person. In 2004 the minimum legal size limit was increased from 350 to 450 mm total length (TL), this change having greatest impact on inshore gillnet catches which are based almost

exclusively on fish smaller than this size (Tracey & Lyle, 2005).

Since 2000, commercial landings of striped trumpeter in Tasmania have averaged about 40 tonnes per annum, down from over 100 tonnes per annum in the late 1990's. Catch reductions have been evident for both inshore gillnet and offshore hook methods. Inshore gillnet catches, of smaller fish, taken prior to the implementation of the new size limit in 2004, had fallen from almost 20 tonnes to around 5 tonnes per annum, with catch per unit of effort (CPUE) trends providing evidence of low recruitment over the past few years (Tracey & Lyle, 2005).

A survey of the recreational fishery during 2000/01 provided an estimated annual recreational harvest of 38 tonnes, which was only slightly less than the commercial catch for the same period (Lyle, 2005). Striped trumpeter are currently the focus of aquaculture trials in Tasmania (Bransden et al., 2005; Trotter et al., 2005). These trials have established that females are multiple or batch spawners and that oocyte development is asynchronous (Morehead, 1998). The rearing of striped trumpeter larvae has indicated a complex and extended larval phase of up to nine months prior to settlement, including a post-larval 'paperfish' stage [1]. Despite an understanding of some of the reproductive capabilities of cultured and captive reared striped trumpeter under various hormonal and photo-thermal regimes (Morehead & Hart, 2003), very little is known about the size or age-at-maturity, reproductive strategies or fecundity of wild striped trumpeter stocks. The absence of key biological information, coupled with recent catch declines, compound concerns for the sustainability of the stock under current levels of fishing pressure in Tasmania.

This study describes the reproductive characteristics of striped trumpeter, with a focus on quantifiable traits necessary for stock assessment modelling: spawning season, batch fecundity and size at maturity. Secondly, this information is incorporated into yield and spawning biomass-per-recruit analyses to assess the impacts of current and alternative minimum size limits on yield and potential egg production.

[1] David Morehead, personal communication

3.2 Materials and Methods

3.2.1 Sampling regime

Striped trumpeter were collected opportunistically between 1991 and 2005 from the north-east, east, south and southwest coasts of Tasmania (Fig. 3.1). Available samples ranged from fish frames collected from fishers and fish processors, where only length and sex and, potentially, gonad stage information could be obtained, to whole fish caught for scientific research, where length, weight, sex, gonad weight and maturity stage were assessed. Otoliths were also collected when possible. Most samples were hook caught from offshore reefs at depths greater than $\simeq$90 m. However, between 1994 and 1999, striped trumpeter were also sampled using gillnets set over shallow inshore reefs ($<$30 m) off the southeast coast of Tasmania, these fish were exclusively juveniles and represented cohorts spawned in 1993 and 1994 (Murphy & Lyle, 1999). This sampling strategy resulted in a patchy dataset for reproductive analysis, in space and time. In 2004, targeted research sampling was undertaken during the spawning period (September – October) to provide gonad samples for histological examination and estimation of fecundity.

3.2.2 Biological data

Fork length ($\pm$1 mm), sex and, where possible, total weight ($\pm$10 grams), gonad weight ($\pm$1 gram) and macroscopic maturity stage (based on criteria outlined in Table 3.2) were recorded. For inclusion in the reproductive assessment an individual had to have at least gonad weight or gonad stage recorded. The relationship between fork length (FL) in mm and total weight (TW) in grams was calculated as:

$$TW = a.FL^b \tag{3.1}$$

where *a* and *b* are constants. Gonadosomatic index (GSI) of each fish that had intact gonads excised was estimated as:

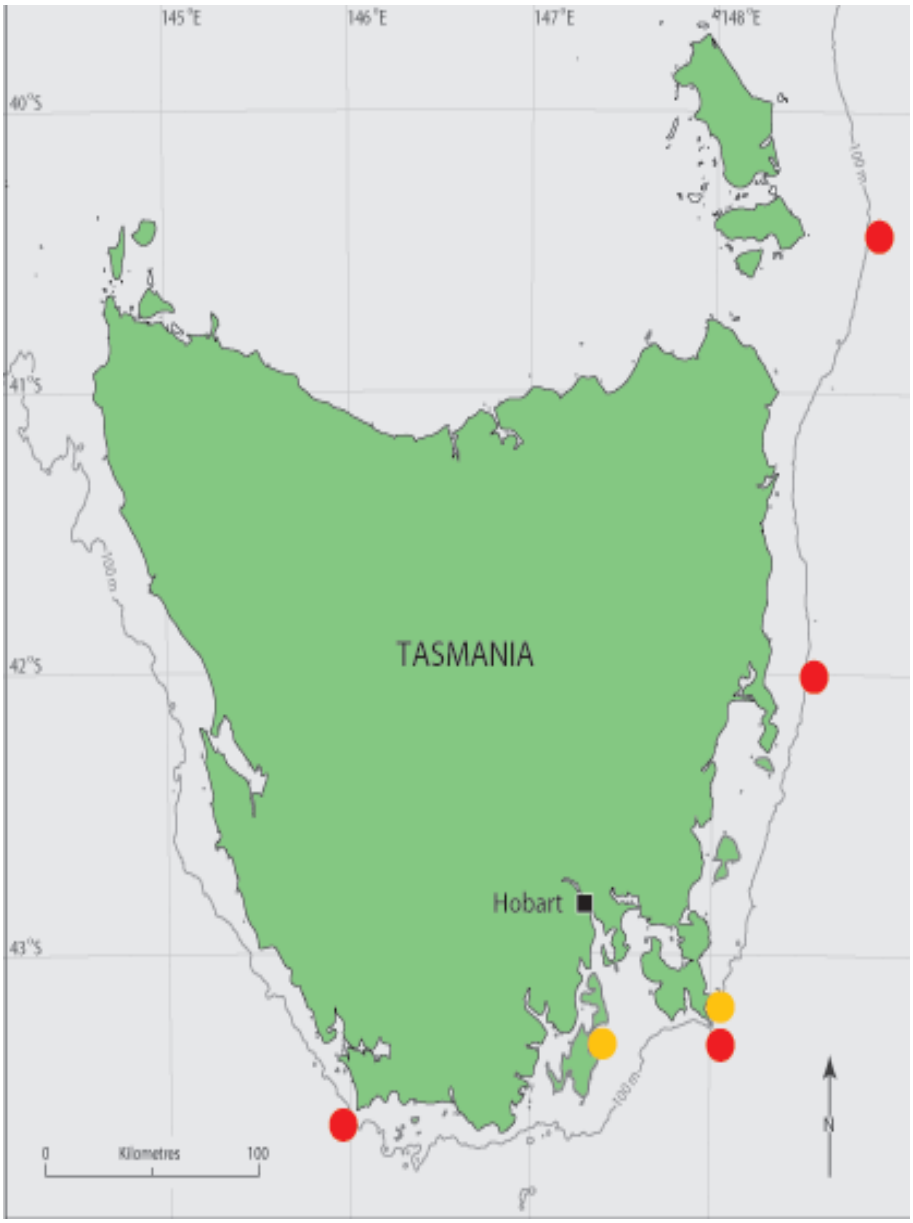

Figure 3.1: Map of Tasmania, Australia showing sampling locations of striped trumpeter (*Latris lineata*) for reproductive assessments. Red dots indicate offshore sites, orange dots indicate inshore sites.

$$GSI = \frac{GW}{TW - GW} \times 100 \tag{3.2}$$

where *GW* is gonad weight. If total weight was not available for an individual (typically the result of sampling filleted fish), total weight was estimated using the length-weight relationship. Mean monthly GSIs were calculated using the dataset pooled over all years.

3.2.3 Histological analysis

Ovaries were removed from females collected between September and October 2004. The gonads were weighed ($\simeq$1 gram) and the whole ovary or a sub-sample (dependent on gonad size) was preserved in 10% formalin acetic acid calcium chloride (FAACC) diluted with seawater. A comparison of the left and right ovary lobe weights was undertaken using a t-test. To avoid possible variation in the developmental stage of oocytes due to their position in the ovary, all tissue samples for histological preparation and oocyte counts were taken

Table 3.1: Sample sizes of striped trumpeter samples collected between 1991 – 2004 around Tasmania, indicating the types of biological information collected

Year	Gonad weight		Gonad stage		Whole weight	
	M	F	M	F	M	F
1991	40	41	–	–	8	12
1992	59	67	–	–	–	–
1993	–	–	–	–	–	–
1994	1	4	1	4	1	4
1995	22	6	22	10	22	8
1996	31	48	35	71	28	48
1997	13	11	13	11	10	11
1998	–	–	–	–	–	–
1999	77	101	146	174	68	78
2000	20	36	20	36	–	–
2001	13	21	13	21	–	–
2002	37	24	42	30	10	10
2003	14	13	14	13	7	10
2004	58	73	60	73	58	69
2005	19	21	20	21	19	21
Total	404	466	386	464	253	294

transversely through the medial section of the ovary. Prior to histological preparation the gonad tissue was dehydrated in 70% ethanol. After infiltrating the samples with wax, two or three 5 μm sections were prepared from each sample and then stained firstly with Mayer's haemalum and then with Young's Eosin.

Diameters for fifty oocytes from each oocyte development phase present were measured for an individual female representing each of the maturity stages II – VI. Oocyte development phases were categorised as: primary oocyte (younger than perinucleolar stage), cortical alveoli, early vitellogenic, late vitellogenic (final oocyte maturation) and hydrated (West, 1990). Only oocytes sectioned through the nucleus were included in the size frequency analysis.

3.2.4 Fecundity

Batch fecundity was estimated for 39 stage V females as the average number of oocytes from sub-samples raised to the total ovarian weight by:

$$N_{tv} = N_{vs} \times \frac{GW}{W_S} \tag{3.3}$$

Where N_{tv} is the total number of late vitellogenic/maturing oocytes in the ovary, N_{vs} is the mean number of late vitellogenic oocytes counted within three sub-samples from the one gonad and Ws is the average weight of the three sub-samples. Approximately 0.30 g of a preserved ovary was blotted on absorbent paper and weighed to the nearest $\pm$0.001 g for each sub-sample. The samples were inspected microscopically at 40x magnification and oocytes were categorized to a developmental phase based on visual characteristics and oocyte diameter. Oocytes categorized as late stage vitellogenic were distinctly larger than pre-and early vitellogenic oocytes and were counted for batch fecundity estimation. Fecundity estimation was determined for ovaries that were not damaged and had no obvious hydrated eggs in the lumen. A power regression was fitted to represent the relationship between batch fecundity and fish length.

3.2.5 Size at maturity

A logistic regression was fitted to the maturity-at-length data (pooled across regions and years) of fish collected throughout the spawning season (August – November). Maturity was described in a binary configuration (0 = immature, stages I – III for females and males; and, 1 = mature, stages IV – VII for females and stage IV – V for males). Due to limited sample sizes in some length classes, data were pooled into 20 mm categories. The logistic model fitted to the data was:

$$P = \frac{e^{\beta_0+\beta_1.FL}}{1+e^{\beta_0+\beta_1.FL}} \tag{3.4}$$

where *P* represents the probability of being mature at size FL, and β_0 and β_1 are the regression coefficients for the intercept and fork length, respectively. The model fit was estimated by maximising the log-likelihood of the binomially distributed error term. The length where 50% of each particular sex was determined to be mature (L_{50}) was derived from the equation:

$$L_{50} = \frac{-\beta_0}{\beta_1} \tag{3.5}$$

Age at 50% maturity ($t_{50\%}$) was calculated using the estimate of length at 50% maturity and the von Bertalanffy growth parameters based on the single phase growth model reported in Tracey & Lyle (2005). The von Bertalanffy growth function (VBGF) re-arranged to the form:

$$t_{50\%} = t_0 + \frac{1}{k}\left[ln\frac{L_\infty}{L_\infty - l_{t50\%}}\right] \tag{3.6}$$

where $t_{50\%}$ is the age at 50% maturity, L_∞ is the asymptotic length in mm, k is the Brody growth parameter, t_0 is the age at zero length, and $l_{t50\%}$ is the length at 50% maturity. Tracey & Lyle (2005) found no significant difference in the von Bertalanffy growth functions (VBGF) describing the growth of males and females, hence, in the present

study the parameters of the VBGF used were of the pooled VBGF, estimated as FL_∞ = 773.27 mm, k = 0.15 yr^{-1} and t_0 = -1.46 years.

3.2.6 Per-recruit analyses

Tracey & Lyle (2005) applied catch curve analysis and estimated that the instantaneous rate of total mortality (Z) for striped trumpeter in 1999 was 0.253 year^{-1}. Two estimates of natural mortality (M) were presented by Tracey & Lyle (2005), one based on Hoenig's (1983) empirical equation of 0.096 year^{-1}, implying an instantaneous fishing mortality (F) rate of 0.16 year^{-1} ($F = Z - M$), and the second based on Pauly's (1980) equation of 0.151 year^{-1}, resulting in F equivalent to 0.102 year^{-1}. For yield and spawner biomass-per-recruit analyses, simulations were undertaken using these values of M. Fishing mortality (F) was assumed to be constant across all ages and size classes above and including the defined legal size and the sensitivity of the analysis to a range of fishing mortality estimates was tested through simulation.

The equation used to estimate the potential yield per recruit (YPR) was based on the method of Thompson & Bell (1934):

$$YPR = \sum_{a=a_c}^{a_{max}} \sum_{t=1}^{t=n} N_{at} \times (TW_{FL_{at}} \times \frac{F_t}{Z_t} \times (1 - e^{-(M_t+F_t)})) \tag{3.7}$$

where $N_a = N_{a-1}e^{(-M+F)}$, FL for each age was estimated from the von Bertalanffy growth curve, TW_{FL} = total weight of fish at length FL estimated from the length-weight relationship, n = the number of equal duration time steps in a year, a_c = the age at recruitment to the fishery, a_{max} = the maximum age considered. The inclusion of a plus group had no noticeable effect and so was omitted. The equation used to estimate spawning stock biomass-per-recruit (SBR) was:

$$SBR = \sum_{FL_M}^{FL_{max}} \left(fr_{FL} \times W_{FL} \times e^{-\sum_{t=t_r}^{t-1}(F+M)} \right) \tag{3.8}$$

where fr_{FL} is the proportion of fish mature at a given FL estimated from the logistic maturation ogive, and tr is the age at which the fish, on average, reaches the minimum legal length.

3.3 Results

3.3.1 Biological data

Males included in the reproductive assessment ranged between 269 – 950 mm FL and females ranged from 269 – 845 mm FL. Length frequency distributions were not significantly different between the two sexes (Kolmogorov-Smirnov; Z = 1.16, p = 0.134). Analysis of residual sums of squares (Haddon, 2001) suggested no significant difference between the sex specific length-weight relationships (F = 0.02, df = 1, p = 0.10); consequently a power regression was applied to the length-weight data of all individuals combined, giving parameter values, $a = 1.0 \times 10^{-5}$ and b = 3.04 (r^2 = 0.99).

Females accounted for 56% of the sample (n = 126) examined during the 2004 spawning period (August – November) however the sex ratio was not significantly different from unity (χ_2 = 1.56, df = 1, p = 0.21).

3.3.2 Spawning period

Only individuals greater in length than the sex specific L_{50} were included in the temporal assessment of GSI values. Monthly GSI estimates for both sexes indicated a discrete seasonal reproductive cycle, with GSIs consistently higher for females (Fig. 3.2). GSIs increased in August, peaked strongly in September, at a mean of 5.58 for females and 3.97 for males, then fell sharply in October and returned to resting levels by December, implying a peak in spawning activity between September and October. Fish of both sexes with developing gonads (>stage III) were caught between July and November. The proportion of each gonad development stage observed for females greater in length than the size at

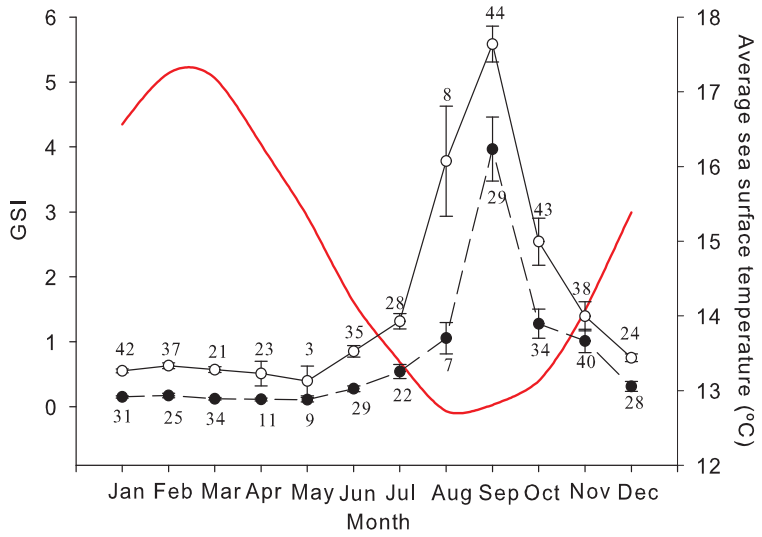

Figure 3.2: The gonadosomatic indices (GSI) estimated for males (•) and females (○) each sampling month, with data pooled across a 15-year period (1991 – 2005). Error bars represent the standard error of the mean. Numbers above and below the mean GSI indicate the number of individuals used for the estimate of females and males respectively. Monthly sea surface temperature (thick red line) has been estimated by averaging weekly remote sensed data swathed over the east coast of Tasmania from 1982 to 2004.

first maturity (505 mm FL) and confirmatory histological evidence of post ovulatory follicles in stage VII females supports a peak in spawning over September and October, with stage V – VII females dominating these months (Fig. 3.3).

3.3.3 Histological analysis

Histological preparations from 49 ovaries collected between September and October 2004 were assessed, 84% of which were in spawning condition (stages V – VI). Examples of oocyte development are shown in figures 3.4 and 3.5). Size frequency distributions of primary oocyte, cortical alveoli, early vitellogenic, late vitellogenic and hydrated oocytes

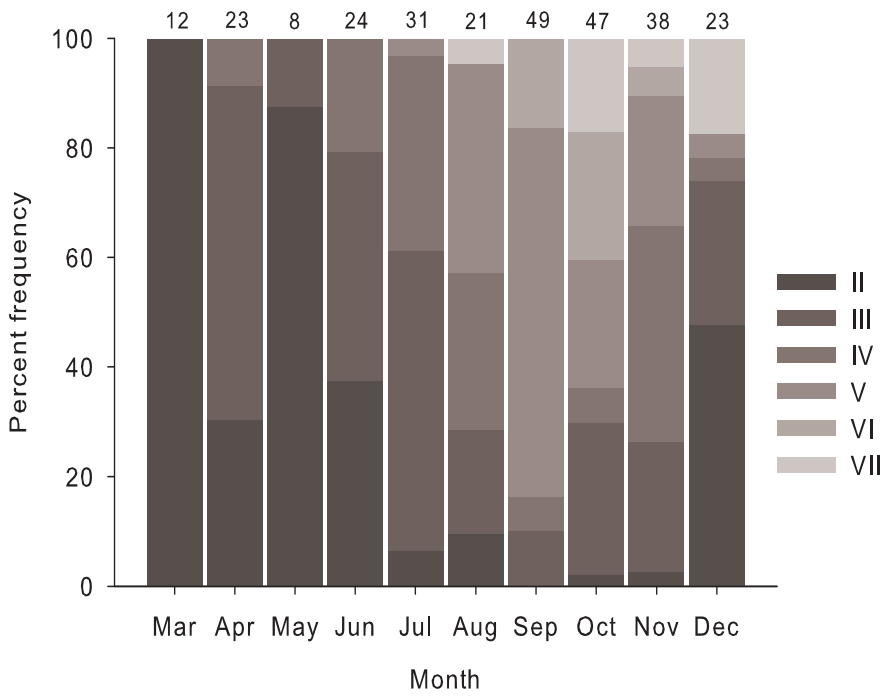

Figure 3.3: Percent frequency of female maturity stage observed in each sampling month, compiled over a 15-year period (1991 – 2005). Maturity stages II – VII are described in Table. 3.2.

from representative females at maturity stages II through VI are shown in Fig. 3.6. Stage II ovaries were dominated by primary oocytes (50 – 110 μm) and cortical alveoli (100 – 220 μm). As ovaries reached stage III a distinct batch of early vitellogenic, yolked oocytes was apparent (280 – 320 μm), stage IV ovaries had a similar distribution of oocytes to stage III though qualitative observations indicated a relative increase in the proportion of yolked oocytes. Stage V ovaries still had a significant standing stock of pre- and early vitellogenic oocytes (the latter had increased in size 280 – 450 μm), as well as a clearly distinguishable clutch of late vitellogenic/maturing oocytes (500 – 790 μm). It was assumed that this clutch would develop into the next batch of hydrated eggs to be spawned. Stage VI ovaries still had a considerable number of primary oocytes and cortical alveoli, early and late-vitellogenic oocytes were present and distinguishable from one another, and hydrated oocytes also sat in an easily distinguishable group based on diameter (720 – 1000 μm) and physical appearance. For fecundity estimation, the late-vitellogenic clutch present in stage V fish was counted. Stage VI fish were not included to reduce the possibility that some of this clutch may have already been expelled from the ovary.

3.3.4 Atresia and post ovulatory follicles

The rate of atresia for both unyolked and yolked oocytes was generally low, however, one ovary presented high level of atresia (above 90%) of yolked oocytes, implying that this individual had either completed spawning or failed to spawn. It is, however, possible that the stress of capture and retention in the wet-well of the fishing vessel for up to 10 days may have contributed to this condition.

Post-ovulatory follicles (POFs) occurred in 89% of the mature ovaries examined, indicating that the majority of females assessed had already released at least one batch of oocytes.

Table 3.2: Female ovary and male gonad development staging criteria modified from the criteria of West (1990) and Knuckey and Sivakumaran (2001), including the GSI range for female striped trumpeter determined in this study

	Maturity stage	Macroscopic appearance	GSI range
Female			
I.	Virgin	Ovary small and thread-like, pink translucent.	0.02 - 0.13 (12)
II.	Developing virgin or resting	Ovaries translucent yellow to creamy colour. Possibly a few ovarian blood vessels. Oocytes not visible.	0.04 - 0.93 (9)
III.	Early developing	Ovaries beginning to swell, under close inspection small oocytes are visible.	0.05 - 2.00 (49)
IV.	Late developing	Ovaries swollen, small oocytes clearly visible.	0.93 - 2.81 (42)
V.	Gravid	Ovaries swollen and large swelling of belly visible externally. Ovaries yellowish to orange. Oocytes clearly visible. Hydration of oocytes may be interspersed through ovary. No eggs released with light pressure on the abdomen.	1.15 - 9.23 (55)
VI.	Running-ripe	Ovaries significantly enlarged, oocytes are translucent orange and freely expressed naturally or with light pressure on the abdomen.	1.91 - 7.75 (10)
VII.	Spent	Ovaries flaccid, yellowish to red and an increase in visible blood vessels. Potentially residual hydrated oocytes. This stage will eventually resemble a stage 2 ovary as atresia resides and the ovary returns to a resting state.	0.59 - 1.91 (14)
Male			
I.	Immature	Testes small, flat and thread-like.	
II.	Early developing	Testes beginning to become rounder in shape, occupy approximately 2/3 the length of the coelomic cavity.	
III.	Developing	Testes lobed, milt sometimes present.	
IV.	Running ripe	Testes large and significantly lobed, milt becomes free flowing. May appear pinkish in colour.	
V.	Spent/resting	Testes appear blood shot, and pinkish to brown in colour, may be residual milt.	

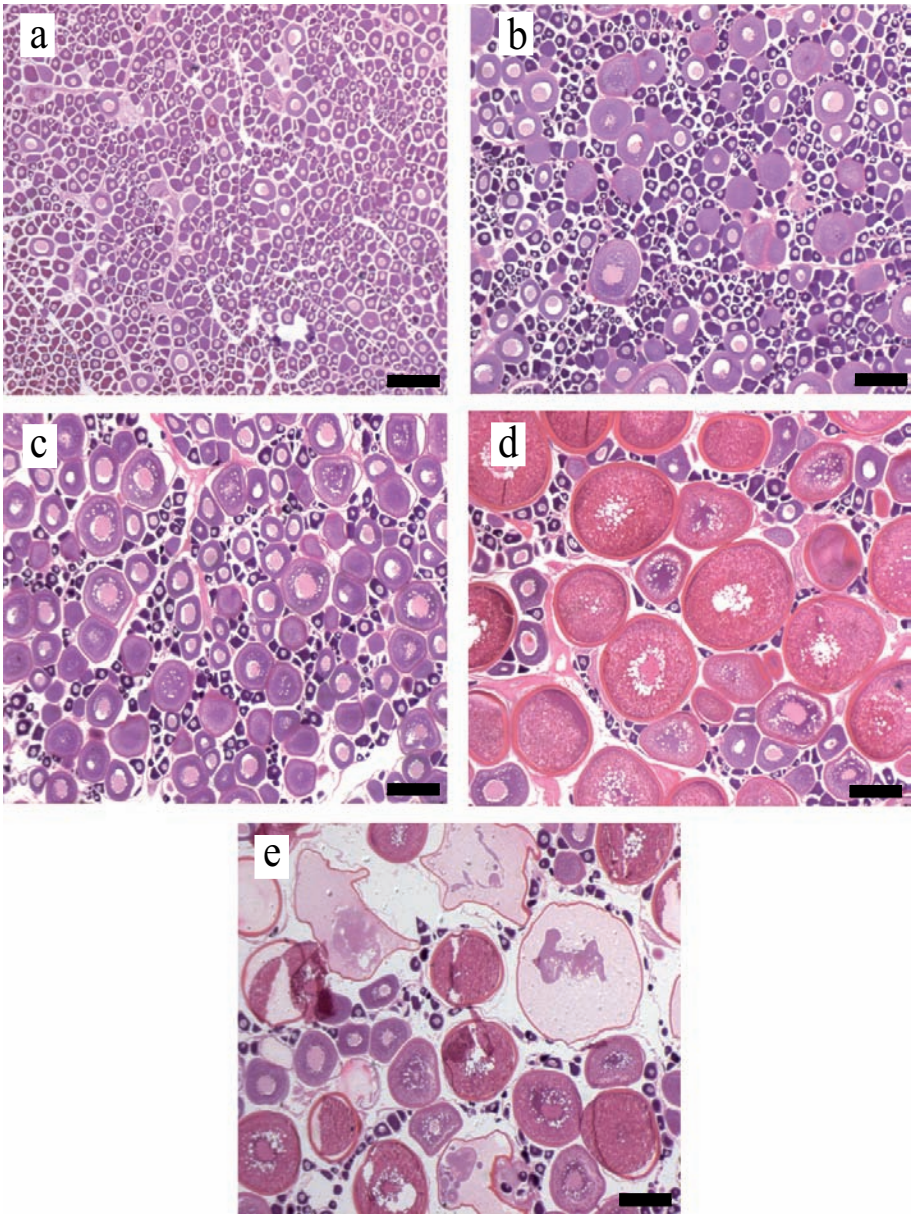

Figure 3.4: Stages of female ovary maturation (a) Stage 2 [immature or mature resting], (b) Stage 3 [First evidence of yolked oocytes], (c) early stage 4 [Females deemed as mature at this stage], (d) Stage 5 [Evidence of germinal vesicle migration], (e) Stage 6 [Abundant in hydrated oocytes]. Scale bar = 500 micron

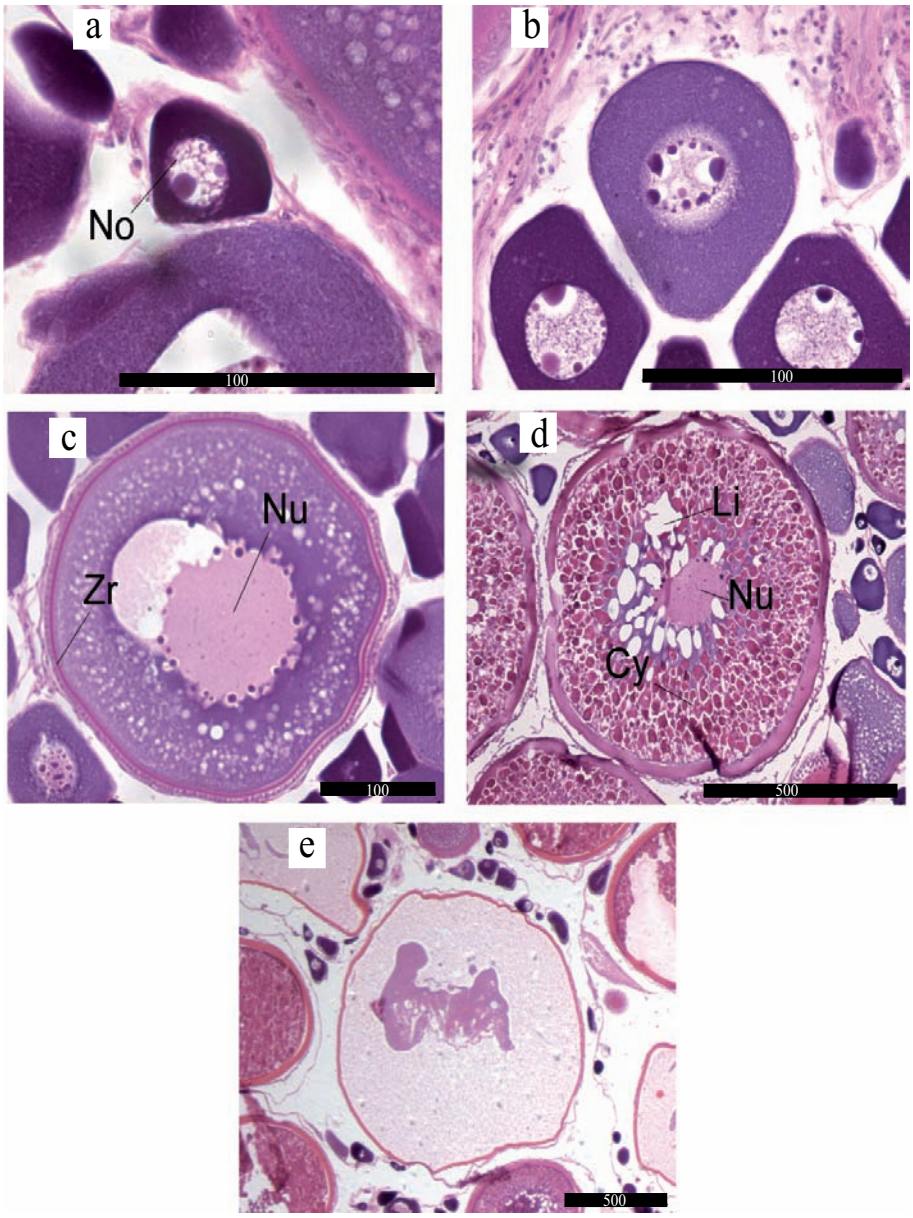

Figure 3.5: Typical stages of oocyte development observed in female striped trumpeter ovaries, (a) Primary oocytes, (b) Cortical Alveoli, (c) early yolked, (d) late yolked, (e) Hydration. No = Nucleoli, Nu = Nucleus, Cy = Cytoplasm, Li = Lipid, Zr = Zona radiata.

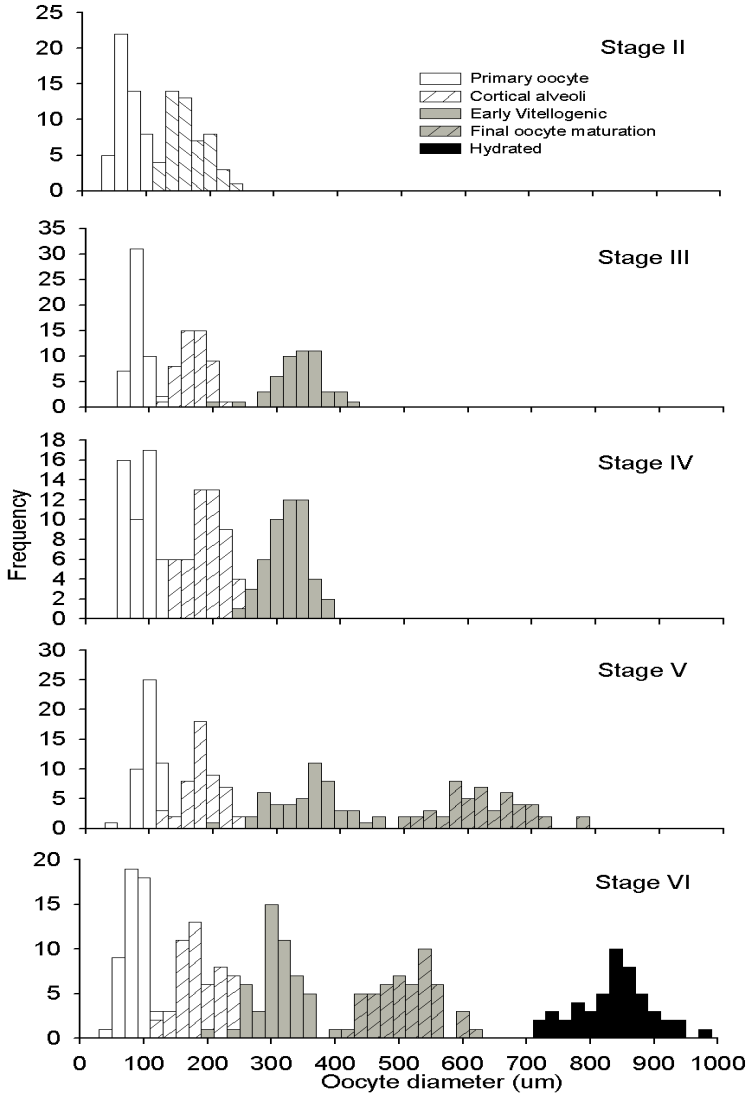

Figure 3.6: Frequency distribution of primary oocytes, cortical alveoli, early vitellogenic, late vitellogenic and hydrated oocyte diameter (μm) for stages II to VI.

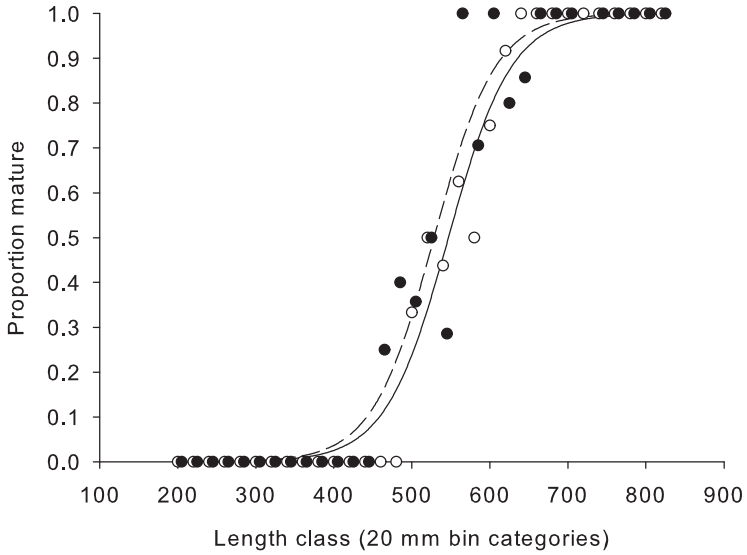

Figure 3.7: Proportion mature of male and female striped trumpeter at 20 mm length intervals. Fitted splines illustrate the optimal logistic maturation ogive determined by the minimisation of the sum of square residuals from each data set. Females = 120 total, 85 mature and males = 195 total, 107 mature. Data restricted to samples caught in the spawning season August – November. Male data points offset by 5 mm to enhance interpretability of figure.

3.3.5 Size at maturity

Parameter values for the length-at-maturity logistic model were β_0 = -15.086, β_1 = 0.0278 for females and β_0 = -15.023, β_1 = 0.0284 for males (Fig. 3.7). The smallest mature fish observed were a 505 mm female (estimated age = 5.5 years) and a 519 mm male (estimated age = 5.9 years) and the estimated mean sizes at 50% maturity were 543 mm for females (estimated age = 6.8 years) and 529 mm for males (estimated age = 6.2 years) (Table 3.3).

Table 3.3: Estimated lengths of males and females at 25, 50 and 75% maturity within the sampled population. Samples were constrained to individuals caught over the spawning period (August – November)

	Male		Female	
Percent mature	Length (mm)	Age (years)	Length (mm)	Age (years)
25%	490	5.2	503	5.6
50%	529	6.2	543	6.8
75%	567	7.4	583	7.9

3.3.6 Fecundity

There was no significant difference between the weights of the left and right lobes of the ovary in 12 females tested using a t-test assuming equal variance ($t = -0.603_{[1,12]}$, p = 0.56). Relative fecundity (number of maturing oocytes per gram of ovary tissue) from the 39 females sampled ranged between 2307 – 3450, with a mean of 2897 $\pm$ 45 (SE), and was not significantly related with body weight (r^2 = 0.0008). The estimated batch fecundity for striped trumpeter ranged from 205,054 for a 540 mm FL (2 kg) fish to 2,351,029 for a 800 mm FL (9.5 kg) fish (Fig. 3.8). Batch fecundity was significantly related to both fish weight ($F_{[1,39]}$ = 318.23, $p < 0.0001$, r^2 = 0.89) and fork length ($F_{[1,39]}$ = 157.98, $p <$ 0.0001, r^2 = 0.80), the relationship between batch fecundity (FEC) and fork length being described by the power function FEC = 4.15 x $10^{-8} FL^{4.69}$ (Fig. 3.8).

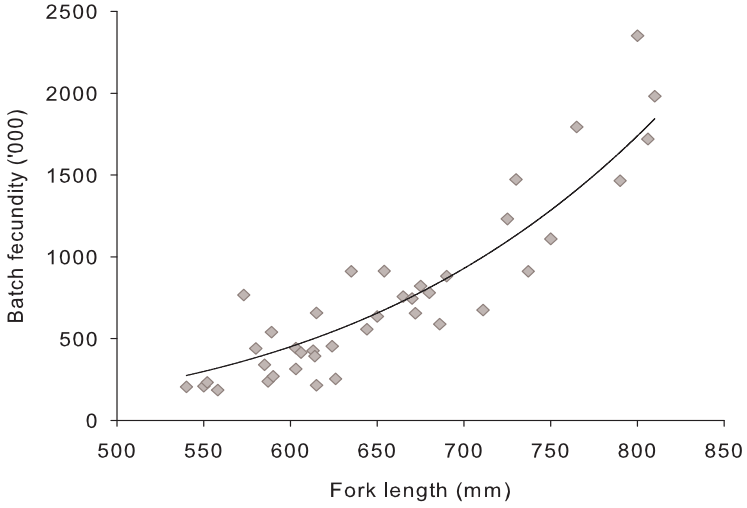

Figure 3.8: Batch fecundity regressed against fork length for female striped trumpeter from the sampled populations around Tasmania. n = 49.

3.3.7 Per recruit analysis

For both models, YPR increased quickly as F increased from zero to 0.22, above this level of F, YPR remained relatively constant (Fig. 3.9a & b). Spawner biomass per recruit (SPR) decreased rapidly as F increased from zero to 0.3, the level of M had little effect on SPR (Fig. 3.9c & d).

As M increased the range of size at first capture (FL_c) values at which YPR maximized decreased. SPR increased rapidly as FL_c exceeded the approximate size at maturity ($\simeq$500 mm FL).

At the current minimum legal size limit (equivalent to 425 mm FL), for model 1, YPR is close to maximum and SPR is at 35% of maximum. Using the parameters of model 2, YPR decreased to approximately 80% while SPR increased to 52% (Fig. 3.10a). At the previous minimum legal size limit (330 mm FL), model 1 predicted YPR to be close to maximum but SPR to be 28%. For model 2 and the current minimum legal size, YPR

decreased to about 90% and SPR increased to 42% (Fig. 3.10b). If the minimum size limit was increased to 525 mm FL, just below the size at maturity, model 1 predicted that YPR would still be close to maximum and SPR would increase to 50%, while model 2 predicts that YPR would decrease to 65% and SPR would increase to almost 70% (Fig. 3.10c).

Assuming the length frequency data presented in Fig. 3.11 is representative of commercial catches it is apparent that at the current FL_c of 425 mm FL, zero percent of the catch would have been returned. In 1991 and 1992 if the size limit was set at 550 mm FL approximately 35% of the catch would need to be returned. In 1999 at FL_c of 550 mm FL, 57% of the catch would have been returned, this year is anomalous in the fact that the strong modal peak around 500 mm is the product of the extreme recruitment pulse spawned in 1993. In 2004, disturbingly, the lack of significant recruitment of young cohorts is evident with only 12% of the catch needing to be returned as below 550 mm FL.

3.4 Discussion

Striped trumpeter exhibit a discrete annual spawning cycle that extends over three months in the late austral winter – early spring period, with peak spawning activity occurring in September and October. Spawning is widespread throughout the distribution range around Tasmania, with females in spawning condition collected from all offshore sampling locations. Furlani & Ruwald (1999) suggested that spawning commenced and finished earlier at higher latitudes off Tasmania, although their study did not present empirical data to support this claim. Due to limited spatial and temporal sampling within the current study it was not possible to test this assertion.

The present findings were generally consistent with those reported by Furlani & Ruwald (1999), who suggested a spawning period from July to early October for striped trumpeter from Tasmanian waters. Aquaria studies suggest that females have an oocyte development period (from cortical alveoli to hydrated stage) of approximately 3 months (June – August) and a spawning period of approximately 2 to 2.5 months (September to early November),

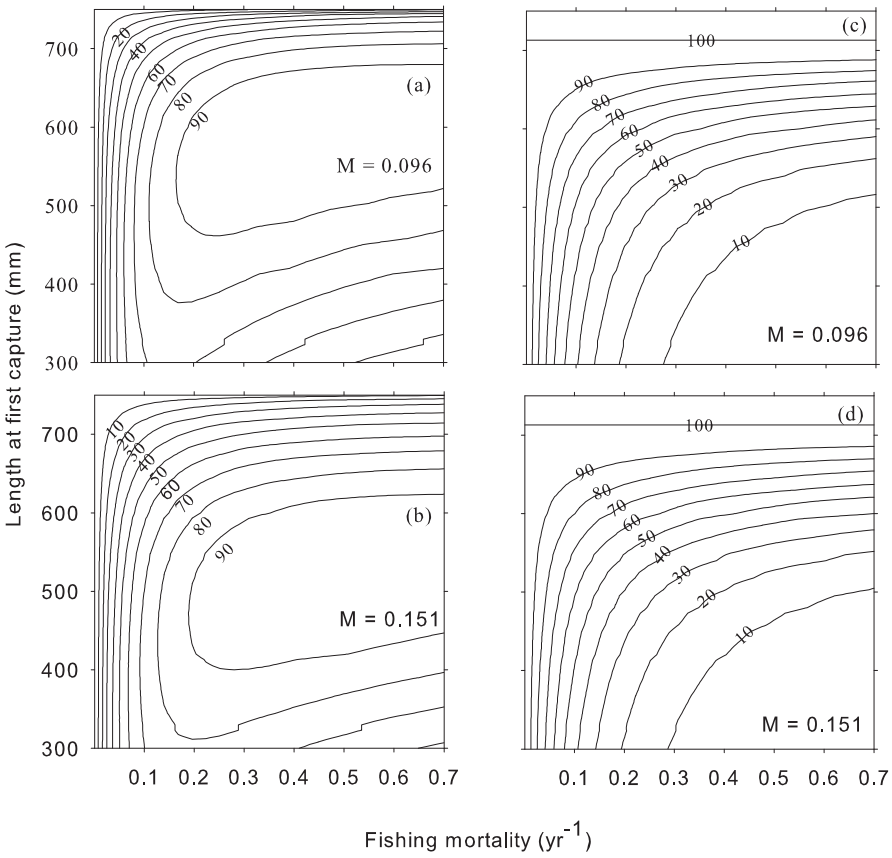

Figure 3.9: Isopleths of yield-per-recruit (% of maximum yield) where natural mortality (M) was set at; (a) M = 0.096 year^{-1}, (b) M = 0.151 year^{-1}, and spawner per recruit isopleths where natural mortality (M) was set at; (c) M = 0.096 year^{-1}, (d) M = 0.151 year^{-1}, both sets of isopleths are represented against successive increases in fishing mortality (F) and length at first capture (FL_c) for striped trumpeter.

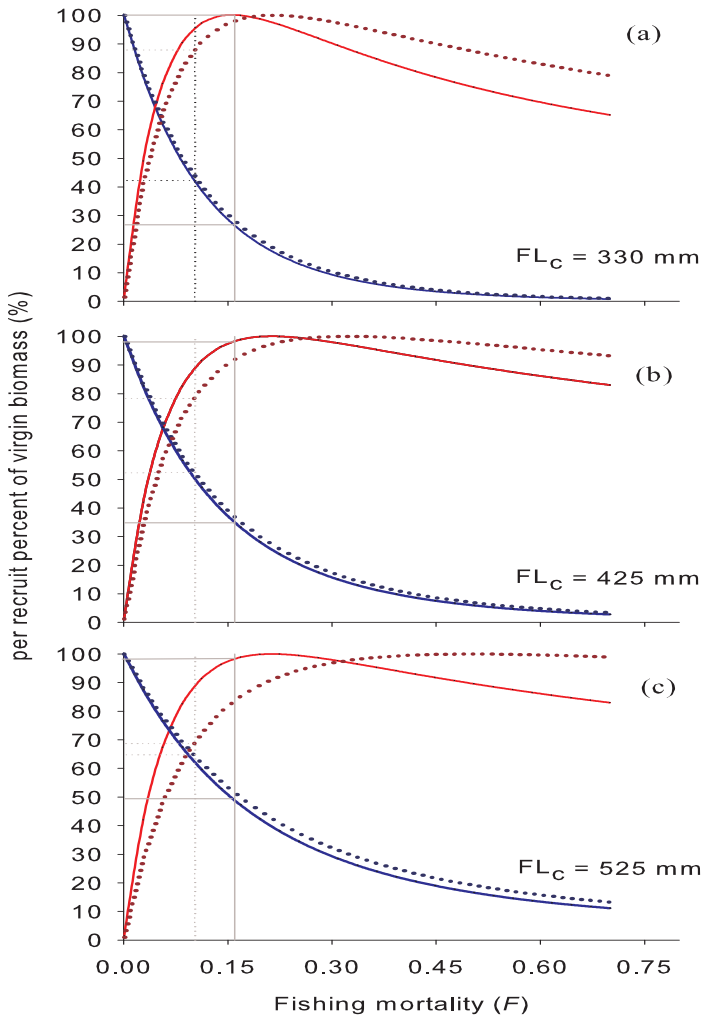

Figure 3.10: Spawner per recruit curves (blue line) and yield per recruit (red line) of striped trumpeter from Tasmanian waters, the solid lines represent Model 1 (M = 0.096, F = 0.160) and the dotted lines represent Model 2 (M = 0.151, F = 0.102), estimated at (a) 330, (b) 425 and (c) 525 mm FL size at first capture (FL_c) for striped trumpeter from the waters around Tasmania. The solid vertical lines represent the estimate of fishing mortality (F) for Model 1 and the dotted vertical line for Model 2.

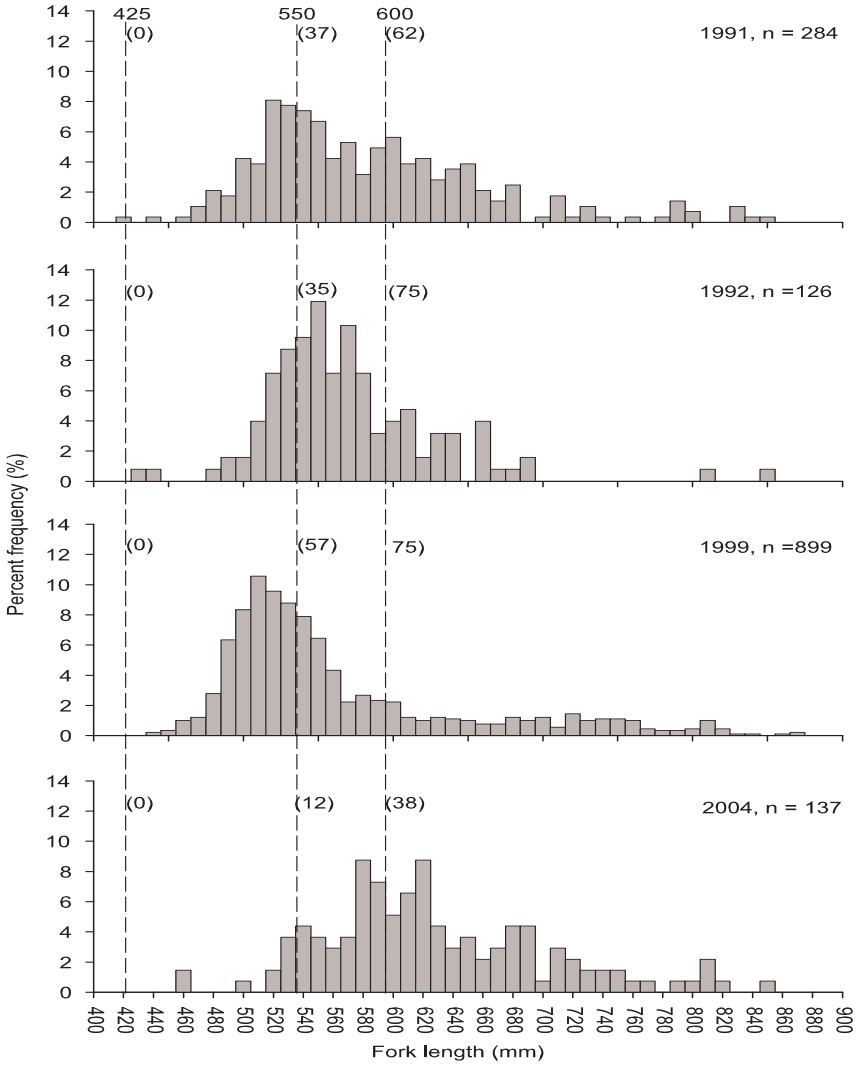

Figure 3.11: Length frequency distributions of all fish sampled in the years 1991, 1992, 1999 and 2004. Dashed lines represent size at entry to the fishery, the numbers above each line represent the proposed size limit and the numbers in the parentheses indicate the proportion of catch below each minimum size limit within each year. 425 mm FL is the estimated current minimum size limit based on the actual minimum size limit of 450 mm TL.

followed by a dormant period of 6.5 to 7 months (December – May) (Morehead, 1998). In New Zealand waters striped trumpeter are thought to spawn earlier in the year, being described as a winter spawner, though no further details are available (Graham, 1956; Francis, 1988). No reports of the spawning cycles could be located for striped trumpeter populations from other areas of its distribution.

All spawning condition fish examined in the present study were caught at depths of between 90 and 180 m, indicating that spawning occurs in relatively deep water along the continental shelf. Once striped trumpeter eggs are fertilized they become positively buoyant (Morehead & Hart, 2003) and are expected to float towards the sea-surface. In a cultured environment, survival of striped trumpeter eggs to hatching was optimised between 13 and 16°C, with the optimal temperature determined as 14°C. The length of hatchlings was also greatest for embryos reared at 14 C (Morehead & Hart, 2003). The timing of spawning in early spring coincides with sea surface temperatures of 13 – 14°C off the east coast of Tasmania (Fig. 3.2) and therefore thermal cycling is likely to be a prominent cue to initiate spawning. Photo-thermal cues of increasing light and temperature have in fact been shown to initiate spawning under aquaria conditions (Morehead & Hart, 2003). Early spring also coincides with an increase in primary productivity off the east coast of Tasmania (Harris et al., 1991), potentially providing young larvae with an abundance of prey.

Size at 50% maturity was slightly larger for females, at 543 mm, compared with 529 mm for males which, based on length at age relationships, indicated that striped trumpeter reach maturity at between 5 and 7 years of age. This is comparable to another related species, blue moki (*Latridopsis cillaris*) from New Zealand, which reach maturity at 5 – 6 years of age and about 400 mm (Francis, 1988). Murphy & Lyle (1999) concluded that maturity in bastard trumpeter (*Latridopsis forsteri*) occurred at lengths greater than 450 mm, since all fish they caught during an intensive inshore net sampling regime off the east coast of Tasmania were immature and below this size.

Oocyte development in striped trumpeter followed the general pattern observed in mul-

tiple spawners, passing through several smaller inner cycles of gonadal development (developed, gravid, running ripe) during the spawning season. Morehead (1998) found that in aquaria this inner phase took between three and 10 days and that the size of each clutch, based on the volume of ripe eggs released, decreased with each subsequent spawning event within a season. The co-occurrence of hydrated oocytes and postovulatory follicles in some histological preparations examined in this study provided evidence that subsequent clutches developed within a reasonably short period of time, given that postovulatory follicles typically persist for only 2 – 3 days after spawning (Macewicz & Hunter, 1993).

By using gravid stage V ovaries, estimates of fecundity are believed to be accurate because at this maturity stage eggs were not expelled with gentle pressure on the abdomen and the clutch of late vitellogenic/maturing oocytes could be readily distinguished from the younger clutches. Relative fecundity did not vary significantly with fish weight and a power function adequately represented the relationship between batch fecundity and length. By comparison with other Perciforms, batch fecundity in striped trumpeter was relatively high (Mertz & Myers, 1996).

Multiple spawning and a relatively protracted spawning period in striped trumpeter presumably act to increase the likelihood of producing offspring during periods of favourable environmental conditions. This bet hedging strategy has been suggested to reduce the impact of environmental variation on reproductive success (Bobko & Berkeley, 2004).

Reducing fishing pressure on pre-adults using minimum size limits is an effective method of increasing the reproductive capacity of a population. The Target Reference Point (TRP) $0.4B_0$ is considered to be a sustainable reference target while $0.2B_0$ is considered to be critical Limit Reference Point (LRP) to be avoided (Mace, 1994). According to these criteria at the previous size limit of 330 mm FL, the level of fishing effort on the population would have been sustainable according to Model 1, however, using Model 2 with the lower value of M the population would have been overexploited. At the current size limit of 425 mm FL the population is considered overexploited using Model 1, however the SPR value (35%) is approaching the TRP that is considered sustainable. If the

size limit was to be increased further, to 525 mm FL, the SPR values using both models are well above the $0.4B_0$ level at 50 and 65% for Models 1 and 2 respectively. The value of M had little effect on the SPR curves, conversely it had a marked effect on the YPR curves, the magnitude of this effect increased as the size limit increased. For all three size limits simulated, YPR was close to 100% using Model 1, however using Model 2, with the higher value of M, yield decreased significantly as the size limit was increased.

When trying to optimize a harvest strategy the trade off between environmental and economic benefits are crucial, in the case of the striped trumpeter per-recruit modelling would suggest that at the current size limit this balance is close to optimum, the SPR value is estimated as between 35 and 52%, with the majority of this range above the TRP considered to be sustainable (40%), while the YPR estimates suggests that the yield available to the fishery is maintained between 80 and 100%. Although it must be kept in mind that per-recruit modelling assumes an equilibrium state in terms of recruitment and biological parameters. Striped trumpeter are known to exhibit strong recruitment variability, with up to a decade between significant recruitment pulses, meaning that the fishery can be reliant on only a few cohorts. Therefore, although the current size limit and mortality estimates are assumed to create a sustainable fishery, a more precautionary approach should still be applied until a more robust assessment is available for the species. The need for such caution is emphasised by the recent declining trend in commercial catches in Tasmania, which have fallen steadily over the past six years, the most recent catch being the lowest recorded for over 20 years (Lyle, 2005).

In practice the commercial fishery is partially self-regulated with an economic trade-off between operating costs and the impact of the 250 kg trip limit. This creates a situation where it may not be economically viable for operators to solely target the species and as such most fishing activity is undertaken as an adjunct to fishing for other species (as is common practice by commercial rock lobster fishers). Such economic imperatives are not important for recreational fishers and through the increasing accessibility of technology (echosounders and global position systems) and growing numbers of recreational vessels

capable of operating in offshore waters, there is a general perception that recreational interest in the species has grown in recent years and thus catches are likely to have increased over time.

Due to the size at recruitment to the offshore fishery (approximately 450 mm FL, age 5) relative to the size at 50% maturity (approximately 530 mm FL, age 6), individuals are vulnerable to the fishery for at least one year before the majority reach maturity. This is a concern especially in light of the apparent lack of significant recruitment over the past decade. Although there are anecdotal reports that suggest that historically juveniles have been abundant on inshore reef habitats; it is unclear whether the recent poor recruitment is the product of environmental factors or overexploitation or a combination of both.

As an example of the potential impact of size limits on the hook and line fishery, in 1999 when the size limit was 350 mm TL, none of the sampled catch (n = 284) would have been undersized at the current FL_c of 425 mm FL, however at a FL_c of 550 mm FL, 57% of the catch by number would have been sub-legal. The 1999 size composition was characterised by a strong mode at around 500 mm, produced by the strong 1993 year class entering the offshore fishery. By 2004, through growth of the 1993 cohort coupled with an apparent lack of significant recruitment, only 12% of the sampled catch (n = 137) was smaller than 550 mm FL.

The current or any future increase in the minimum size limit not only has implications for catch rates but raises concern about the survival of sub-legal individuals that are released, especially where fish are caught at depth. A range of factors have been shown to influence survival, the most important being hooking damage (Muoneke & Childress, 1994) and, in deep water species, the impact of barotrauma (St John & Syers, 2005). While there are no data on hooking damage for existing commercial and recreational fishing practices for this species and their potential impacts on survival, anecdotal evidence suggests that barotrauma stress is unlikely to be a significant factor. For instance, it is common practice for commercial fishers to hold striped trumpeter alive in onboard fish wells until they are landed for sale, without the need to vent the air bladder. Similarly, many fish used

in this study were held live and unvented in wet wells for several days prior to biological examination. Rates of onboard mortality for these fish were not specifically quantified but were typically very low (well under 10%). Therefore, any further increase in size limits is unlikely to result in barotrauma stress being a significant factor in any incidental mortality of released fish.

In the absence of a more robust stock assessment we have employed per-recruit analysis, a relatively simplistic approach that does not incorporate information about recruitment variability, one of the main sources of variability in the total yield from a fishery (MacLennan, 1993). Although this assessment suggests that the current size limit is appropriate, we conclude that there remains some risk to the stocks due to recruitment variability, in particular prolonged poor recruitment, which is likely to be the primary factor influencing the populations biomass, this issues needs to be addressed in future assessments and in the development of management strategies.

Chapter 4

Application of elliptical Fourier analysis of otolith form as a tool for stock identification.

Abstract. Geometric morphometrics is a relatively new tool to fisheries research showing promise as a means of enabling researchers to cheaply and quickly categorise fish to individual stocks based on variations in otolith form, most commonly size and shape. In this study we introduce the method of elliptical Fourier analysis using two widely separated populations of striped trumpeter (*Latris lineata*) as a case study and compare the interpretation of results based on both unconstrained and constrained ordination techniques. There were no significant differences in otolith morphometrics between sex or age classes within each region. All form descriptors were standardised for fish length, thereby minimising confounding effects on any potential inter-regional otolith form differences. Non-metric multidimensional scaling was not sufficient to elucidate differences in otolith form between populations. However, using constrained canonical analysis of principal coordinates and canonical discriminant analysis, regional differences became evident with allocation success of 75 and 87% respectively. Based on this study differences in otolith form reflect that the two tested striped trumpeter populations have reasonable phenotypic anonymity. This study further supports the usefulness of shape analysis and constrained non-parametric statistical tests as tools for stock

discrimination and introduces elliptical Fourier analysis to the study of otolith morphometrics.

4.1 Introduction

Stock identification is an integral component of modern fisheries stock assessment (Begg et al., 1999) and also for understanding the population dynamics of a species in an ecological sense. There are many techniques appropriate for studying stock structure, for review see: Ihssen et al. (1981); Begg et al. (1999). Geometric morphometrics is one such technique. It is a robust tool for analysing both physiological and morphological form, and has been used to discriminate between unique fish stocks or populations (Adams et al., 2004).

Form, defined as a model composed of various attributes of an object, is an aspect of fundamental importance in morphological investigations Chen et al. (2000). Within ichthyology a range of form components; for example, size, colour, shape, and patterning, are used to identify and classify individuals into particular groups (Ihssen et al., 1981; Adams et al., 2004). Otoliths contain characteristics that are stock specific (Ihssen et al., 1981) and are regarded as an ideal subject for morphometric analysis due to their species specificity and limited extent of individual variability in growth, relative to variability in somatic growth (Campana & Casselman, 1993).

Several methods have been used to describe and compare form in morphological studies including ratios of linear dimensions, biorthogonal grids, Euclidean distance matrix analysis, thin plate splines, Eigen shape analysis, and several variations of Fourier analysis (Chen et al., 2000). Elliptical Fourier functions represent a precise method for describing and characterizing outlines, efficiently capturing outline information in a quantifiable manner (Kuhl & Giardina, 1982; Lestrel, 1997). The method does not require equal intervals along the outline and therefore can accommodate significantly more complex shapes than polar Fourier functions, the approach that has traditionally been used in stock discrimination studies based on fish otoliths (Bird et al., 1986; Castonguay et al., 1991; Campana & Casselman, 1993; Torres et al., 2000). Polar Fourier functions are constrained to a series of radii originating from a barycentre of the study specimen, each radii can only intersect the perimeter once. In the case of otoliths that are particularly convoluted, or where the

rostrum has significant curvature it would be possible for radii to intersect the perimeter at more than one point. Elliptical Fourier functions alleviate this problem by not relying on a radiating centroid, instead generating harmonics by calculating x and y co-ordinates as a function of a third variable (t).

In this paper we compare otolith form and somatic growth in two widely separated striped trumpeter (*Latris lineata*) populations. The study species has a broad geographic distribution, occurring around the temperate latitudes of southern Australia, New Zealand (Last et al., 1983), including the sub-Antarctic Auckland Island (Kingsford et al., 1989), the Gough and Tristan Da Cunha Island groups in the southern Atlantic Ocean (Andrew et al., 1995), the Amsterdam and St. Paul Island groups in the southern Indian Ocean (Duhamel, 1989) and the Foundation seamount in the southern Pacific Ocean (Roberts, 2003). Samples of fish from Tasmania, Australia and the St. Paul/Amsterdam Island regions were examined and compared and we demonstrate the advantages of using elliptical Fourier analysis and constrained ordination techniques to assess differences in otolith morphology.

4.2 Materials and Methods

4.2.1 Sampling and otolith collection

Latris lineata from the east coast of Tasmania (TAS: 40°36'S, 148°47'E) were sampled between the years 2000 and 2003 (n = 199). Two collections were also made from the St. Paul and Amsterdam Islands (SPA: 37°50'S, 77°30'E), one collected between December 2001 and January 2002 (n = 206) and the second during April 2003 (n = 200) (Fig. 4.1). All fish were caught using line-fishing methods from depths greater than 90 m and were either examined fresh or frozen prior to processing. Fork length (mm) and sex were recorded from each individual, and the sagittal otoliths (herein referred to as otoliths) were removed. Otoliths were cleaned, air-dried and stored in individually labelled vials.

Table 4.1: Description of sample and variable details for each analysis performed in this study and adjustments made to the data. Ordination refers to the suite of multivariate tests used; non-metric multidimensional scaling (MDS), canonical discriminant analysis (CDA) and canonical analysis of principal co-ordinates (CAP). SPA and TAS refer to the two case study locations, St. Paul/Amsterdam Islands and Tasmania respectively.

Analysis	Test	Variables[a]	Samples	Group				
		SPA	TAS	SPA	TAS	SPA	TAS	
Within population	PERMANOVA	Size[b]	5	5	72(m=36,f=36)	72(m=36,f=36)	2	2
Sex	PERMANOVA	Shape	37	37	72(m=36,f=36)	72(m=36,f=36)	2	2
Age	PERMANOVA	Shape	37	37	66(10/age class)[b]	66(10/age class[e])	6	6
Between populations[c]	PERMANOVA	Size[b]	5	5	66[d]	66	1	1
	PERMANOVA	Shape	37	37	66[d]	66	1	1
	PERMANOVA	Size[e]/shape	42	42	66[d]	66	1	1
	Ordination	Size[b]	5	5	113	113	1	1
	Ordination	Shape	37	37	113	113	1	1
	Ordination	Size[b]/shape	42	42	113	113	1	1

[a] All variables were log transformed.
[b] Age classes defined in methods section.
[c] Samples within each population were pooled by sex and age for between population analyses.
[d] Fourty-seven individuals were randomly removed from the SPA sample to create a balanced sample design for the PERMANOVA tests.
[e] Size variables were standardised for fish length (see methods for details).

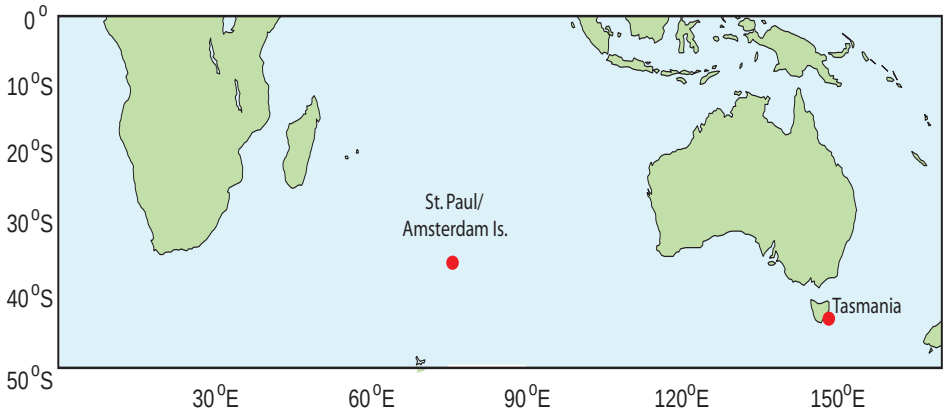

Figure 4.1: Map of the Indian ocean and bordering continents illustrating the sampling locations.

4.2.2 Age and Growth

Individuals were aged by interpreting the increment development on transverse sections of their respective otolith (Tracey & Lyle, 2005). All counts and increment measurements were made without knowledge of fish size, sex or date at capture.

Validation of first increment and annual periodicity of subsequent increment deposition for *Latris lineata* from TAS has been shown by Tracey & Lyle (2005). In this study it was assumed that the incremental structure was analogous for individuals sampled from SPA. A random sub sample of 335 otoliths was read a second time by the primary reader, and a sub sample of 50 otoliths by a second reader experienced in otolith interpretation. The precision of repeated reads was assessed by determining percentage agreement between repeated reads and calculation of the average percent error (Beamish & Fournier, 1981).

A re-parameterised version of the von Bertalanffy growth function (VBGF) was fitted to the length-at-age data (Francis et al., 1999), using least squares regression:

$$L_t = l_\tau + \frac{(l_\nu - l_\tau)\left(1 - r^{\frac{2(t-\tau)}{\nu-\tau}}\right)}{1 - r^2} \tag{4.1}$$

where

$$r = \frac{l_\tau - l_\omega}{l_\omega - l_\nu} \tag{4.2}$$

and where l_τ, l_ν and l_ω are the mean lengths at ages τ, ν and $\omega = (\tau + \nu)/2$. The re-parameterised version of the VBGF was preferred as it allows the model parameters to be directly observable from the data, the parameters are also statistically favourable to the standard VBGF parameters (Francis et al., 1999; Cerrato, 1990), by removing the inverse relationship between L_∞ and *k*. To determine whether growth displayed regional disparity, likelihood ratio tests (LRT) were conducted on both the predicted growth curves estimated by the re-parameterised VBGFs and the individual parameters calculated for each curve (Kimura, 1980).

4.2.3 Otolith form analysis

Complete otoliths were viewed under a dissection microscope at 12 x magnification (Fig. 4.2). Where possible the left otolith was used, however, if unavailable the right otolith was used. If both otoliths were broken (*Latris lineata* otoliths are particularly brittle) the fish was discarded from otolith form analysis. Otoliths were positioned with the concave side up (sulcal groove down) and the rostrum pointing to the right before image capture. A digital camera was used to capture a binary silhouette of the otolith shape. When the right otolith was used, the image was horizontally flipped using standard image analysis techniques, to ensure that the rostrum was orientated to the right of screen for each specimen. Using image analysis software (SigmaScan©) the longest axis, perimeter and area of each otolith were measured. The program 'SHAPE' (Iwata & Ukai, 2002) used a 'chain coding' algorithm (Freeman, 1974 cited in Iwata & Ukai, 2002) to extract the contours of the otolith outline in preparation for elliptical Fourier analysis.

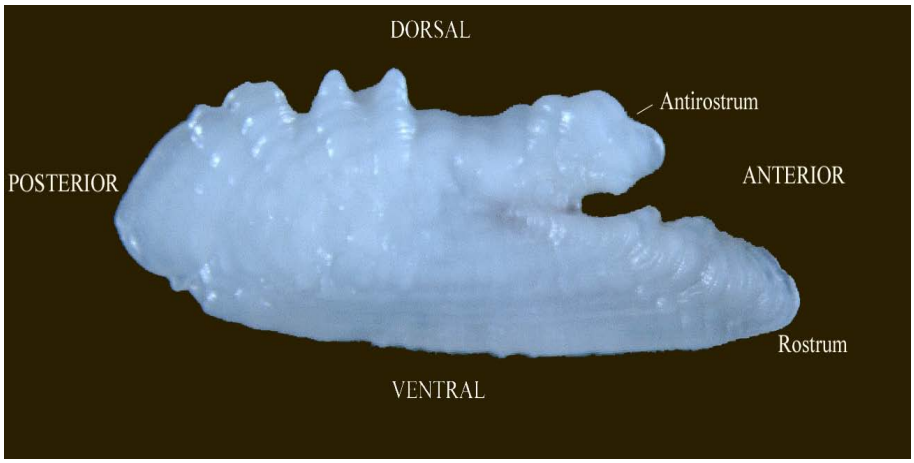

Figure 4.2: Photomicrograph showing external face of a striped trumpeter sagittal otolith

4.2.4 Elliptical Fourier analysis

An elliptical Fourier function fits a closed curve to an ordered set of data points in a two-dimensional plane (Kuhl & Giardina, 1982). It uses an orthogonal decomposition of a curve into a sum of harmonically related ellipses. These ellipses can be combined to reconstruct an arbitrary approximation of the closed curve for striped trumpeter (Fig. 4.3).

Two elliptical Fourier series are parameterised as the functions $x(t)$ (for the x axis and $y(t)$ (for the y axis):

$$x(t) = a_o + \sum \left[a_j \cos\left(\frac{2j\pi t}{T}\right) + b_j \sin\left(\frac{2j\pi t}{T}\right) \right], \tag{4.3}$$

$$x(t) = c_o + \sum \left[c_j \cos\left(\frac{2j\pi t}{T}\right) + d_j \sin\left(\frac{2j\pi t}{T}\right) \right], \tag{4.4}$$

where k is the number of harmonics used, j is the order of the harmonic, and T is the outline perimeter.

The derivation of Fourier coefficients for the jth harmonic of the trace's x and y-projection as described by Kuhl and Giardina (1982), are:

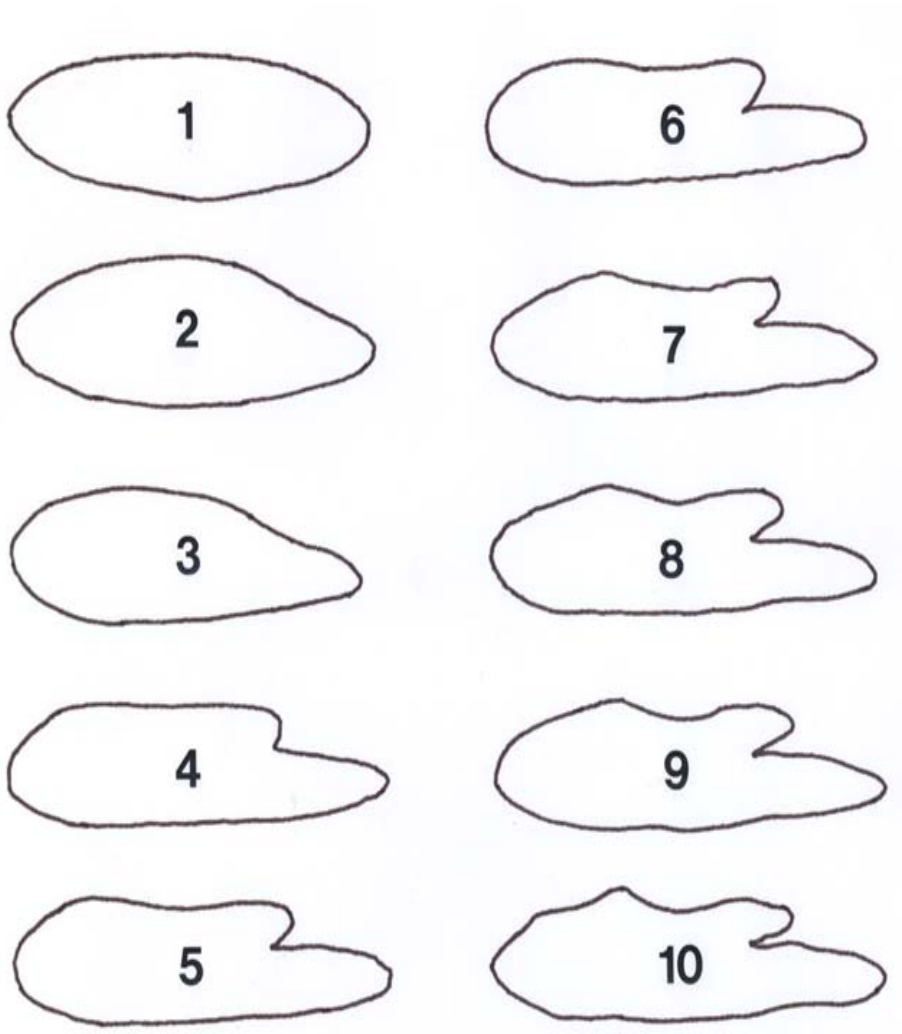

Figure 4.3: Elliptical Fourier reconstruction of otolith outline by summation of harmonics one to ten.

$$a_j = \frac{T}{2j^2\pi^2}\sum\frac{\triangle x_i}{\triangle t_i}\left(\cos\frac{2j\pi t_i}{T} - \cos\frac{2j\pi t_{i-1}}{T}\right), \tag{4.5}$$

$$b_j = \frac{T}{2j^2\pi^2}\sum\frac{\triangle x_i}{\triangle t_i}\left(\sin\frac{2j\pi t_i}{T} - \sin\frac{2j\pi t_{i-1}}{T}\right), \tag{4.6}$$

$$c_j = \frac{T}{2j^2\pi^2}\sum\frac{\triangle x_i}{\triangle t_i}\left(\cos\frac{2j\pi t_i}{T} - \cos\frac{2j\pi t_{i-1}}{T}\right), \tag{4.7}$$

$$d_j = \frac{T}{2j^2\pi^2}\sum\frac{\triangle x_i}{\triangle t_i}\left(\sin\frac{2j\pi t_i}{T} - \sin\frac{2j\pi t_{i-1}}{T}\right). \tag{4.8}$$

where k is the number of steps in the trace (indexed by i), $\triangle x_i$ is the displacement along the x-axis of the contour between steps i - 1 and i, $\triangle t_i$ is the length of the linear segment between these steps, t_i is the accumulated length of such segments at step i.

Terms a_0 and c_0 are constants, computed as:

$$a_0 = \frac{1}{T}\sum\frac{1}{2}\frac{\triangle x_i}{\triangle t_i}(t_i^2 - t_{i-1}^2) - \frac{\triangle x_i}{\triangle t_i}t_i, \tag{4.9}$$

$$c_0 = \frac{1}{T}\sum\frac{1}{2}\frac{\triangle y_i}{\triangle t_i}(t_i^2 - t_{i-1}^2) - \frac{\triangle y_i}{\triangle t_i}t_i, \tag{4.10}$$

As otolith size parameters have been dealt with as a separate issue in this study, the elliptical Fourier coefficients were made invariant to size and also rotation so as the only variables being considered were shape characteristics of the otoliths. Kuhl & Giardina (1982) provided a method to achieve this by the matrix transformation:

$$\begin{pmatrix} a_j & b_j \\ c_j & d_j \end{pmatrix} = \frac{1}{E^*}\begin{pmatrix} \cos\phi & \sin\phi \\ \sin\phi & \cos\phi \end{pmatrix}\begin{pmatrix} a_j & b_j \\ c_j & d_j \end{pmatrix}\begin{pmatrix} \cos\phi & \sin\phi \\ \sin\phi & \cos\phi \end{pmatrix}, \tag{4.11}$$

where j is the harmonic order of the quadruple of coefficients, E is the magnitude of

the semi major axis of the best fitting ellipse

$$E^* = (a^{*2} + c^{*2})^{0.5} \tag{4.12}$$

ϕ is the orientation of this ellipse in radians

$$\phi = arctan(c^*/a^*), 0 \leq \phi < 2\phi \tag{4.13}$$

and θ is the rotation of the starting point from the end of the ellipse (standardised for a set of pictures to one of the pair of ends)

$$\theta = \frac{1}{2} arctan\big[2(A_1B_1 + C_1D_1)/(A_1^2 + C_1^2 - B_1^2 - D_1^2)\big], 0 \leq \phi < 2\pi \tag{4.14}$$

The values of a^* and c^* above are given by

$$a^* = A_1 \cos\theta + B_1 \sin\theta \tag{4.15}$$

$$c^* = C_1 \cos\theta + D_1 \sin\theta \tag{4.16}$$

The program 'SHAPE' (Iwata & Ukai, 2002) was used to generate ten harmonics for each individual. Each harmonic is composed of four coefficients and therefore 40 coefficients per individual. The size and orientation normalizations cause the degeneration of the first three coefficients: a_1 = 1, b_1 = c_1 = 0. Therefore each individual is represented by 37 coefficients.

4.2.5 Morphological data analysis

To determine whether there were differences between length frequency distributions a Kolmogorov-Smirnov (KS) test was conducted between regions and between sexes within

regions.

Morphometric variables were examined for homogeneity of variance using Levene's test and all variables were log transformed. ANCOVA was used to determine the effect of fish length on the magnitude of each size variable. If significant interactions between 'region-fish length' were identified the dependent variable was excluded, as they could not be corrected for fish length. Variables that were significantly correlated with fish length were standardised using the common within-group slope, that is the slope was multiplied by fork length, and the product subtracted from the observed variable (Bolles & Begg, 2000; Cardinale et al., 2004).

Size and shape were treated separately and combined for comparative analysis. The assumption of normal distribution common to parametric tests such as ANOVA and MANOVA was redundant in this study, as the non-parametric permutation tests used maintain type I error and are robust when data are non-normal (Willis & Anderson, 2003). Variables were initially transformed to a Euclidean distance dissimilarity matrix. Non-metric multidimensional scaling (MDS), canonical discriminant analysis (CDA) and canonical analysis of principal coordinates (CAP, Anderson & Willis, 2003) were then used to ordinate the matrices. These three methods were used to compare results and assess which would be most appropriate for this analysis. Permutational multivariate analysis of variance (PERMANOVA, Anderson, 2004), previously known as non-parametric multivariate analysis of variance (NP-MANOVA, Anderson, 2001) was used as a probabilistic hypothesis test to assess differences in otolith shape for: (1) sex within region, (2) age within region, where age was categorised into classes 5-9 and 10+ years, and (3) overall regional differences in respect to otolith form characteristics. However, PERMANOVA does not accommodate unbalanced sample designs, therefore individuals were randomly removed from the SPA sample to balance the sample sizes for PERMANOVA tests only, a description of sample and variable details, including adjustments, is shown in Table 4.1. For all CAP and PERMANOVA tests we used 4999 unrestricted random permutations of the raw data (Anderson, 2001). A 'post hoc' test identified the contribution of individual variables to

differences among groups, variables with correlations (r) to the canonical axis greater than $r = \pm 0.20$ were identified.

To determine the level of misclassification between intraspecific sampling regions the method of 'leave-one-out' (Lachenbruch & Mickey, 1968 cited in Anderson & Willis, 2003) was applied to the variables in canonical space. As CAP analysis is a constrained ordination with region known *a priori*, the 'leave-one-out' approach gives a reasonable and unbiased measure of how distinct the groups are in multivariate space (Anderson & Willis, 2003).

4.3 Results

Tasmanian *Latris lineata* otoliths were on average significantly larger than the St. Paul/ Amsterdam Island otoliths (Table 4.2), however, there was no significant difference between length frequencies by region (Kolmogorov-Smirnov (KS); $Z = 0.433$, $p > 0.05$). The samples from both regions did not include individuals less than 405mm, smaller (juvenile) individuals typically occur associated with shallow inshore reefs (Tracey & Lyle, 2005).

Table 4.2: Summary of somatic and otolith size variables and age recorded from *Latris lineata* collected in Tasmania (TAS) and St. Paul/ Amsterdam Island (SPA). SE = standard error.

	TAS			SPA		
Variable	Mean	SE	Range	Mean	SE	Range
Otolith length (mm)	8.58	0.10	7.15–11.31	8.31	0.09	6.59–10.67
Otolith perimeter (mm)	21.21	0.33	16.89–29.30	20.37	0.30	16.30–31.04
Otolith area(mm^2)	47.74	0.75	38.01–65.95	45.69	0.67	36.47–70.99
Otolith weight (mg)	26.58	1.18	13.10–65.20	23.21	0.84	12.39–56.30
Fork length (mm)	589.47	5.90	405.00–845.00	566.64	3.64	420.00–800.00
Age (years)	9.42	0.44	4.00–35.00	7.55	0.20	4.00–33.00

4.3.1 Ageing

Overall, 176 (89%) of TAS and 335 (83%) of SPA samples were successfully allocated an age estimate. Precision of repeated readings by the primary reader was 75% in agreement between first and second reads with an average percent error of 0.97. Agreement between two readers yielded an average percent error of 1.59.

Two versions of the length-at-age data were analysed; firstly, the entire data set encompassing all represented ages from both regions, where the age parameters; τ = 5, ω = 17.5 and ν = 30 were chosen arbitrarily for the re-parameterised $VBGF_S$.

A likelihood ratio test (LRT) on the growth curves fitted to the complete data sets showed significant differences in somatic growth between regions. The curves diverged at approximately age 8 years, and then re-converged at approximately age 25 years (Fig. 4.4a). Testing individual parameters highlighted the difference between the midsections of the curves; the parameter $l_{17.5}$ (the midpoint of selected parameters) was the only one to yield a statistical difference (Table 4.3). However, statistical differences, both overall and of the midpoint parameter, appeared to be strongly influenced by a lack of data in the older age classes. Therefore, we re-analysed the length-at-age data using a subset truncated to a maximum age of 10 years, below this age we had sufficient representation from above 4 years of age (Fig. 4.4b). The truncated data represented 91% and 77% of the entire SPA and TAS data sets respectively. Parameters for the model fitted to the truncated data were again chosen arbitrarily to represent the entire subset of ages (τ = 4, ω = 7 and ν = 10). The LRT on the growth curves generated from the truncated data showed no significant overall difference in somatic growth between regions (Table 4.3).

After consideration of age sample size biases, we resolve that the truncated data set was more representative of regional growth, hence based on the truncated data set no significant difference in growth rates between regions was detected.

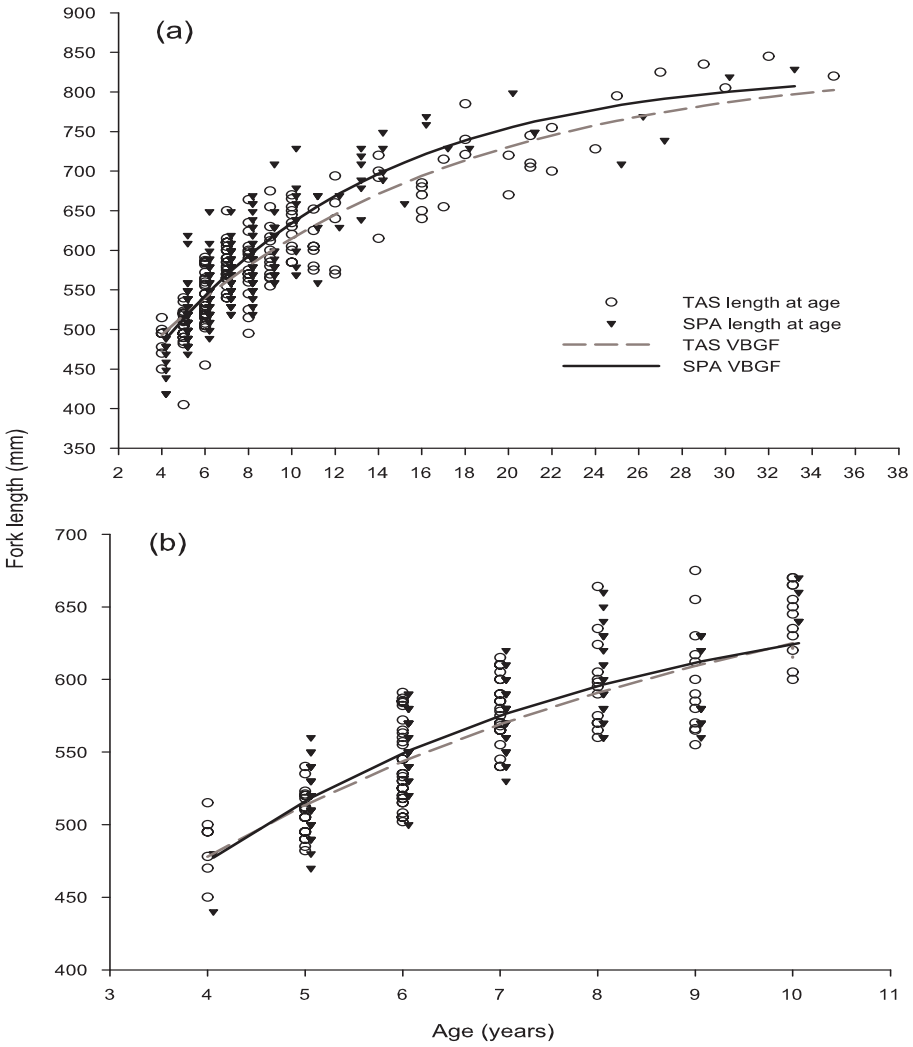

Figure 4.4: (a) Length-at-age data of all *Latris lineata* that were aged using otoliths (Tasmania (TAS); n = 176, St. Paul/Amsterdam Islands (SPA); n = 335) and, (b) the same data set truncated to a maximum age of 10 years (TAS; n = 134, SPA; n = 303). The data from each region is fitted with a re-parameterised von Bertalanffy growth function (VBGF).

4.3.2 Within region variations in otolith form

Levene's test for homogeneity of variance was not satisfied for fork length (FL), otolith area (OA), otolith length (OL), otolith perimeter (OP), otolith weight (OW) and elliptical Fourier coefficients 6, 10, 11, 14 and 17. Logarithmic transformation resulted in homogeneity in the variance of OA, OL and OP. Therefore we ran all the statistical tests twice, first excluding variables with heterogeneous variance and a second time including them. The results were almost identical and therefore the results including all the variables are reported so as not to lose any information on otolith size or shape. No significant relationship was shown between any of the morphometric size variables and the fork length-region interactions; however, all were correlated with fork length and thus were corrected using the common within group slope of the respective regression (Table 4.4).

Table 4.3: Likelihood ratio test results of regional differences in the re-parameterised von Bertalanffy growth functions fitted to the complete dataset and to a subset truncated to a maximum age of 10 years. RSS = residual sums of squares. ns = not significant.

Hypothesis	RSS	χ^2	df	p	
Complete data					
Base case	339,381	–	–	–	
Coincident curves	360,476	30.81	3	<0.001	
l_5	348,852	0.12	1	0.724	ns
$l_{17.5}$	359,163	314.06	1	<0.001	
l_{30}	339,435	30.08	1	0.776	ns
Truncated data					
Base case	231,455	–	–	–	
Coincident curves	255,016	30.81	3	0.148	ns
l_4	231,488	30.81	1	0.802	ns
l_7	233,257	30.81	1	0.065	ns
l_{10}	250,943	30.81	1	0.503	ns

Size component

There were no significant differences between size variables by sex from within either region (PERMANOVA; TAS: $F_{[1,71]}$ = 0.470, p = 0.514; SPA: $F_{[1,71]}$ = 1.37, p = 0.242).

Shape component

Otolith shape characteristics explained by the Fourier coefficients did not differ significantly between males and females from within either region (PERMANOVA; TAS: $F_{[1,71]}$ = 0.280, p = 0.970; SPA: $F_{[1,71]}$ = 0.262, p = 0.939). There were also no significant differences in otolith shape between age classes within region from TAS or SPA (PERMANOVA; TAS: $F_{[6,66]}$ = 5.201, $p > 0.05$; SPA: $F_{[6,66]}$ = 1.364, $p > 0.05$). Since there was no statistical sex specific difference in size variables, and little sex or age specific differences in shape descriptors, data were pooled within each region to increase sample sizes to 66 from TAS and 113 from SPA for regional comparisons.

4.3.3 Between region otolith form variations

Size component

The ordination of coefficient data by MDS from the two sampling regions showed little difference between the groups (Fig. 4.5a), with no statistical difference in otolith size characteristics between regions (PERMANOVA; $F_{[1,132]}$ = 0.47, p = 0.323). CDA analy-

Table 4.4: Otolith morphometric variables correlated with fork length (mm) and the within-group slope coefficient (b) used to standardise each variable for fish length.

	Fork length x region (df=2,178)		Fork length (df=1,179)		
Variable	F	p	F	p	b
Otolith length (mm)	0.74	0.392 ns	266.58	<0.001	0.622
Otolith area (mm^2)	0.48	0.488 ns	320.86	<0.001	0.753
Otolith perimeter(mm)	0.34	0.560 ns	364.29	<0.001	0.775
Otolith weight (mg)	0.07	0.780 ns	688.55	<0.001	2.082

sis was in agreement with the MDS indicating no significant difference between regions (56.11%, Hotelling's t^2: p = 0.854) (Fig. 4.5b). The CAP was also unsuccessful at allocating individuals to *a priori* groups (Table 4.4) (Fig. 4.5c).

Shape component

When shape variables were plotted by MDS no regional separation was evident (Fig. 4.5d). However, there was a statistical difference in shape between regions (PERMANOVA; $F_{[1,132]}$ = 3.58, p = 0.02). Both the CDA (84.44%, Hotelling's t^2: $p < 0.0001$) and CAP (Table 4.4) supported the difference between regions and were more successful in allocating individuals to groups than the analysis based on otolith size, both constrained methods were also successful in increasing the visual separation between regions compared to the MDS, Figures 4.5e and 4.5f respectively.

Size and shape combined

There was little difference in the scatter of points for sampling region using MDS (Fig. 4.5g) but, when otolith size and shape characteristics were analysed using PERMANOVA, a significant regional difference was detected ($F_{[1,132]}$ = 2.59, p = 0.01). Again the constrained ordination techniques identified significant differences between the two regions and the highest percent allocation success of all tests, CDA (87.22%, Hotelling's t^2: $p < 0.0001$) and CAP (Table 4.4). Both the CDA (Fig. 4.5h) and CAP (Fig. 4.5i) plots of canonical variate frequency successfully enhanced the visual separation between regions.

The mean values of Fourier amplitudes calculated from each region are illustrated in Fig. 4.6. The lower order harmonics contribute greatly to the gross shape of the otolith and as the harmonic number increases they have less impact on the gross shape and begin to define the finer details such as denticulations. Of the eight coefficients that were strongly correlated with the canonical axis (-0.20 $< r >$ 0.20), five were related to the first five harmonics and three were related to harmonics six and seven. This would suggest that the

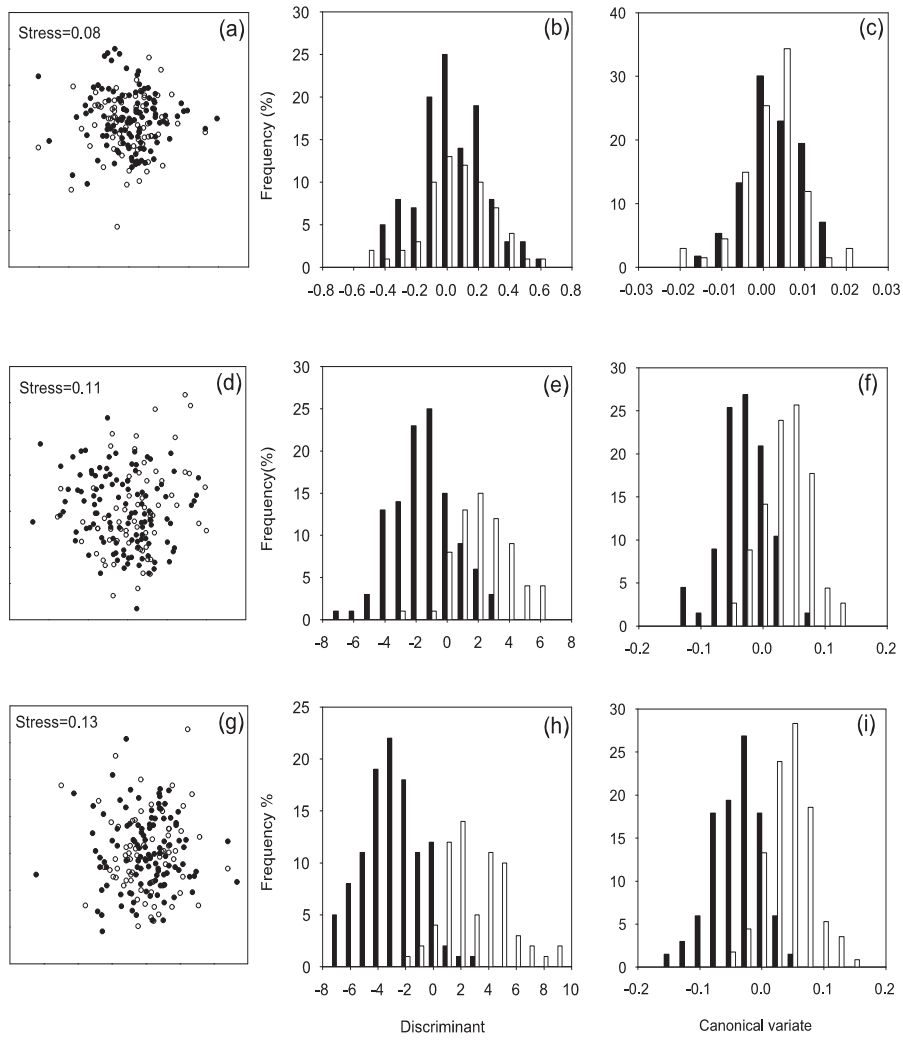

Figure 4.5: Non-metric multidimensional scaling (MDS) ordination plot of otolith (a) size variables, (d) shape variables, and (g) size and shape variables combined. Discriminant analysis plots of otolith (b) size variables, (e) shape variables and (h) size and shape variables combined. Discrimination of otolith morphometrics variables based on the canonical variates obtained from canonical analysis of principal co-ordinates (CAP) for (c) size variables, (f) shape variables and (i) size and shape variables combined of *Latris lineata* collected from Tasmania (TAS: hollow symbols) and St. Paul/Amsterdam Islands (SPA: solid symbols).

majority of influence in the discrimination based on otolith shape is derived from the lower order harmonics, hence morphological differences are more related to gross shape rather than the finer characteristics (Table 4.6).

Table 4.5: Canonical analysis of principal coordinates (CAP) examining the differences in Latris lineata otolith size and shape characteristics between Tasmanian (TAS) and the St. Paul/Amsterdam Islands (SPA). %var = percentage of the total variation explained by the first (m) principal coordinate axes; Allocation success is the percentage of points correctly allocated into their respective group; δ^2 = squared canonical correlation; ns = not significant

			Allocation success (%)				
Factor	m	%var	TAS	SPA	Total	δ^2	P
Shape	34	49.46	67.16	75.22	72.22	0.419	$<$0.001
Size	3	75.55	43.28	49.56	47.22	0.008	0.670 ns
Shape + Size	40	55.10	68.65	78.76	75.00	0.530	$<$0.001

Table 4.6: Correlation values (-0.20 $<$ r $>$ 0.20) relating elliptical Fourier shape coefficients with the canonical axis for effects of sampling region

Coefficient	Correlation	Related harmonic
4	0.2372	1
6	0.3604	2
9	0.2063	3
12	-0.3619	3
14	0.2314	4
24	0.3431	6
25	0.2660	7
27	-0.3354	7

4.4 Discussion

Combining image analysis techniques with elliptical Fourier analysis provided an efficient method for describing otolith shape. There was no significant variability in otolith size

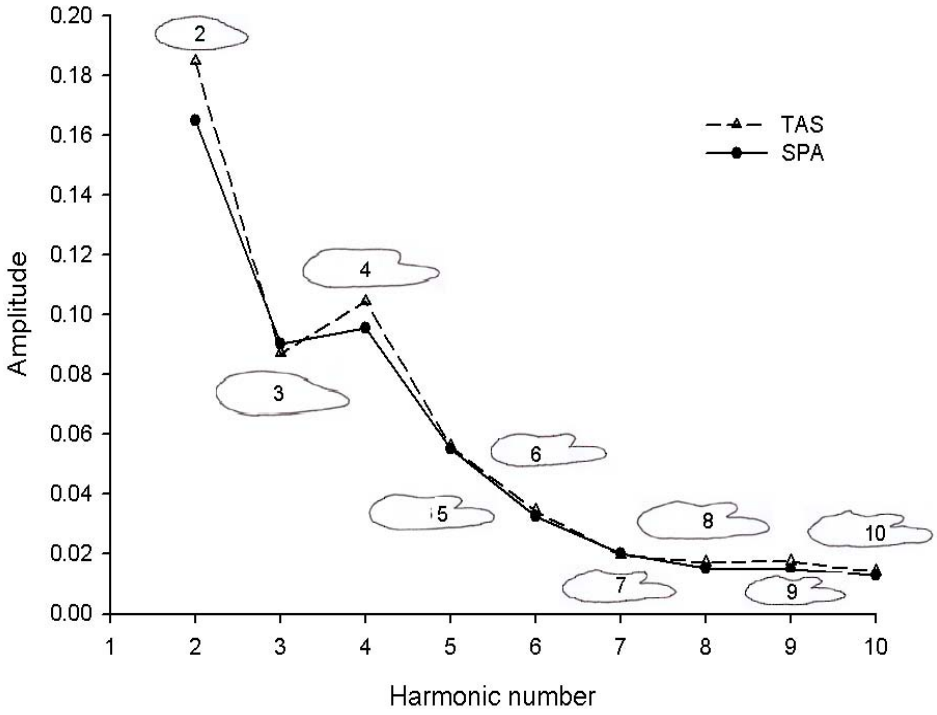

Figure 4.6: Mean amplitudes of elliptical Fourier harmonics 2 – 10 derived from striped trumpeter otoliths, sampled from Tasmania and St. Paul/Amsterdam Islands (SPA).

or shape between sexes or between age classes within each region. Several authors have identified variability in the latter as a disadvantage when using otolith morphometrics as a discriminatory tool (Bird et al., 1986; Castonguay et al., 1991; Campana & Casselman, 1993). The lack of significant intra-regional variability of these two factors enabled the data to be pooled, increasing sample size and consequently avoiding complications when comparing morphometrics between populations.

Another confounding issue that needs to be considered when using otolith morphometrics to identify stock structure is the effect of allometric growth (Cardinale et al., 2004). In this study the sampled length frequency distributions were not significantly different between the two populations, minimising any potential otolith variations arising from length distribution sample biases. To further reduce any possible fish length effect, the otolith size variables were corrected for fish length and the elliptical Fourier functions standardised to a common otolith length.

Comparing the re-parameterised VBGF's indicated no significant difference in length at age (based on ages 4 – 10 years) between the two regions. Furthermore, neither the unconstrained or constrained ordinations could separate the two regions based on the otolith size variables. The close relationship between somatic and otolith growth is well understood (Messieh, 1975; Lou et al., 2005) and may provide a reasonable explanation as to why the results based on somatic growth and otolith size where consistent in that the two populations could not be discriminated. Interestingly sea surface temperature estimates, based on remotely sensed data[1], suggest that the two sample populations live in similar temperature regimes with less than a 1°C difference averaged over the last 20 years[2].

In contrast, otolith shape analyses did significantly discriminate between the two populations. Initially the unconstrained MDS analysis did not reveal any visual separation, but the PERMANOVA test suggested a significant difference. Anderson & Willis (2003) discuss how certain patterns of overall dispersion can sometimes mask real differences

[1]Data sourced from the NOAA-CIRES Climate Diagnostics Centre, Boulder, Colorado, USA, 80305-3328, http://www.cdc.noaa.gov/. [Accessed on: 15 September 2002]

[2]Tracey, unpublished data

in multivariate space among groups in an unconstrained ordination, such as MDS. Constrained methods on the other hand use an a priori hypothesis to generate plots, which can highlight dispersion trends overlooked by unconstrained methods, as appears to be the situation in this study. Using both CDA and CAP as a comparison of constrained ordination methods the populations were discriminated with allocation success as high as 87 and 75% respectively to a priori groups. This is taken to imply that phenotypic differentiation reflected in the otolith shape of the samples examined from each region is sufficient to distinguish the two populations, when considering Booke (1981) definition of a phenotypic stock as a group of individuals within a species that maintain some common characters that are environmentally dependent.

Although the CDA was better at discriminating between the two groups this method is susceptible to the violation of multivariate non-normality within the datasets, whereas the permutational format of CAP bypasses this issue. CAP is also more robust than CDA when the number of variables is close to the number of observations (Anderson & Willis, 2003), as was the case in this study. A further benefit of the CAP is its potential for identifying those variables that are responsible for dispersive patterns in multivariate space, this proved useful in allowing us to identify that the lower order harmonic coefficients, hence gross otolith morphology rather than finer characteristics were contributing more to the regional differences in otolith morphology.

Identifying how otolith form differs between regions is complex, understanding the reason for subtle, but statistically significant shape differences using geometric morphometrics is abstract (Cadrin & Friedland, 1999), and hence the morphometric variables should be considered 'biologically arbitrary' (Rohlf, 1998). Unfortunately shape analysis does not have sufficient power to determine whether individuals are misclassified because of imprecision of the methodology, individual variability or because the fish has migrated from another area (Campana & Casselman, 1993). The possibility of migration between sites was raised through the chance recovery of a fish tagged initially off Tasmania and recaptured from St. Paul Island some three years later (Lyle & Murphy, 2002). Therefore

it can only be speculated as to the reason for misclassification, although, it is most likely to be a combination of inherent error introduced through the methodology, true 'outliers' within each population and individuals migrating from other areas.

In relation to the latter, the concept of acclimation represents a possible avenue for misclassification. For example, if a fish migrates from one location to another does its otolith form acclimate to the new environment, and in turn bias the results. The application of morphometric characteristics is to our favour as they are less likely to be refined by acclimation than other physiological characteristics (Ihssen et al., 1981). Furthermore, it has been reported that there are minimal changes in otolith shape after maturity is reached (Bird et al., 1986). Opportunistically constraining the analysis to adult stocks, which are past their fast growing phase (to 4 – 5 years of age; Tracey and Lyle, 2005) and assuming that migration would only occur post-maturity, we would expect that if there were any acclimated differences in otolith form they would be slow to develop and thus unlikely to be the cause of significant misclassifications.

Based on the results of this study elliptical Fourier analysis had reasonable success in demonstrating that the two case study populations are discrete based on otolith shape. However with any new technique it is always wise to corroborate the findings with more established techniques where possible, a preliminary phylogenetic assessment of mitochondrial DNA sequences between the two populations has also supported this discreteness between the two populations[3].

This study provides support of the potential benefits of otolith shape analysis as a stock discrimination tool. Shape analysis is a cheaper and more time efficient method than either genetic or microchemical discrimination techniques. By introducing elliptical Fourier functions this study adds another tool to the suite of shape descriptive methods already in use for fish otoliths. The study also highlights the need to carefully select the most appropriate statistical tests when analysing results.

[3]Tracey, unpublished data

Chapter 5

Population structure of *Latris lineata*: localised and trans-oceanic scales

Abstract. Striped trumpeter (*Latris lineata*) is a demersal teleost distributed around the temperate clines of all the major oceans in the southern hemisphere. Within Tasmanian waters the species is managed as a single stock, although no studies have been performed to confirm genetic panmixia. A protracted pelagic larval phase and a recent transoceanic tag recapture of an adult fish suggest significant potential for genetic mixing between widely separated populations. Phylogenetic analysis of mitochondrial DNA control region sequences suggested no genetic mixing between Tasmania, New Zealand and St. Paul/Amsterdam islands, evidence for the first time that there is population structure at a transoceanic scale for this species. In addition, an analysis of molecular variance coupled with phylogenetic analyses suggested no significant structuring of striped trumpeter from three locations around Tasmania. The information provided in this study is useful for the design of modern fisheries management techniques such as spatially implemented marine reserves. In addition, species-by-species knowledge about population structures of marine species facilitates ecologically useful generalizations concerning their population dynamics and key issues on the broader ecology of the oceans.

5.1 Introduction

Striped trumpeter (*Latris lineata* Forster in Bloch and Schneider 1801) are widely distributed around the temperate clines of the southern hemisphere. The species has been identified in Australia, New Zealand (Last et al., 1983), including the sub-Antarctic Auckland Island (Kingsford et al., 1989), the Gough and Tristan Da Cunha Island groups in the southern Atlantic Ocean (Andrew et al., 1995), the Amsterdam and St. Paul Island groups in the southern Indian Ocean (Duhamel, 1989) and more recently the Foundation seamount in the southern Pacific Ocean (Roberts, 2003). Their distribution in Australia extends from the mid-coast of New South Wales to Kangaroo Island in South Australia as well as Tasmania (Kailola et al., 1993). From these accounts their distribution is limited to a latitudinal belt spanning from 35°S to 51°S (Fig. 5.1). At present the Australian population is assumed to consist of a single stock although no studies, genetic or otherwise, have been performed to corroborate this. For management purposes striped trumpeter populations from New South Wales, Victoria, South Australia and Tasmania are considered separate management units and are controlled independently by each state.

There are two main conduits for genetic mixing of marine organisms, larval dispersal and adult migration. Many demersal fish species have an extensive larval phase, a trait that may lead to high levels of dispersal and result in a lack of stock structure at various spatial scales. The extent of spatial structuring arising from larval dispersal is dependent on the pelagic larval duration (Cowen et al., 2006). Striped trumpeter are known to exhibit an extended offshore neustonic larval phase believed to be approximately nine months in duration based on aquaculture studies (Furlani & Ruwald, 1999). This extended pelagic larval phase combined with the mesoscale current systems flowing, generally, west to east in the southern hemisphere suggests potential for transoceanic mixing between striped trumpeter populations.

Active migration between reproductive populations of mature individuals is another source of population mixing. This mechanism is reasonably common for pelagic species

but active migration over large distances particularly at an oceanic scale is not considered a common trait of demersal fish. However, the recapture of a striped trumpeter three years after tagging adjacent to the southeast coast of Tasmania approximately 5800km to the west at the Amsterdam Island group represents an obvious anomaly (Tracey et al., 2006). This finding not only revealed that they are capable of transoceanic journeys but also provided another possible mechanism for the wide dispersal and genetic mixing of this species. Of particular interest was the counter current direction of this migration, assuming the migratory path followed the shortest route. If this tag recapture is evidence of a migratory ability previously unobserved for this species, it provides an alternative potential source of mixing.

The mitochondrial genome is particularly suited to genetic studies of intraspecific population structure as it has a rapid rate of evolution and is predominately maternally inherited (Alvarado Bremer et al., 1995). The degree of geographic structuring of mitochondrial DNA (mtDNA) polymorphism in highly mobile vertebrate species reflects both historical and current levels of gene flow among intraspecific populations (Avise et al., 1992; Alvarado Bremer et al., 1995). The d-loop control region is of particular use in phylogenetic studies due to its hypervariability (Wenink et al., 1994).

In this study we have presented the analysis of striped trumpeter mtDNA control region sequences. Firstly, to determine whether striped trumpeter from Tasmania, New Zealand and the St. Paul/ Amsterdam Island regions are a panmictic unit or structured into distinct phylogeographic populations. Secondly, to test for genetic homogeneity of the Tasmanian population.

5.2 Material and Methods

5.2.1 Sampling

Skeletal muscle tissue was collected from 104 striped trumpeter between 1995 and 2004. Thirty individuals were collected from the St. Paul and Amsterdam Islands (SPA) and

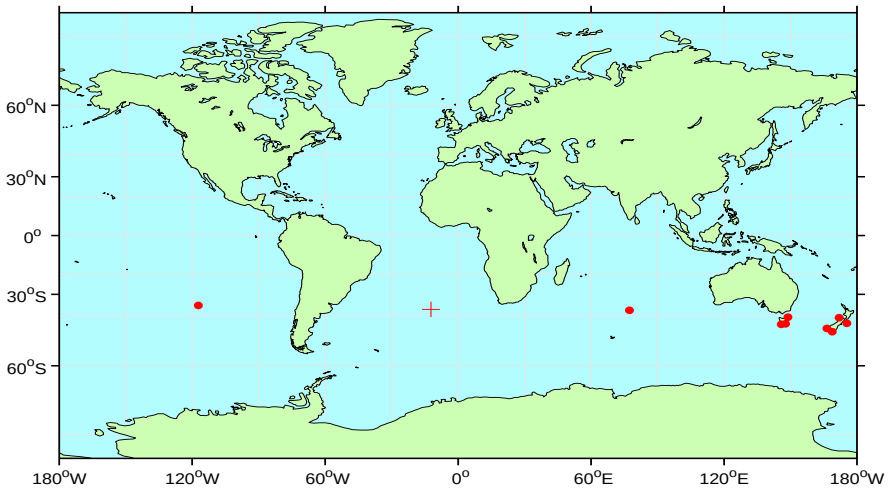

Figure 5.1: Current known distribution of *Latris lineata*. This map has been adapted from Roberts (2003). Circles indicate sample sites for this study.

stored in formalin. Twelve were collected from New Zealand (NZ), 62 individuals from around Tasmania (TAS) and one from the Foundation seamount. These were stored in 70% ethanol. As there was only one sample available from the Foundation seamount it was excluded from most analyses with the exception of the neighbour-joining and maximum likelihood phylogenetic trees. The TAS samples were further divided into three specific areas: northeast, southeast and southwest (Table 5.1).

5.2.2 DNA extraction and amplification

Approximately 50 mg of muscle tissue from each individual was digested for two hours at 65°C in a 1.5 ml micro centrifuge tube containing 500 μl of CTAB and 5 μl of 20 mg/ml Proteinase K. The homogenate was extracted with 500 μl of chloroform-isoamyl alcohol 24:1 v/v followed by extraction with phenol/chloroform-isoamyl alcohol 25:24:1 v/v/v, before a final extraction with chloroform-isoamyl alcohol to remove traces of phenol prior to precipitation in isopropanol. Following centrifugation at 3100 g for 15 minutes, DNA

Table 5.1: Collection details of samples and the number of haplotypes within each sample.

Region	Location	Latitude	Longitude	*n*	Collection year	No. of haplotypes
Pacific Ocean						
	Northeast Tasmania	40°36 S	148°47 E	15	2003 – 2004	15
	Southeast Tasmania	43°32 S	147°55 E	23	2003 – 2004	20
	Southwest Tasmania	43°33 S	145°56 E	23	2003 – 2004	20
	Sub-total			55		61
Indian Ocean						
	St. Paul Island	37°50 S	77°30 E	30	2003	29
	Sub-total			30		29
Tasman Sea						
	Fiordland, New Zealand	45°46 S	166°38 E	5	1995 – 1996	5
	Stewart Is., New Zealand	46°53 S	168°06 E	4	1995 – 1996	4
	Tolaga Bay, New Zealand	38°17 S	177°30 E	1	1996	1
	Kapiti Is., New Zealand			1	1996	1
	Mernoo Bank, New Zealand	43°16 S	175°26 E	1	1996	1
	Sub-total			12		12
Pacific Ocean						
	Foundation seamount	35 27 S	117°20 W	1	1995	1
	Sub-total			1		1

pellets were washed with 500 μl of 70% ethanol, before being dried and resuspended in 100 μl of deionized water.

5.2.3 PCR and automated sequencing

Polymerase chain reaction (PCR) was used to amplify a section of the mtDNA control region. PCR's were conducted in 50 μl volumes, each containing 1 unit of Taq DNA polymerase (Promega, Madison), 5 μl of 10$\times$ reaction buffer (67 mM Tris-HCL (ph 8.8), 16.6 mM $(NH_4)_2$ SO_4, 0.4% Triton X-100 and 0.2 mg/ml Gelatin), 200 μM dNTPs, 0.5 μM of each oligonucleotide primer, 1.5 mM $MgCl_2$, and 20 – 100 ng of suspended genomic DNA. The primer pair L15995 (5ÁAC TCT CAC CCC TAR CTC CCA AAG 3́) and 16498H (3ĆCT GAA GTA GGA ACC AGA TG 5́) (Kocher *et al.* 1989) were used to amplify a 403 base pair region of the d-loop. Thermal cycling conditions for the amplification of the control region consisted of an initial denaturation period of 94°C/3 min, followed by 35 cycles of 94°C/30 sec, 52°C/30 sec, and 72°C/60 sec, and a final extension of 72°C/5 min. To determine successful amplification, the PCR products were separated by electrophoresis in a 1.0% agarose gel with 0.5 μg/ml ethidium bromide, and visualised under ultraviolet (UV) light. Prior to sequencing the target PCR products were purified using an UltraClean PCR Clean-up kit (MO BIO Laboratories, Inc.).

Sequencing reactions were performed using the CEQ Dye Terminator Cycle Sequencing Kit (Beckman Coulter) by following the manufacturer's instructions, and sequencing extension products were separated on a Beckman Coulter CEQ 8000 DNA analysis system.

5.2.4 Phylogenetic analysis and population structure

All sequences were aligned using ClustalW (Higgins et al., 1994) within BIOEDIT version 5.0.9 (Hall, 1999). The vertebrate mtDNA control region is known to have a high rate of nucleotide substitution that can be modelled by a gamma distribution specified by the parameter α (Wakeley, 1993). The parameter $\alpha$was empirically estimated from the sequence

data using DAMBE version 4.0.98 (Xia & Xie, 2001). Values of haplotypic diversity (h) (Nei & Tajima, 1981) and nucleotide diversity (π) (Nei, 1987) based on the Tamura-Nei distances (Tamura & Nei, 1993) were computed with ARLEQUIN version 2.0 (Schneider et al., 2000). The 104 samples were used to calculate a mean transition: transversion ratio in MEGA version 2.1 (Kumar et al., 2001). Geographical structuring within TAS samples and between TAS and SPA samples was tested using a hierarchical analysis of the molecular variance (AMOVA), based on the gamma corrected Tamura-Nei distances. The significance of the tests was determined by a non-parametric permutation procedure (Excoffier et al., 1992) conducted in ARLEQUIN. The pairwise matrix of Fixation index values was also conducted in ARLEQUIN.

The phylogenetic relationship among haplotypes was reconstructed using two methods: neighbour-joining (Saitou & Nei, 1987), using the gamma corrected Tamura-Nei distance matrix reconstructed in MEGA version 2.1, and maximum likelihood analysis performed using the heuristic search algorithm of PAUP version*4.0b10 (Swofford, 2002). Pairwise distances for the maximum likelihood analysis were calculated under the general time reversible model GTR+I+G (Lanave et al., 1984) as calculated by MrModeltest version 2.1 (Nylander, 2004), with resulting trees being unrooted. Nonparametric bootstrap analysis was performed based on 35000 replicate data sets using the fast stepwise addition in PAUP* retaining groups compatible with the 50% majority-rule consensus.

5.3 Results

5.3.1 Sequence variation

Analysis of 104 striped trumpeter control region sequences identified 115 polymorphic sites within a 403 bp fragment. These polymorphisms yielded 96 haplotypes from the five sample areas. This large number of haplotypes gave a high global value of haplotypic diversity (h = 0.9970 $\pm$ 0.0020), therefore the probability that two specimens drawn at random will have differences in this particular section of the control region is greater than

99%. Such a high rate of diversity agrees with other studies of teleost fish (Dudgeon et al., 2000; Aboim et al., 2005). The sequence from the most common haplotype was registered in GenBank - accession reference: DQ845246. Nucleotide frequencies were T = 33.7%, C = 18.3%, A = 34.7% and G = 13.2%, revealing a clear A-T bias in base composition (68%). The observed transition:transversion ratio was 2.9:1.0. These mutations were heterogeneously distributed through the selected mtDNA segment resulting in a low alpha parameter estimate (α = 0.227). The estimated global mean nucleotide diversity was 0.029 $\pm$ 0.004.

5.3.2 Population variability

Within the NE-TAS sample no haplotype occurred more than once resulting in the highest possible haplotypic diversity estimate (h = 1.000 $\pm$ 0.024). This may be due to the low sample size from this area. Within both the SE and SW-TAS samples 20 different haplotypes were identified yielding the lowest haplotypic diversity (h = 0.988 $\pm$ 0.016). The NZ samples also yielded the highest possible value of haplotypic diversity (h = 1.000 $\pm$ 0.03) with each of the sampled individuals having a unique haplotype. From the SPA sample, 29 haplotypes were identified (h = 0.998 $\pm$ 0.09).

5.3.3 Phylogenetic analysis

From the neighbour-joining tree (Fig. 5.2) it is clear that Tasmanian striped trumpeter dominate clade I (81%). By removing clade Ia, which includes several of the SPA samples present in clade I, the percentage of Tasmanian samples was further increased to 88%. The St. Paul/Amsterdam Island samples dominate clade II (61%). Again the removal of clade IIa increased the uniqueness of clade II to the SPA region (76%). Within both clades I and II there was no significant structuring of the Tasmanian samples, supporting the theory that the Tasmanian population is homogenous. Interestingly, of the 11 Tasmanian striped trumpeter occurring in clade II, 10 were from the southern regions of Tasmania.

The model of best fit calculated by MrModeltest version 2.1 was the GTR+I+G with a log likelihood score of 2076.99. The maximum likelihood tree derived from this substitution model (tree score 2040.88) suggested transoceanic structuring, with greater than 50% bootstrap support for clade II which represented the majority of the St. Paul/Amsterdam islands sample.

5.3.4 Phylogeographical relationships of populations

The pairwise matrix of Φ_{ST} values and their corresponding p-values shows a low level of genetic differentiation between all of the TAS samples and a highly significant level between all three TAS samples and the SPA sample (Table 5.2). This absence of genetic heterogeneity between the three TAS sites was confirmed by AMOVAs (Table 5.3) with no significant difference detected ($p < 0.05$). The Tasmanian sites were pooled for further analysis. The subsequent AMOVA performed on the TAS, NZ and SPA data again identified a highly significant divergence between these three populations (Table 5.4).

5.4 Discussion

5.4.1 Transoceanic mixing of striped trumpeter

There are many studies with contrasting findings related to genetic mixing of demersal species associated with seamount and oceanic islands. Some suggest that populations exhibit panmixia across large geographical distances on oceanic scales. These include: slender armourhead *Pseudopentaceros wheeleri* and wreckfish *Polyprion americanus*, while others demonstrate evidence of gene divergence among populations at the trans-oceanic, oceanic and regional scales. These include: roundnose grenadier *Coryphaenoides rupestris*, ling *Genypterus blacodes*, hoki *Macruronus novaezealandiae*, and oreos *Allocyttus niger* (reviewed in Creasey & Rogers, 1999). The concept that the temperate latitudes of

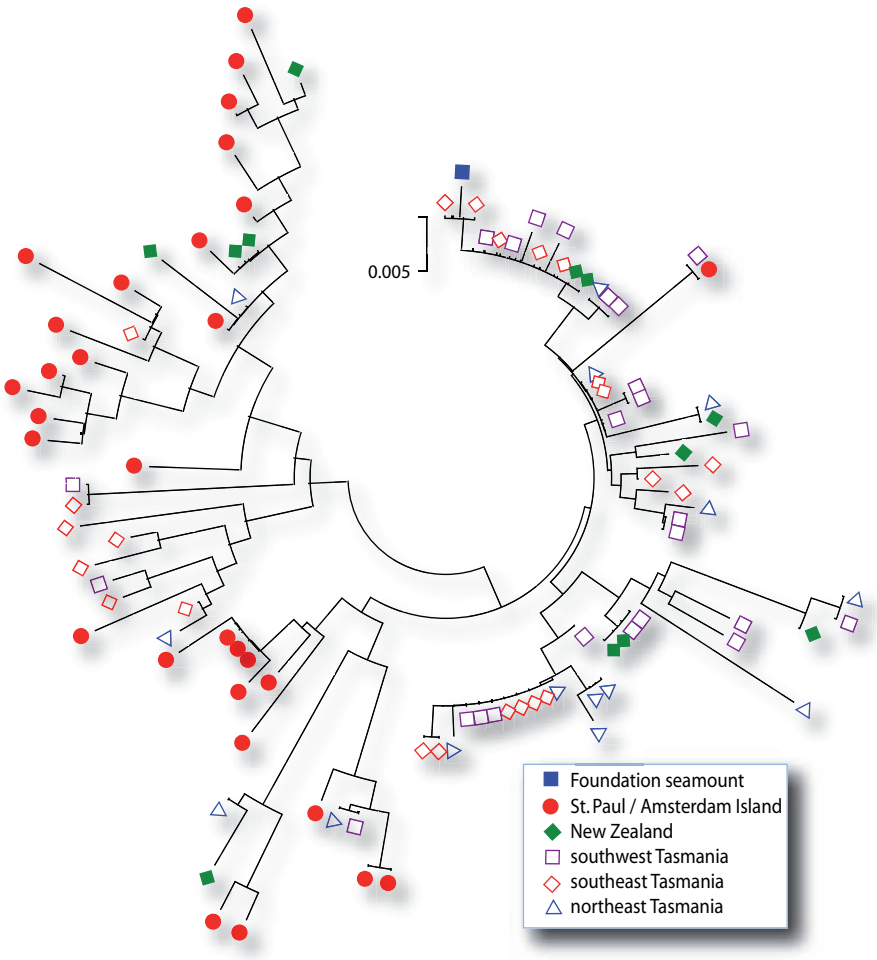

Figure 5.2: Unrooted neighbour-joining tree of the 104 samples based on the gamma corrected Tamura-Nei distance matrix of mtDNA control region sequences. Symbols refer to the geographical origin of the haplotypes.

Table 5.2: *Latris lineata*. Genetic differentiation matrix of sampled striped trumpeter populations. Φ_{ST} below diagonal line, *p*-values above line; number of permutations = 3024.

Population	TAS_{NE}	TAS_{SE}	TAS_{SW}	NZ	SPA
TAS_{NE}	–	0.334 ± 0.008	0.171 ± 0.007	0.000 ± 0.000	0.000 ± 0.000
TAS_{SE}	0.004	–	0.090 ± 0.005	0.000 ± 0.000	0.000 ± 0.000
TAS_{SW}	0.017	0.024	–	0.000 ± 0.000	0.000 ± 0.000
NZ	0.194	0.201	0.222	–	0.002 ± 0.000
SPA	0.195	0.216	0.248	0.143	–

Table 5.3: Tests of genetic diversity between striped trumpeter populations from different locations around Tasmania (NE= Northeast, SE= Southeast, SW= Southwest) using hierarchical analysis of molecular variance (AMOVA) on mtDNA control region sequences.

Test	Source of variation	*df*	Variance component	Percent variation	fixation index	*p*-value
NE v SW	Among populations	1	0.0973	1.66	Φ_{ST} = 0.0166	0.167 $\pm$ 0.011
	Within populations	36	5.7680	98.34		
NE v SE	Among populations	1	0.0228	0.38	Φ_{ST} = 0.0038	0.355 $\pm$ 0.015
	Within populations	36	5.9504	99.62		
SE v SW	Among populations	1	0.1422	2.44	Φ_{ST} = 0.0244	0.121 $\pm$ 0.009
	Within populations	36	5.6848	97.56		

the southern hemisphere may be a broad zoogeographic province for ichthyofauna has also been presented in several studies (Collette & Parin, 1991; Duhamel, 1997; Burridge & White, 2000).

Striped trumpeter are distributed widely around the temperate latitudes of the southern hemisphere. They are found as far north as 35°S at the Foundation seamount and as far south as 51°S at Stewart Island, located below the southern island of New Zealand. If the striped trumpeter populations are linked genetically there are two conduits that would facilitate such a state, adult migration and larval advection. Based on its life history traits larval advection would be the most obvious for striped trumpeter. However, adult migration cannot be discounted for this species due to a transoceanic migration identified by a tag recapture caught at Amsterdam Island which was originally tagged adjacent to Tasmania. Although this was only one individual the exchange of ecologically insignificant numbers of migrants between populations can maintain apparent panmixia (Utter, 1991; Dudgeon et al., 2000). This migration was particularly unusual as the maximum depth that striped trumpeter has been recorded at is 350 m (Last et al., 1983). As the species is demersal it would be reasonable to assume that the depths of the southern ocean basins would constitute barriers for adult migration.

Adjacent to Tasmania female striped trumpeter are highly fecund with a relatively protracted spawning period (Tracey et al., 2007). The larvae are also characterized by a pelagic larval duration of approximately 7 – 9 months. These characteristics combined

Table 5.4: Test of genetic diversity between striped trumpeter populations from Tasmania (TAS), New Zealand (NZ) and the St. Paul/Amsterdam Island group using hierarchical analysis of molecular variance (AMOVA) on mtDNA control region sequences.

Source of variation	*df*	Variance component	Percent variation	fixation index	*p*-value
Among populations	2	1.85	21.87	Φ_{ST} = 0.219	$\ll$ 0.0001
Within populations	100	6.60	78.13		

with the predominant westerly wind drift that occurs in the southern hemisphere provide the most likely mechanism for transoceanic population connectivity.

If larval migration were to occur between the landmasses and seamounts which striped trumpeter inhabit the most likely location would be between Australia and New Zealand as this is the shortest stretch of Ocean between two populations at approximately 1700 km. This is compared with approximately 5600 km between St. Paul/Amsterdam Island and Tasmania and 6000 km between the Foundation seamount and New Zealand.

An oceanographic transport mechanism between Australia and New Zealand exists in the form of the Tasman Front which meanders west to east, diverging off the north-south flowing East Australian Current (EAC) as it reaches about 35°S (Chiswell et al., 2003). However, the time frame for passive drifters deployed off the coast of Australia to reach New Zealand is approximately 15 – 24 months (Cresswell et al., 1994), much longer than the nine month pelagic larval phase of striped trumpeter.

F-statistics, AMOVA and phylogenetic analyses all indicated genetic structuring of striped trumpeter populations at the transoceanic scale. This suggests little recent gene flow between the SPA, NZ and TAS populations arising from either larval dispersal or active migration of adult fish. The sample from the Foundation seamount was allocated to Clade I of the neighbour-joining tree which contained the majority of the Tasmanian samples. Although, as there was only one sample from the Foundation seamount it was impossible to draw any conclusions as to the relationship of the Foundation seamount with the other sampled populations. The fixation index was relatively consistent and highly significant between the three transoceanic populations. Surprisingly, the lowest F_{ST} value was recorded between the NZ and SPA populations, the locations with the greatest geographic separation. It is quite likely that the low sample size from New Zealand has contributed to this apparent homoplasy. The phylogenetic trees do not give any indication of the New Zealand population forming a cohesive unit, again the low sample size is assumed to have contributed to this outcome. Another potential source of error arising from the New Zealand sample is the issue of temporal genetic stability as the New Zealand

sample was collected eight years prior to the other samples. A collection of both temporal and spatial replicates allows a quantitative evaluation of the importance of sampling error and signal:noise ratio in gene diversity analysis (Waples, 1998), although this was beyond the scope of this study.

The structuring between the TAS and SPA samples concurs with a previous study based on otolith morphometrics which found otolith form differences between striped trumpeter collected from TAS and SPA were indicative of two distinct populations (Tracey et al., 2006).

The genetic differences between the Tasmanian and New Zealand populations of striped trumpeter indicated in this study also concur with other studies exploring the genetic divergence of marine species between Australian and New Zealand populations. Jackass morwong *Nemadactylus macropterus*, a demersal perciform with an extended larval phase, showed distinct separation between Australian and New Zealand populations (Grewe et al., 1994). The lobster species *Sagmariasus verreauxi* also was shown to have populations genetically different between the two countries (Brasher et al., 1992). *S. verreauxi* also has an extended pelagic phyllosoma phase estimated to be between 8 and 12 months (Booth, 1986). In contrast to these studies the southern rock lobster *Jasus edwardsii* is genetically indistinguishable between Australia and New Zealand (Ovenden et al., 1992). It has a larval phase estimated to be between 12 to 24 months (Booth & Phillips, 1994), concurring with the time taken for the remote drifters to advect from one country to the other. This synthesis would suggest that pelagic larval duration is a major determinant in transoceanic gene mixing. Although the consideration of active versus passive migration cannot be discounted, for example, the lobster species use passive migration for the duration of their larval phase with the exception of diurnal vertical migration (Bradford et al., 2005), while the fish species are capable of extensive active migration once they are post-flexion, which occurs after approximately one month for striped trumpeter (Furlani & Ruwald, 1999).

In terms of fishery stock assessment identifying the lack of significant migration to or from the Tasmanian stock is of significant benefit, satisfying the assumption of a ‘closed’

population, common to many fishery stock assessment models.

5.4.2 Structuring of the Tasmanian striped trumpeter population

The results from this study indicate that adjacent to Tasmania, striped trumpeter constitute a single homogenous stock. Although the F_{ST} values between the Tasmanian samples are large enough to represent isolation in an ecological if not evolutionary sense, considering the scale of the fixation index is from 0 to 1, with the lower the value the less the genetic difference, therefore it is possible that if the sample sizes were increased these values could become significant and the finding of a homogenous stock would be compromised. Data from striped trumpeter larval dispersal modeling (Tracey and Hobday, *unpublished data*) indicate that the extended larval dispersal coupled with the oceanographic features common to Tasmania create a situation where there is a high probability of significant mixing of larvae from isolated spawning grounds adjacent to Tasmania. This supports the case for homogeneity of the Tasmania population.

A similar finding indicating the absence of sub-structuring across southern Australia based on genetic analysis has been reported for *N. macropterus* (Grewe et al., 1994), although there is conjecture as to this finding. An otolith microchemistry study suggested a more complex population structure around the same area (Thresher et al., 1994). The discrepancy between these two findings has been ascribed to the relative sensitivities of the two techniques to the rates of exchange between the two populations (Thresher et al., 1994).

Identifying stock structure is an essential component to understanding a population's ability to maintain viability under fishing pressure. The apparent homogenous structure of the population adjacent to Tasmania would indicate that a single stock management strategy is appropriate for this species. However, the caveat as to the robustness of the findings based solely on genetic analysis would warrant further study to confirm this finding. This could include analysis of: sequence data from the entire control region, sequence data from another coding region of the mitochondrial genome, a nuclear marker or even a divergent

technique such as otolith microchemistry. All of these techniques have potential to further resolve the phylogenetic relationship between the Tasmanian sample locations. However, given the small-scale of the striped trumpeter fishery adjacent to Tasmania it would be difficult to warrant management in the traditional sense as anything other than a single stock based on genetic divergence alone. If it was shown that the life history parameters of the discrete sub-populations were different then separate stock assessments would be warranted and potentially alternate size limits, etc could be imposed to regulate exploitation of the sub-populations.

Genetic data relating to the structure of marine population on a species-by-species basis is important from both an ecological and a conservation perspective. This information enhances the design of modern fisheries management techniques such as spatially implemented marine reserves. In addition, only by gaining knowledge about the population structures of a greater number of marine species can we make ecologically useful generalizations concerning their population dynamics and key issues such as the impact of pelagic larval duration on the broader ecology of the oceans.

Chapter 6

Shallow inshore reefs as juvenile habitat for a temperate fish displaying an ontogenic depth-stratified migration

Abstract. An understanding of critical life history requirements, including habitat utilisation, is important when managing fish populations. Striped trumpeter (*Latris lineata*) support small, but iconic commercial and recreational fisheries in Tasmania, characterised by exceptional recruitment variability and apparent ontogenic habitat preferences. Using otolith microchemistry we estimated the comparative contribution to the adult population of juvenile striped trumpeter from shallow inshore habitats. Juvenile striped trumpeter from a strong recruitment pulse (1993 cohort) were collected at age two from inshore reefs and as adults at age six from deeper offshore reefs around the coast of Tasmania. Natural variations were identified in the concentrations of lithium and strontium within the incremental structure of the observed otoliths. Discriminant analysis suggested that 70% of adults sampled originated from an inshore juvenile habitat, 13% were from deeper reefs and 17% could not be statistically allocated with confidence. These results emphasise the importance of shallow inshore reefs for this species by quantifying the disproportionate contribution of juveniles to the adult biomass from these areas.

6.1 Introduction

Understanding the links between different life history stages and habitat utilisation is essential for the effective management and the sustainable utilization of aquatic resources, particularly when juvenile and/or nursery habitats are distinct from adult habitats. There are many examples of coastal fish species that utilise discrete habitats, such as estuaries, seagrass meadows, kelp forests and reef systems, as juveniles before being recruited to adult populations (for review see Gillanders et al., 2003). An understanding of the spatial complexities and habitat utilisation involved in successful recruitment is particularly important for species that display strong recruitment variability, a characteristic of many temperate marine species (Rothschild, 1986).

Striped trumpeter (*Latris lineata*) is a demersal teleost that is widely distributed throughout the southern hemisphere including Australia, New Zealand and several isolated island locations in the Indian, Pacific and Atlantic Oceans (Tracey et al., 2006). Aquaculture trials have found that for the first nine months of life striped trumpeter are pelagic larvae/paperfish, before undertaking a significant morphological change thought to coincide with settlement to demersal reefs based on the observation of juveniles in the field (Tracey unpublished data). Evidence from fishery and scientific sampling in southeast Australia suggest that throughout the juvenile phase striped trumpeter have a distinct preference for shallow inshore rocky reefs (<50 m in depth) (Tracey & Lyle, 2005). As striped trumpeter approach maturity at approximately five years of age, they move offshore to reef areas on the upper edge of the continental shelf at depths ranging from 80 – 250 m (Tracey & Lyle, 2005). The affinity of juveniles to these inshore habitats around Tasmania was most evident through 1995-1998 when juvenile striped trumpeter were unusually abundant in inshore gillnet catches, this cohort was back-calculated to a particularly strong recruitment pulse spawned in 1993 (Tracey & Lyle, 2005).

These observations pose the question of whether inshore reefs do in fact represent a crucial habitat in the life history of striped trumpeter or whether juveniles also recruit to

the adult population from other areas, including deeper reefs.

Determining previous juvenile habitats of individual adults using conventional tagging techniques is difficult because of the small size of larvae and juveniles, high rates of mortality at early life history stages, and the large numbers that need to be tagged in order to recover a sufficient sample size (Gillanders, 2005). Otolith microchemistry is perhaps the most successful method available to date to back-classify individuals to particular juvenile nursery areas (Gillanders & Kingsford, 2000; Forrester & Swearer, 2002). The method depends on environmental gradients between locations producing differences in the suite of elements incorporated into the otoliths of individuals from each of the environments.

Our underlying hypothesis is that the apparent depth-based distribution by life history stage displayed by striped trumpeter will be reflected in otolith microchemistry such that the relative importance of the inshore reefs as nursery habitats can be evaluated.

In this study a single cohort was monitored at various times over a seven year period with microchemical changes in the otolith structure associated with movement from shallow coastal reefs to deeper offshore reefs. The specific objective was to estimate the proportion of adults from the 1993 cohort that could be linked back to inshore reefs as juveniles, and hence evaluate the significance of shallow, inshore reefs as juvenile habitat for striped trumpeter.

6.2 Material and Methods

6.2.1 Sample collection

Sampling was focused on striped trumpeter specimens from a single large cohort spawned in the austral spring of 1993 (September – October), referred to here as the 1993 cohort. Age estimates were determined using the validated protocol described by Tracey & Lyle (2005). Two distinct reef habitats are referred to throughout this study. The first, ‘shallow inshore’ we define as less than 30 m in depth and the second, ‘deeper offshore’ are reefs located on the continental shelf break at a minimum depth of 120 m and a maximum of

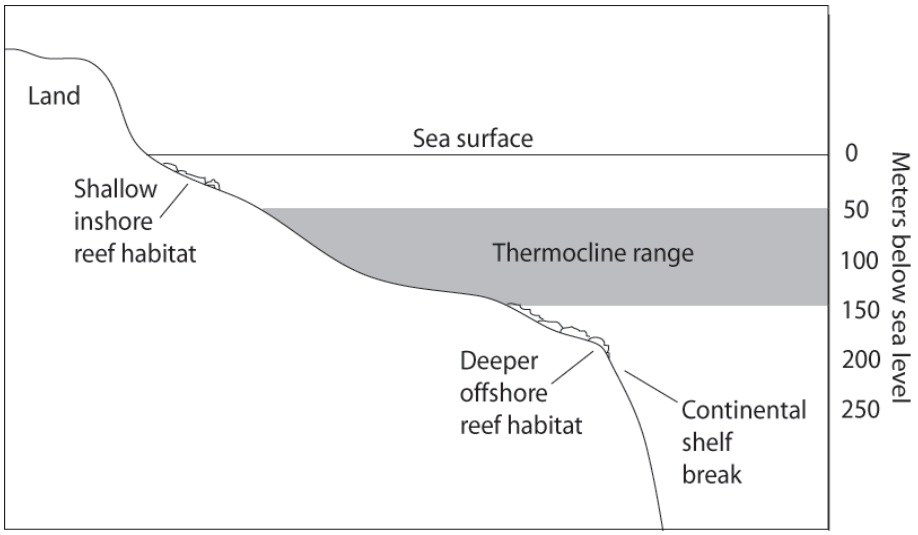

Figure 6.1: Schematic profile illustrating the habitats defined through this study as 'shallow inshore reefs' and 'deeper offshore reefs' and there location in relation to the depth range of the thermocline.

≃200 m (Fig. 6.1). Juveniles (used to characterise the shallow habitat signature) from the 1993 cohort were sampled as two-year old fishes from shallow inshore reefs using gillnets from the Tasman Peninsula and Bruny Island, and then again as three-year olds from the Tasman Peninsula (Fig. 6.2). Adults from the 1993 cohort were sampled as five-year olds in 1999 and as six-year olds in the following year from an offshore reef on the southeast coast of Tasmania, at a depth of ≃150 m. Six-year olds were also collected from offshore reefs on the southwest coast (depth ≃140m) and the east coast (depth ≃120 m) (Fig. 6.2). Sample details are summarised in Table 6.1.

Five adults were also sampled from deeper offshore reefs on the southeast coast of Tasmania around the same time as the two years olds were collected in 1996. These adults were included to examine whether there was a temporal rather than a depth effect on otolith microchemistry.

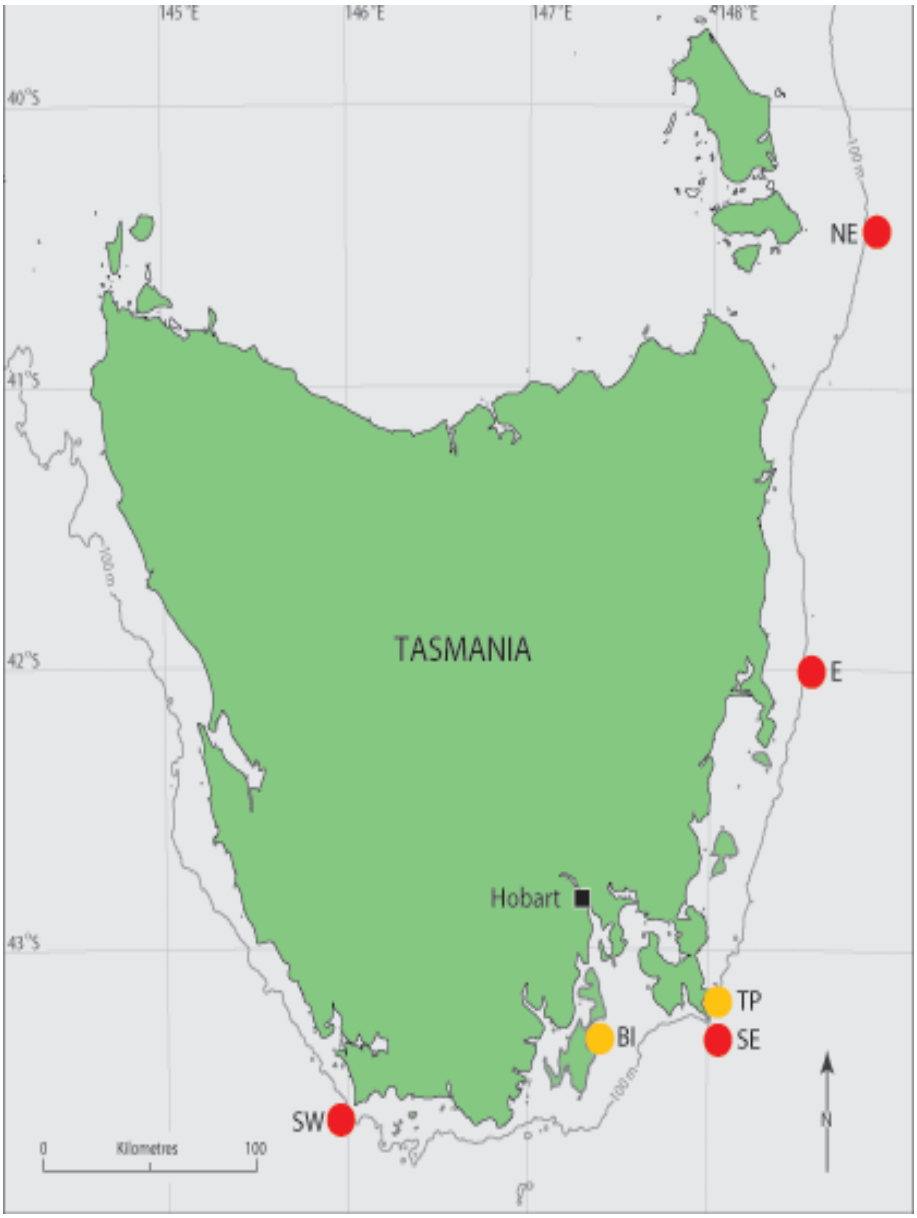

Figure 6.2: Map of Tasmania, Australia showing sampling locations for juveniles (yellow dots) from Tasman Peninsula (TP) and Bruny Island (BI), and adult (red dots) striped trumpeter (*Latris lineata*).

6.2.2 Sample preparation and analysis

Otoliths were mounted in epoxy resin and transversely sectioned through the primordium ($\simeq$300 micron thickness) to expose the increment structure. Otolith mounts were ground using 1500 grit carborundum paper lubricated with 18.2ΩMcm^{-1} Milli-Q water and polished using 0.3 μm alumina powder on a suede polishing disc. Once final polishing had been completed the mounts were ultrasonically cleaned for five minutes in Milli-Q water and then rinsed further using Milli-Q water to remove surface contaminants before being allowed to air dry.

Microchemical analysis was conducted by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) using a New Wave UP-213 Nd:YAG Q-switched Laser Ablation System controlled by the MEOLaser 213 software package, coupled with a Agilent HP4500 Quadrapole ICP-MS. Ablation was conducted in the spot mode, where the sample is stationary under the pulsating laser beam resulting in a continuous deepening in the form of a cylindrical hole during ablation. The ablations were carried out in an atmosphere of ultra-high purity Helium using a custom designed small-volume ($\simeq$3 cm^3) sample cell. This cell ensured rapid transport of the ablated material to the mass-spectrometer, resulting in accurate documentation of chemical variations with depth.

Table 6.1: Capture location, date, age (years), fork length range (FL) (mm) and sample size of individuals analysed using laser ablation inductively coupled plasma-mass spectrometry.

Capture location	Sample	Collection year	Age (years)	FL range	Sample size
Inshore sites					
Tasman Peninsula	TP_2	1996	2	311 – 382	11
Tasman Peninsula	TP_2	1997	3	346 – 417	15
Bruny Island	BI_2	1996	2	298 – 384	12
Offshore sites					
Southeast shelf	SE95_2/E	1995	8 – 10	610 – 740	5
Southeast shelf	SE_2/E	1999	5	460 – 537	9
Southeast shelf	SEb_2/E	2000	6	499 – 592	12
Southeast shelf	SW_2/E	2000	6	515 – 721	12
East shelf	E_2/E	2000	6	495 – 545	5

For sample analysis the beam diameter was nominally set at 80 μm with a pulse rate of 5Hz, each sample was also pre-ablated at 2Hz for two seconds to minimise the effect of surface contamination. All otoliths were ablated on the growing edge to determine the elemental signature assumed to represent the fish's habitat at the time of capture. Otoliths of adults (age five or older) were also spot ablated on the incremental zone corresponding to the second growth increment, to determine the elemental signature assumed to represent the fish's environment at age two (juvenile).

The following suite of elements was recorded for each analysis: ^{7}Li, ^{24}Mg, ^{43}Ca, ^{45}Sc, ^{47}Ti, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{85}Rb, ^{88}Sr and ^{137}Ba. Of the 15 elements recorded only seven provided readings where greater than 95% of the samples were greater than the limits of detection (Table 6.2). Only these elements were used for subsequent analysis: Li, Mg, Fe, Ni, Sr, Ba, and the internal standard Ca.

The average laser energy output was $\simeq$12 J/cm^2. At the beginning of each ablation background levels were collected for 30 seconds, and the average of these measurements was subtracted from the following sample measurement to correct for background levels. The acquisition time for each sample ranged between 40 – 60 seconds.

The results were quantified using an international standard NIST612 following the procedure of Longerich et al. (1996). To account for instrument drift the standard was ablated regularly throughout the analytical sessions, and drift corrected using linear interpolation. The concentration of Calcium in the otoliths was assumed to be constant at 40.04 wt% CaO, based on $CaCO_3$ stoichiometry. The detection limits (DL) of each element were calculated from three standard errors of the background signal.

Analytical precision is dependent on the concentration level and varies from $\simeq$2% (1 standard error) at high concentrations to $\simeq$30 – 50% at concentrations near the DL. Accuracy is difficult to estimate due to the lack of international secondary standards, but is believed to be $>$50% based on our experience with analysis of other materials.

6.2.3 Statistical analysis

Elemental concentrations were examined for univariate normality and homogeneity of variances using Kolmogorov-Smirnov tests and Levene's statistic respectively, and then transformed to ln (x + 1) to conform to statistical assumptions. Univariate and multivariate methods were used to test hypotheses pertaining to differences in the elemental composition between samples. Due to difficulties in procuring samples from particular sites, replication between samples was unbalanced (Table 6.1). Classification of samples was assessed by step-wise linear descriptive discriminant analysis and multivariate differences were visualised by producing canonical discriminant plots. Equal prior probabilities were assumed as sampling was not proportional to population priors (Huberty, 1994). Classification of samples was assessed by linear descriptive discriminant analysis and multivariate differences were visualised by producing canonical discriminant plots. Prior probabilities of the discriminant analyses were based on the sample size of each test group. The classification accuracy of each discriminant function was determined by comparing the jackknife (leave-one-out) predicted group membership and calculating the percentage of individuals that were correctly classified.

For predicting retrospective adult membership to juvenile habitats, discriminant function coefficients were derived for the elemental signatures of individuals collected from inshore and offshore habitats and the discriminant function scores (D) calculated for elemental signatures of the 2^{+} increment of offshore adults using the linear discriminant equation:

$$D = B_0 + B_1 X_1 + ...B_\psi X_\psi \tag{6.1}$$

where X is the value of each independent variable and B is the coefficient estimated from the data for each element (B_0 is a constant). Normal probability theory was used to explain the distribution of the 'D' score for the inshore and offshore elemental signatures. The posterior probability that an adult as a juvenile belonged to either of these groups was

determined using Bayes' rule:

$$P(G_i/D) = \frac{P(G/D_i)P(G_i)}{\sum_{i=1}^{g} P(G/D_i)P(G_i)} \tag{6.2}$$

Where $P(G_i)$ is the prior probability (weighted by sample size, inshore = 0.37, offshore = 0.62), and $P(D/G_i)$ is the conditional probability of obtaining a particular discriminant function value of D.

Statistical analysis was conducted using SPSS ver.13 and Microsoft Excel.

6.3 Results

6.3.1 Mass spectrometer precision tests

In order to assess the internal precision of the LA-ICPMS analyses, ablations were performed twice, approximately three months apart, on the two-year and three-year-old juveniles collected inshore from the Tasman Peninsula. Significantly different tuning parameters of the mass-spectrometer were used for the two measurements to assess the effects of plasma conditions and ion extraction parameters on the concentrations obtained. For this test, all the predictive elements above detection limits were included.

The MANOVA performed on the two-year-olds showed a significant difference between the treatments (Pillai's Trace: $F_{[6,17]} = 173.647, p < 0.001$), although, of the seven individual elements only Fe showed a significant difference. After the removal of Fe from the suite of predictors the MANOVA result was non-significant (Pillai's Trace: $F_{[5,18} = 1.173, p = 0.360$).

The MANOVA conducted on the repeated analysis of the three-year-olds concluded no significant difference between the two treatments (Pillai's Trace: $F_{[6,19]} = 1.515, p = 0.227$), although the results of Fe were significantly different. The observed differences in Fe concentrations are likely to have been caused by CaOH interference, as the CaOH compound is formed in the plasma, and its production is likely dependent on the tuning

parameters. As a consequence Fe has been excluded from subsequent data treatment.

6.3.2 Elemental synthesis

Mg and Ni concentrations varied significantly between test groups (listed in Table 6.1) although no clear trend based on spatial or temporal effects was evident (Fig. 6.3). Ba concentrations varied little for the majority of test groups with the exception of the growing edge ablations of the southwest and northeast samples. Of the six elements above detection limits only Li and Sr showed a systematic trend based on the depth-stratified groupings (Fig. 6.4). Therefore, only these two elements were used for subsequent descriptive and predictive analyses.

Table 6.2: Limits of detection (LOD) and percentage of ablations above LODs (%>LOD) of all otoliths analysed. Otolith elemental concentrations were determined using laser-ablation ICPMS. Bolded values indicate that this isotope met our selection criteria (>95% of samples above DOL) for inclusion in comparative analysis

Isotope	LOD	%>LOD
^{7}Li	0.0493	**100.0**
^{24}Mg	0.1034	**100.0**
^{45}Sc	0.0493	70.0
^{47}Ti	0.2535	16.7
^{53}Cr	0.5288	15.8
^{55}Mn	0.2082	35.8
^{57}Fe	7.7307	**100.0**
^{60}Ni	0.1342	**96.7**
^{65}Cu	0.7179	6.7
^{66}Zn	0.3417	32.5
^{75}As	1.0363	88.2
^{85}Rb	0.0547	64.2
^{88}Sr	0.0355	**100.0**
^{137}Ba	0.0277	**100.0**

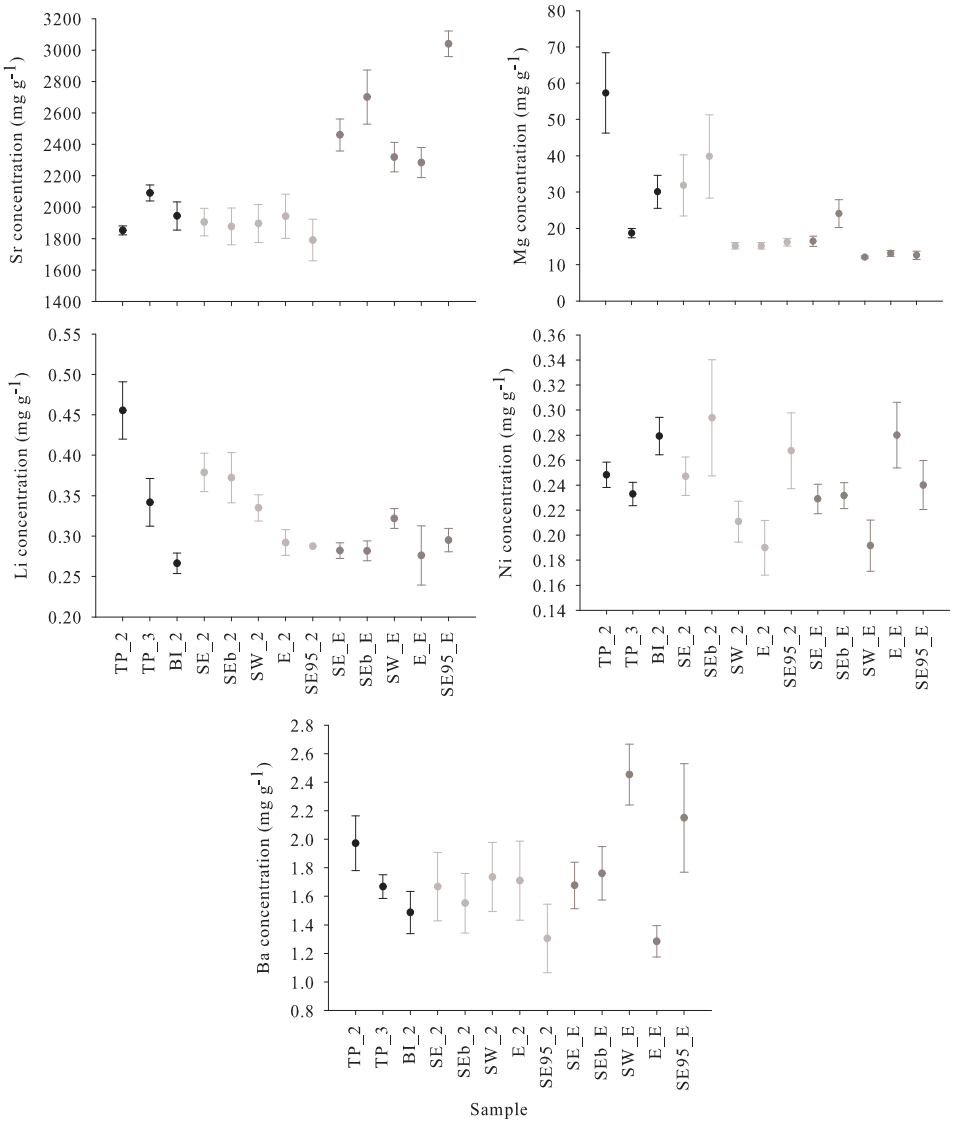

Figure 6.3: Sample means (± SE) of each of the six elements above detection limits, See Table .1 for sample descriptions. Sample descriptions ending in (_2) indicate that the ablation was conducted on the 2nd annual increment, while (_E) indicates that the ablation was conducted on the growing edge of the otolith.

6.3.3 Inshore temporal and spatial variability

To assess inshore temporal stability in elemental concentrations, otoliths from two-year-olds (n = 11) were compared with three-year-olds (n = 15). A discriminant function was able to accurately separate the two groups (Wilk's $\lambda : F_{[2,22]} = 0.518, p < 0.001$). A univariate test showed that there were significant variations between years for both Li (p = 0.029) and Sr (p = 0.003). The jackknife classification correctly classified 81.8% (n = 9) of two-year-olds and 80% (n = 12) of the three-year-olds, with an overall classification accuracy of 80.8% (Fig. 6.5a).

To identify inshore spatial stability in elemental concentrations the otoliths of two-year-olds (n= 11) from the Tasman Peninsula were compared with fish of the same age (n = 12) from Bruny Island, these two 'inshore habitats' are approximately 55 km apart. A discriminant function was able to accurately separate the two groups (Wilk's $\lambda : F_{[2,21]} = 0.454, p < 0.001$). A univariate test showed that there was significant variation between locations for Li (p = 0.008), however, there was no significant difference in Sr concentration (p = 0.359). The jackknife classification correctly classified 63.6% (n = 7) of fish from the Tasman Peninsula and 83.3% (n = 10) from Bruny Island, with an overall classification accuracy of 73.9% (Fig. 6.5b).

6.3.4 Offshore temporal and spatial variability

To assess temporal stability of the microchemical signature on the growing edge of adult striped trumpeter from offshore reefs we sampled otoliths from five-year-olds (n = 9) and compared these results with six-year-olds (n = 13) from the same area sampled approximately one year later. A discriminant function was not able to statistically separate the two groups (Wilk's $\lambda : F_{[2,19]} = 0.970, p = 0.750$). Univariate tests showed no significant variations between ages for either Li (p = 0.473), or Sr (p = 0.812). The jackknife classification could not correctly classify any five-year-olds and only 69.2% (n = 9) of the six-year-olds were correctly classified, with an overall classification accuracy of 40.9%.

To assess spatial stability of the signatures on the growing edge of individuals caught on the offshore reefs, six-year-olds from the southeast (n = 12) were compared with fish of the same age from the southwest (n = 12) and the east coasts (n = 5) of Tasmania. A discriminant function was not able to statistically separate the three regions on either the first (Wilk's $\lambda : F_{[4,25]} = 0.701, p = 0.060$) or second (Wilk's $\lambda : F_{[1,28]} = 0.953, p = 0.266$) canonical axis. Univariate tests showed that there was no significant variations between locations for either Li (p = 0.169), or Sr (p = 0.143). The jackknife classification could not correctly classify any fish from the east coast, 75% (n = 9) from the southeast and 66.7% (n = 8) from the southwest coasts, with an overall classification accuracy of 58.6% (Fig. 6.6).

6.3.5 Contribution of inshore/offshore juvenile habitats

A significant difference was detected between the two-year-olds from the 1993 cohort caught inshore and the adults caught in the same year offshore (Wilk's $\lambda : F_{[2,25]} = 86.47, p < 0.0001$). A canonical plot shows that the offshore sample from 1995 was more closely aligned with the offshore samples collected four and five years later than the inshore samples caught in the same year (Fig. 6.7). These results provide no evidence for temporal variability that could account for misclassification when hind-casting adults from the 1993 cohort.

In order to assess the relative contribution to the adult population that was derived from the inshore reefs, the two 2^+ increment inshore samples and the four offshore edge samples were pooled into two baseline groups; even though the trace element signatures of the inshore samples were deemed significantly different it was assumed that the differential gradient between the inshore and offshore signatures would out-weigh within group differences. The inshore temporal sample (TP_3) was excluded from this analysis as we only analysed the microchemical signature for the 2^+ increment region in the adult samples. This created three groups for back-classification analysis; one representing the inshore signal of two year old fish (n = 23), the second representing the offshore signal (ablated from

the growing edge of the otolith) for the offshore adult fish (n = 39), and a third representing the signal from the 2^+ growth increment zone for the offshore adult fish (n = 39).

The elemental signature of juveniles collected inshore had a higher mean concentration of Li (0.326 $\pm$ 0.026 μg g^{-1}) and a lower mean concentration of Sr (2.029 $\pm$ 0.052 mg g^{-1}) compared with the signature from the growing edge of adult otoliths collected from deeper reefs (0.292 $\pm$ 0.008 μg $^{-1}$ and 2.467 $\pm$ 0.070 mg $^{-1}$, respectively) (Fig. 6.7). Univariate tests showed highly significant variation between groups for Li (p = 0.002), and Sr ($p < 0.001$). Based on these differences in elemental concentrations a descriptive linear discriminant function was able to accurately separate the two groups (Wilk's $\lambda : F_{[2,60]} = 0.488, p < 0.001$). The jackknife classification correctly classified 82.6% (n = 19) of the inshore group and 92.3% (n = 36) from the offshore group, with an overall classification accuracy of 88.7%.

Cumulative normal distributions were generated from both the inshore (mean = -1.31, SD = 0.89) and offshore (mean = 0.78, SD = 1.06) canonical discriminant scores derived from each group, p = 0.05 was calculated as 0.3 and -1.0 for each group respectively. Discriminant scores were then calculated for the group representing the 2^+ increment signatures from offshore adults using the coefficients estimated for the first two groups and substituting them into the equation, $D = 44.359 - 9.282Li + 6.093Sr$. Posterior probabilities were generated from these scores as to group membership to the inshore and offshore groups. The percentage allocation to each group for the 2^+ increment in the adult sample was calculated as 70% (n = 27) and 13% (n = 5) for shallow and deep signatures respectively, the remaining 17% (n = 6) could not be statistically assigned to either group with greater than 75% confidence.

6.4 Discussion

The main objective of this study was to assess the importance of shallow inshore reefs as a nursery habitat for striped trumpeter using otolith microchemistry. The fundamental

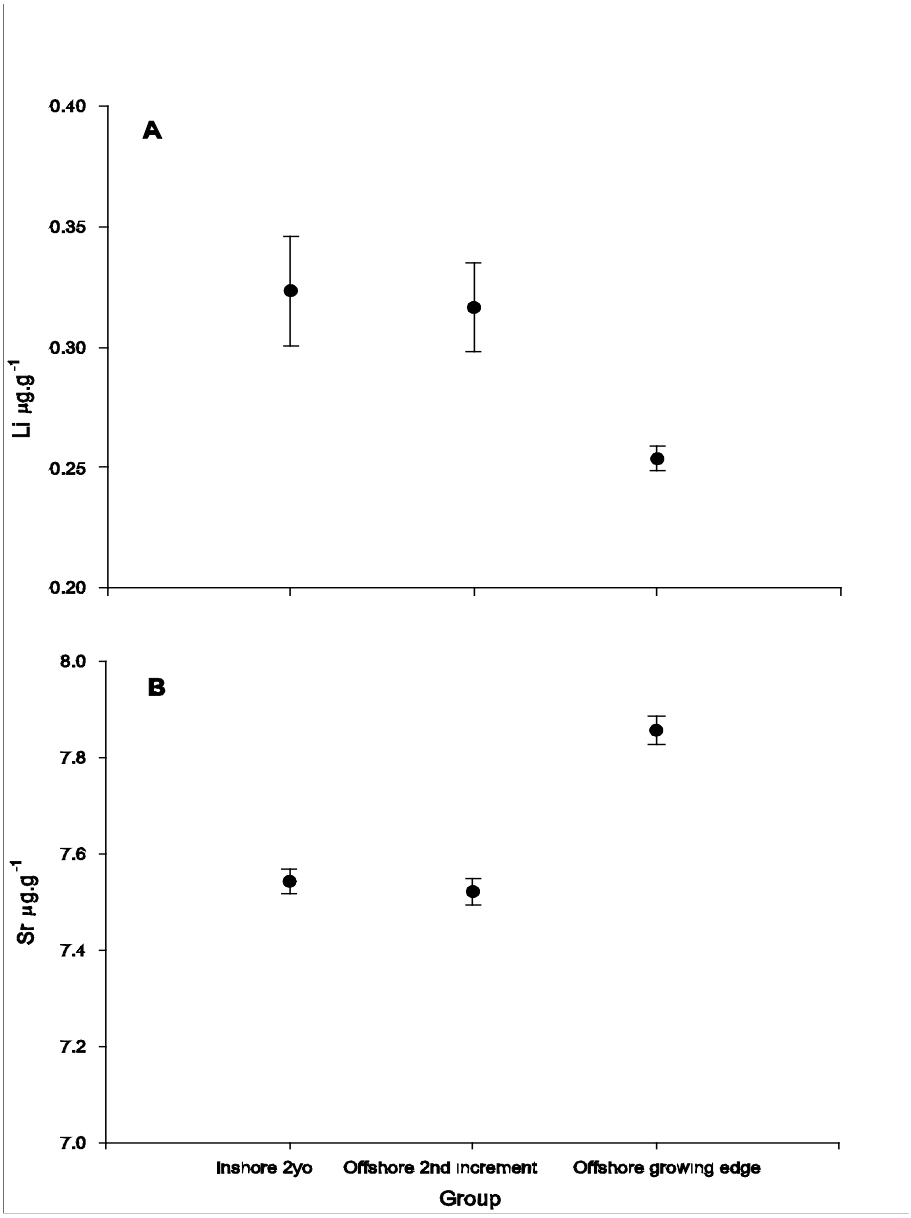

Figure 6.4: Mean (± SE) elemental concentration of (A) Li and (B) Sr, for the three pooled test groups. Concentrations Ln (x +1) transformed.

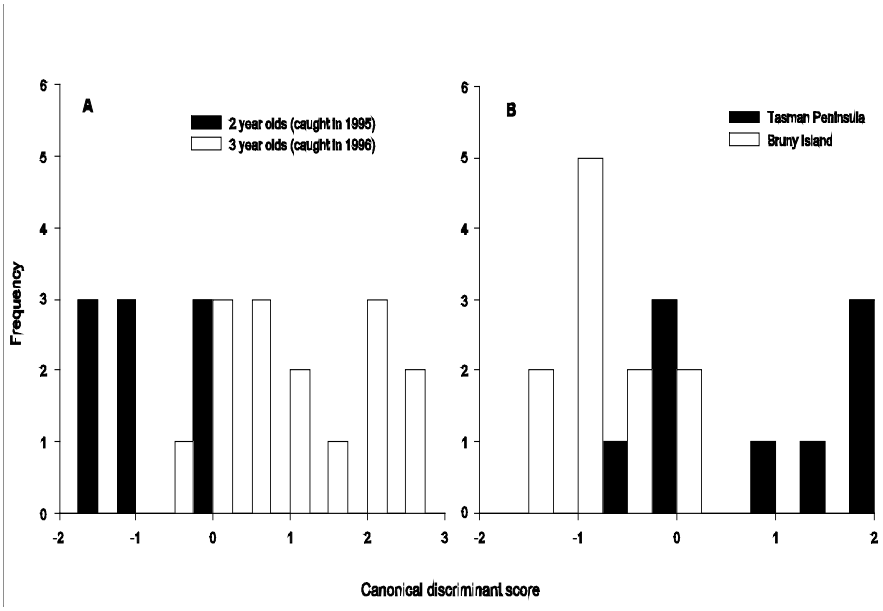

Figure 6.5: Plots of trace element signatures (^{7}Li, ^{88}Sr) from otoliths of striped trumpeter, canonical scores determined by discriminant function analyses, data were ln $(x + 1)$ transformed. (a) Illustrates the results of a temporal test between ablations conducted on the growing edge of otoliths from juveniles collected from shallow inshore reefs on the Tasman peninsula in 1995/96 (age = 2 years) and 1996/97 (age = 3 years); and, (b) the results of a spatial test between ablations conducted on the growing edge of otoliths from juveniles collected in 1995/96 from the Tasman Peninsula and Bruny Island.

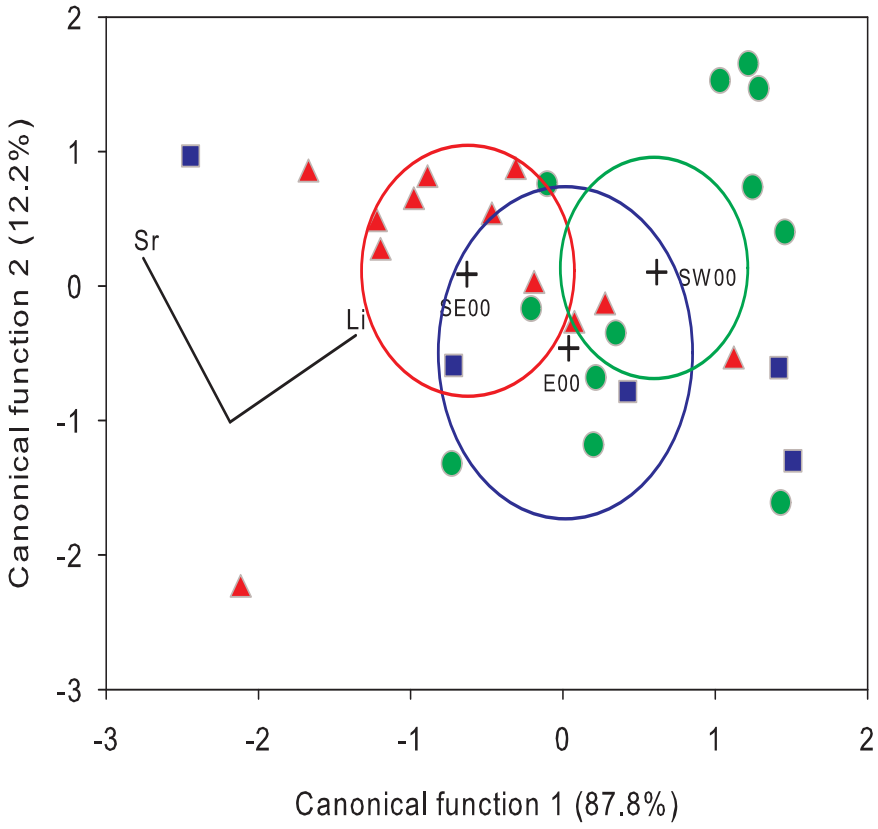

Figure 6.6: Ordination plot of trace element signatures (^{7}Li, ^{88}Sr) from spatially segregated adult striped trumpeter samples, canonical scores determined by discriminant function analyses, data were ln (x + 1) transformed. Samples were collected in 1999/00 at offshore reefs from the (1) southeast [red triangle], (2) east [blue square], and southwest coasts of Tasmania [green circle]. Group centroids are represented by the symbol [+] and ellipses represent 95% confidence limits around the mean. Numbers in parentheses on axis titles show the percent of variance explained by that axis discriminant function.

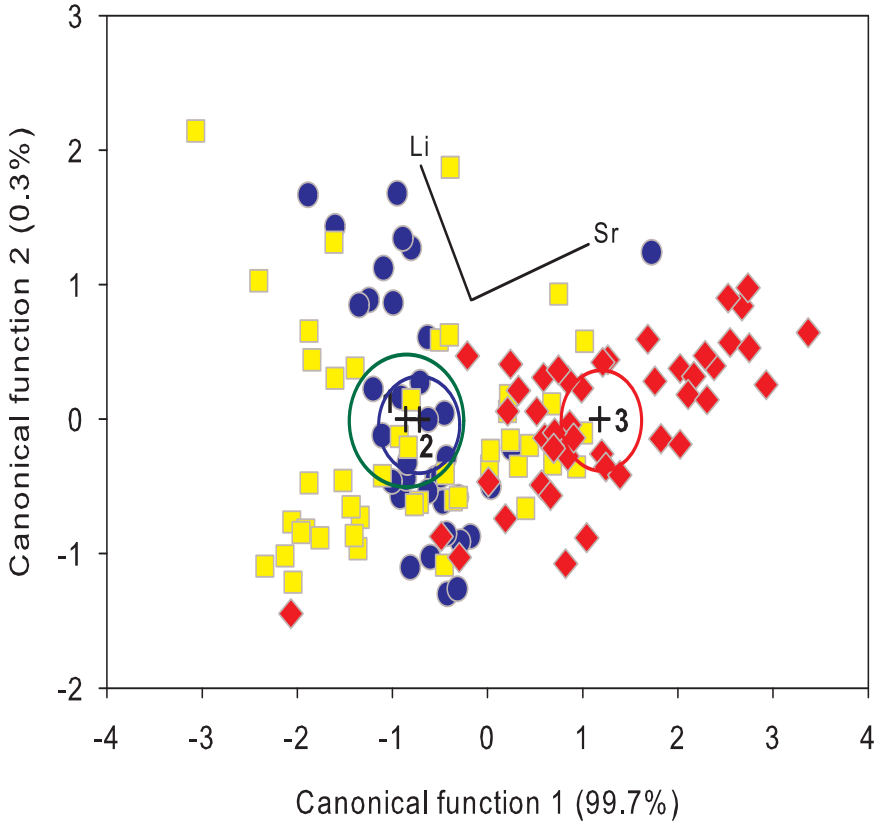

Figure 6.7: Ordination plot of trace element signatures (^{7}Li, ^{88}Sr) from the pooled groups, canonical scores determined by discriminant function analyses, data were ln (x+1) transformed. Groups are categorised as (1) juveniles at age two-years on the growing edge (2nd increment) [yellow squares], (2) Adults on the 2nd increment [blue circles] and (3) Adults on the growing edge [red diamonds]. Group centroids are represented by the symbol [+]. Numbers in parentheses on axis titles show the percent of variance explained by that axis discriminant function.

assumption underpinning this approach was that significant variation exists in the levels of trace elements deposited to otoliths of fish residing at either shallow inshore or deeper offshore reef habitats, and that these trace element signatures remain stable in the otolith matrix over time. Site-specific elemental compositions within otoliths can be influenced by a number of factors, including variation in elemental concentrations in seawater, water temperature, salinity, and genetics (see Campana (1999) for review of confounding factors). The ontogenic migration from shallow inshore to deeper offshore reefs means that striped trumpeter traverse the thermocline, typically found at approximately 50 – 150m depth (Hobday et al., 2006) (Fig. 6.1). Correlations between Sr concentration and ambient water temperature have been identified in the calcified tissue for a range of marine organisms, such as; corals (Smith et al., 1979), bivalves (Masuda, 1976), squid (Ikeda et al., 1999) and fishes (Gauldie et al., 1986), so not surprisingly Sr was the variable that was identified as having the greatest discriminating power between chemical composition of otoliths collected from either the shallow inshore or deep offshore habitats.

The proximity of the shallow inshore reef habitats to the coast also means that the otolith microchemistry of fish caught in such areas are more likely to be influenced by anthropogenic factors and geochemistry related to landmasses than those fish caught offshore in deeper water. However, for studies that establish connectivity between juvenile and adult habitats it is not necessary to know what processes could be contributing to observed differences but rather that the differences do exist (Campana & Thorrold, 2001).

There are some important considerations that need to be addressed when applying otolith microchemistry to determine associations with different habitats. Possible temporal variability in elemental signatures within an area need to be assessed as samples taken in one particular time period might not be representative of samples taken from the same area during another period (Gillanders, 2002). In this study no temporal or spatial variation was evident in the offshore signatures. There was, however, some temporal and spatial variability in the signatures derived from the shallow samples, with correct classification estimated as 81 and 74% respectively. By focusing ablations on the 2^{+} increment of otoliths from

the adult offshore samples we were able to eliminate the temporal effect on otolith microchemistry. As for spatial variability, we have assumed, and confirmed through this study, that the shallow-deep comparison would result in far greater variation in the elemental signatures than any spatial variation between the samples collected from the shallow habitats. However, one assumption that has not been addressed in this study, and as such is a limitation to the results, is the effect of ontogenic change in otolith microchemistry. We have used adults to generate an offshore signal from the time that the juveniles were collected inshore, rather than juveniles from offshore. So, there was no direct way to assess whether an ontogenic change in elemental composition takes place over the four year period between two and size years of age. Previous studies have shown that in some cases this ontogenic effect can be pronounced and have a confounding effect on subsequent results (Ludsin et al., 2004). Another limiting factor of this study is the small sample sizes used in some of the statistical analysis, this can have an adverse effect on statistical probabilities and needs to be considered when interpreting the results.

Using the elements Li and Sr it was possible to predict juvenile membership to either shallow or deep habitats for 83% of the adults tested. Although sample sizes are small, the evidence for the significance of the inshore reefs to subsequent recruitment to the adult population is compelling (implying at least 70% of the adults derived from inshore reef areas). Since gillnet fishing effort is focussed on inshore reefs and as such fishing mortality was likely to be higher on the inshore segment of the juvenile population, the actual significance of inshore areas may well be underestimated (due to removals prior to recruitment offshore). We conclude that the shallow inshore reefs were important as a juvenile habitat for the strong 1993 recruitment pulse of striped trumpeter.

The inshore reefs of Tasmania, characterised by complex geomorphic systems abundant with kelp beds and a diverse array of fauna can be categorised as nursery habitats for striped trumpeter and more than likely provide a similar role for a large number of other species, for example the confamilial bastard trumpeter (*Latridopsis forsteri*) which is commercially exploited and also abundant as immature juveniles on inshore reefs (Murphy &

Lyle, 1999).

Consideration of nursery habitats is necessary for the development of ecosystem-level management of fisheries and other coastal resources (Beck et al., 2001). Around Tasmania there has been a long-term decline in the abundance of the dominant kelp species Macrocystis pyrifera on inshore reefs (Edyvane, 2003). A positive correlation between kelp abundance on reefs (in particular *Macrocystis spp.*) and the abundance of juvenile fish has been described from inshore habitats in the northern hemisphere (DeMartini & Roberts, 1990). In addition, urchin barrens have become established in some areas off the east coast of Tasmania through the range expansion of *Centrostephanus rodgersii*, which has dramatically reduced the diversity of marine flora and fauna at affected sites (Johnson et al., 2005). The potential impacts of climate induced range shifts on the spatial structuring of the biological components of a reef are an important but understudied issue (Edgar et al., 2005). The compounding nature of these threats raises concern about the ongoing health of Tasmania's inshore reef systems and their resilience to perturbations.

There is a suite of spatial management measures available to protect the juvenile population and inshore reef habitats through sectoral fisheries management and biodiversity conservation arrangements. Integrating the protection of inshore reef habitats important to multiple species, or nursery/habitat 'hot spots' into marine planning and broader fisheries reform and ongoing management regimes, would be a step towards a more ecosystem based fisheries management strategy.

In the case of striped trumpeter the recent implementation of a 450 mm minimum size limit in Tasmania has significantly increased the protection of the crucial juvenile life stage from the gillnet fishing that occurs over shallow inshore reefs. Ongoing monitoring of the populations in conjunction with further implementation of environmental regulations when required, to ensure these inshore reef habitats remain of adequate quality to provide suitable habitat for reef fish is a positive direction for fisheries management in Tasmania.

Chapter 7

Putting the ‘Bio’ into a bio-physical larval disperal model

Abstract. The larval dispersal phase of fish species can have significant effects on the population structure of marine systems. These patterns of dispersal and the factors influencing recruitment success are poorly understood for many marine species. To date most models of larval dispersal have focused on advective processes driven by oceanographic features but have lacked sufficient data to couple biological responses of the larvae to ocean variables such as temperature. This is primarily due to a lack of relevant biological knowledge for key parameters for the particular species. In this study we develop a hydrodynamic dispersal model and couple it with data gained through research into the spawning behaviour from the wild striped trumpeter fishery, and temperature-based responses to growth and mortality from extensive aquaculture trials for the striped trumpeter larvae. Comparisons of modeled versus measured recruitment success indicates that the model is unable to recreate the observed peaks in interannual recruitment success. However, the model did provide insight into the importance of the timing and intensity of the spawning season as well as the effects of temperature on larval growth and survival.

7.1 Introduction

Variability in the recruitment of the pelagic young of marine fishes, which is often extreme, has major biological effects and substantial implications for management of fish stocks (Rothschild, 1986). Such temporal fluctuations in settlement of post-larvae to nursery habitats can significantly affect the size and structure of adult populations (e.g. Thresher et al, 1989). In fisheries models such variability is difficult to explain as generally little is known about the physical and biological processes controlling recruitment patterns, and as such is a significant source of uncertainty when modelling population dynamics. In recent years attention has been directed toward understanding the role of recruitment in structuring marine populations (Carr, 2000; Sponaugle & Cowen, 1997; Roughgarden et al., 1988). However, the process of recruitment in marine organisms is a complex, multidimensional phenomenon. The complexity of the recruitment process is emphasised by the myriad of factors that influence recruitment success; such as the size of the adult spawning biomass, environmental conditions associated with spawning, the effects of ocean currents on larval dispersal, and temperature and prey availability on growth and survival, (for review see Sponaugle & Cowen, 1997). The majority of these factors have some degree of bio-physical coupling that can contribute in either a positive or a negative manner. Through advances in remote sensing technology and computational power it is now possible to build models that provide a mechanistic understanding of marine population connectivity by resolving the biological and physical processes involved in larval dispersal and transport, (e.g. Griffin et al., 2001; Chiswell et al., 2003; Paris & Cowen, 2004).

Past studies of larval recruitment processes have focused on the physical factors that either advect or retain larvae. More recently, there is a growing body of evidence suggesting that biological components may have a greater effect on the proportion of propagules successfully recruiting to a population than the vagaries of the physical environment. For example, growth rates of pelagic larvae have been argued to be one of the fundamental determinants of the recruitment success of temperate marine fish (Anderson, 1988; Miller

et al., 1988; Cushing & Horwood, 1994). This supports the growth-predation hypothesis in which larval mortality is size-dependant (Bergenius et al., 2002). The logical next step of modelling the recruitment processes of pelagic larvae is to integrate biological modules into models designed to simulate particle dispersal, including coupling knowledge of larval growth and survival with physical properties such as temperature.

Striped trumpeter (*Latris lineata*) is a long-lived demersal teleost, distributed around several southern hemisphere temperate island groups and continents (Higgins et al., 1994). The species is found throughout southern Australia and constitutes a small but iconic fishery around Tasmania. The striped trumpeter population of Tasmania exhibits a typical dispersive strategy of reef fish, with the potential for larval dispersal over great distances. The duration of the larval-paperfish stage of striped trumpeter is relatively protracted; hatchery-raised larvae require approximately nine months in a simulated ambient environment before a dramatic transformation into the adult morphology (Ruwald, 1992). For wild striped trumpeter this transformation is concurrent with settlement to inshore reefs with a small proportion possibly settling on deeper reefs characteristic of adult habitat (Tracey, Chapter 6). Although few wild larvae have been captured, it is assumed that the eggs and larvae remain in a pelagic/neustonic environment based on their behaviour in aquaria and their morphological form pre-transition.

The recruitment of striped trumpeter to inshore juvenile habitats in Tasmania is highly variable. Over the last decade there has been low recruitment with one exception, an exceptionally strong recruitment pulse back-calculated to the 1993 spawning season (August – November). This cohort was still conspicuous in the striped trumpeter catch in 2006 as 12 year olds (Ziegler et al., 2006).

It is likely that the oceanographic variability around Tasmania, the Tasman Sea and Great Australian Bight combined with the extended pelagic larval stage contributes to the observed recruitment variability. Advection modelling suggests that the strong currents of the region have a major influence on the dispersion of larval fish (Thresher et al., 1988; Young et al., 1993; Bruce et al., 2001) and invertebrates (Chiswell et al., 2003). Environ-

mental conditions have also been shown to influence the recruitment variability of fish in New Zealand at similar latitudes to Tasmania (Francis, 1993).

Tasmania lies in a complex oceanographic and meteorological zone. The shelf waters of eastern Tasmania have a seasonal cycle of warming and cooling. This cycle is primarily driven by the changing position of the subtropical convergence zone separating the East Australian Current (EAC) and the subantarctic waters (Harris et al., 1987). This in turn is linked to westerly wind stress dictated by El Niño-Southern Oscillation events adjusting the continental high-pressure area over Australia (Thresher, 2004; Cai et al., 2005). The EAC forms a potential advective pathway between Australia and New Zealand as it tends to meander eastward across the Tasman Sea at about 35°S where it is commonly known as the Tasman Front (Chiswell et al., 2003).

An understanding of the factors influencing recruitment variability would be of significant benefit to the management of striped trumpeter. Aquaculture trials for striped trumpeter have provided a timely and unique insight into the effects of various abiotic factors on larval biology, and as such this species is a suitable study species for a model integrating larval biology within a physical model.

Thus, the aim of this study is to utilise a bio-physical model that incorporates passive dispersal forced by ocean currents coupled with temperature-dependant estimates of growth and survival for striped trumpeter based on biological data collected from aquaculture trials. The model predictions can be tested against historical recruitment data to assess the performance of the model and determine which factors are having the greatest influence on striped trumpeter recruitment variability. Such a model would have potential as a predictive management tool providing information on recruitment pulses before they enter the fishery allowing for pre-emptive, adaptive management strategies.

7.2 Data source and methods

7.2.1 Model structure

In this study an individual-based bio-physical model is developed (Fig. 7.1). Two stages of the model are relevant here. Stage 1 uses 'virtual larvae parcels' (VLPs) to simulate dispersal and subsequent settlement success of each VLP with limited biological parameters. The second stage of the IBM incorporates the survival of individual larvae within each VLP. Therefore, for stage 1 the VLP can be considered individual particles and in stage 2 the VLP are a collection of individual larvae that have been subjected to the same environmental conditions along their dispersal trajectory. The primary reason for this model structure was to reduce computational investment by reducing the number of individuals yet allow sufficient sample sizes to calculate survival rates.

7.2.2 Hydrographic component and particle transport

The model domain encompasses the known striped trumpeter regions of potential relevance to the Tasmanian population; New Zealand, the Tasman Sea and southeast Australia, including Tasmania (Fig. 7.2). The current fields of the model are generated by geostrophic velocities, calculated from sea surface height (SSH) anomalies added to mean current fields from the CSIRO Atlas of Regional Seas (CARS) dataset (Ridgway et al., 2002). Spatial resolution of the current fields is 0.2°. Virtual larvae parcels are released from coastal spawning nodes and are advanced by a Lagrangian code module developed to track their trajectory. At each time step (daily) a random displacement (10% of current velocity) was added to account for effects of possible physical processes occurring at scales smaller than the resolution of the ocean model.

At the conclusion of each daily timestep a sea-surface temperature (SST) value for each new VLP location was obtained from 3-day composite sea surface temperature (SST) images, derived from NOAA AVHRR LAC data received and processed in Australia by

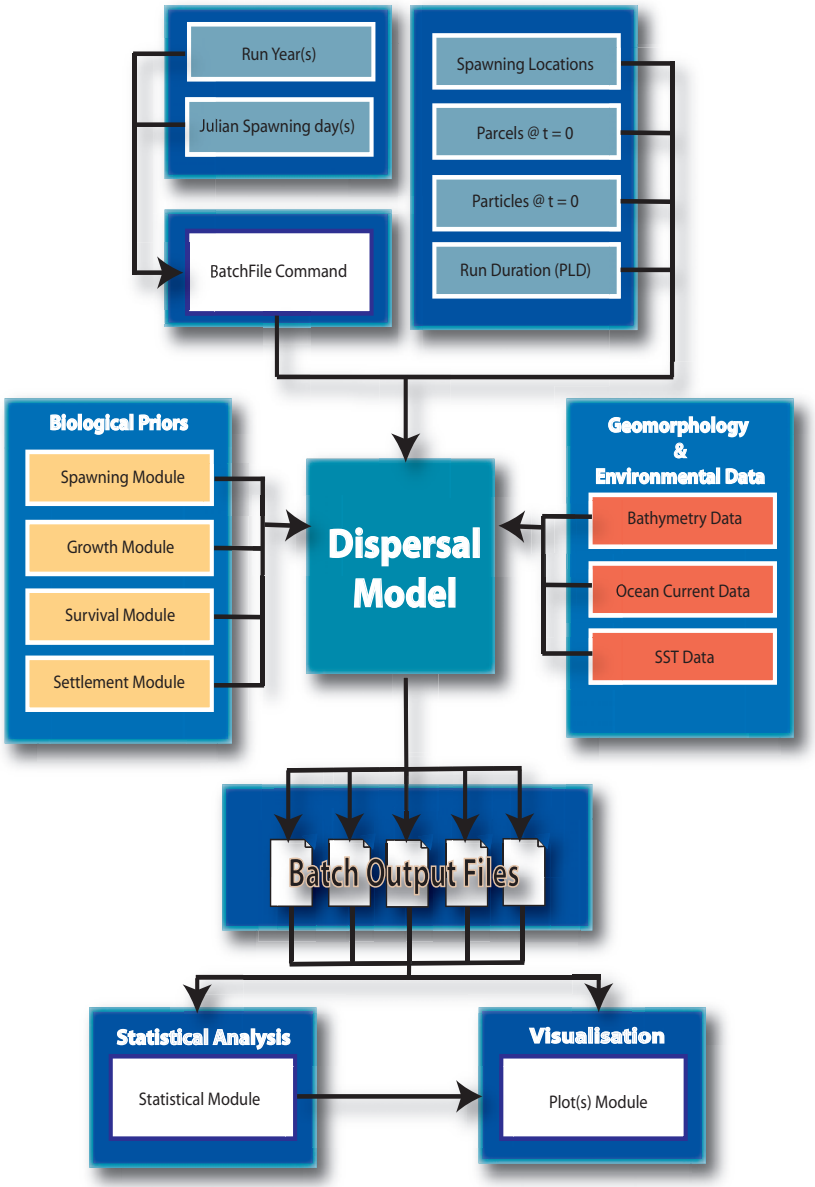

Figure 7.1: Schematic illustrating model structure.

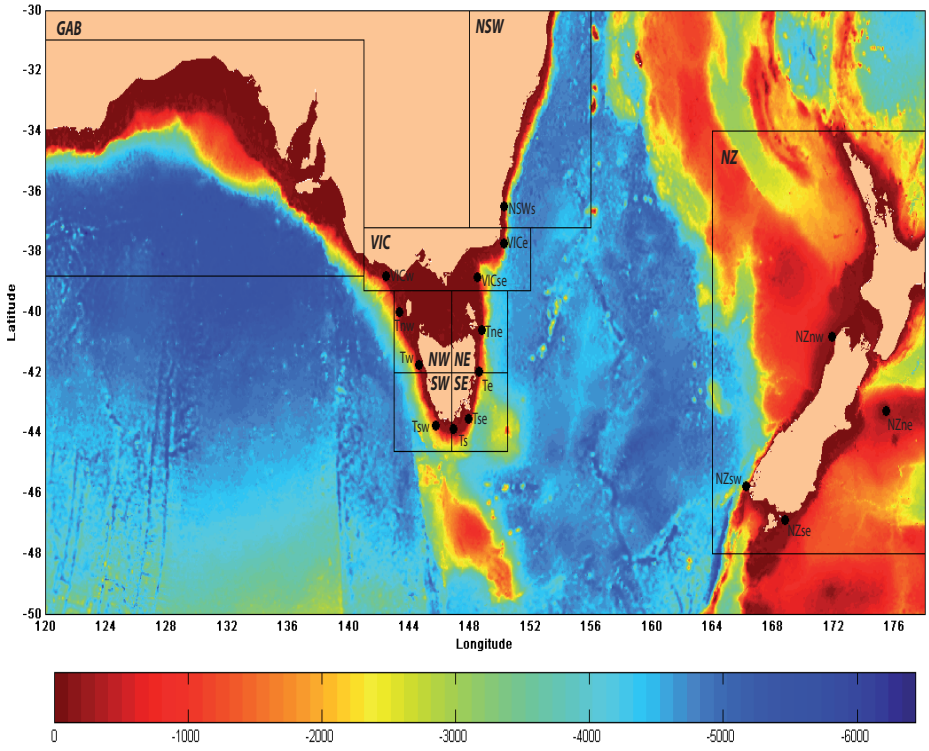

Figure 7.2: The location of the physical model domain showing (i) the sites of virtual larval parcel release (spawning nodes) (●), and (ii) settlement regions [black bounding boxes]. Background colour shows the bathymetric profile of the region, depth increases from red to blue. Acronym labels are as follows: GAB = Great Australian Bight, NSW = New South Wales, VIC = Victoria, NZ = New Zealand, T = Tasmania, and subscripts are acronyms for direction, i.e. ne = north east.

CSIRO[1]. This process is repeated until the VLP either settles, exits the model domain or the model run is completed (at 360 days).

Bathymetric depths for the model domain were obtained by extracting digital bathymetry from the AGSO 2005 dataset. The spatial resolution of this data is 0.01°.

7.2.3 Life history and larval behaviour component

Spawning structure

Striped trumpeter are known to spawn over rocky reef located on the edge of the continental shelf around the east, south and west coasts of Tasmania (Tracey et al., 2007). Adult striped trumpeter are also caught around Victoria, southern New South Wales and New Zealand, although little is known about the spawning locations in these areas. The known information was used to identify spawning nodes (VLP release sites) as shown in Figure 7.2. Around Tasmania the spawning season for striped trumpeter begins in August and continues through to the end of November. The spawning season was modelled using 15 unique release days spread evenly across the spawning season. To weight the number of VLP's to be released from each spawning node, a Gaussian distribution was fitted to the mean temporal trend of the gonadosomatic index (GSI) of Tasmanian striped trumpeter (Fig. 7.3). The number of VLP's released from a particular node was determined by the Gaussian probability at time t relative to the nominally defined maximum number of VLPs released at the time of peak spawning. Several assumptions were made based on the spawning strategy integrated into the model, namely; (1) that there was no inter-annual spatial variability in spawning locations, (2) that there was no inter-annual temporal variability in the timing of the spawning season, and (3) that there was no inter-annual variability in the spawning production of the adult biomass.

[1]Griffin et al., unpublished data

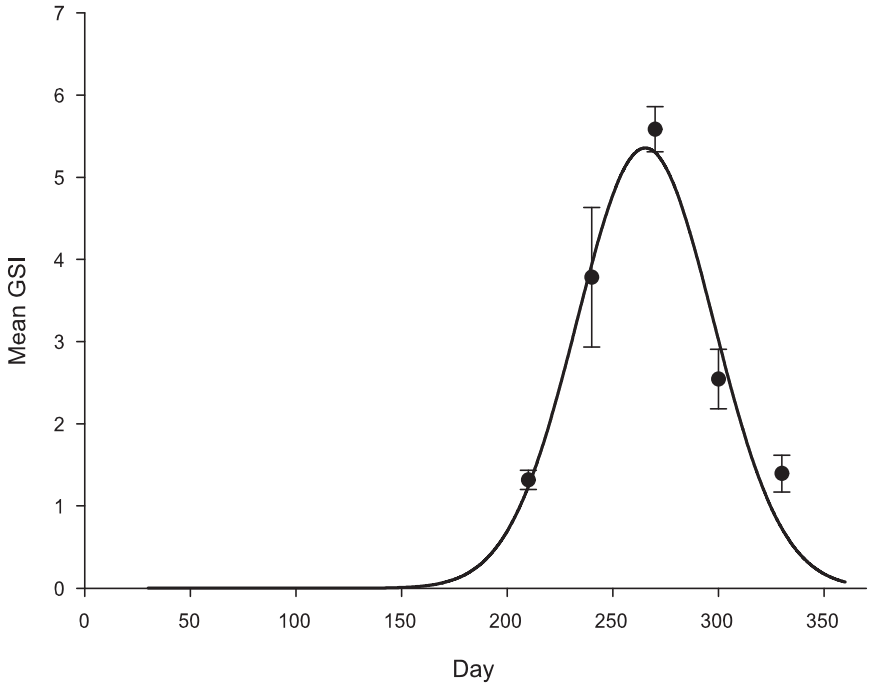

Figure 7.3: Gaussian distribution [N(265.38, 32.34)] fitted to the temporal trend of female striped trumpeter (*Latris lineata*) gonadosomatic values from field sampling in Tasmania (1991 – 2005) n = 161. Error bars represent the standard error of the means.

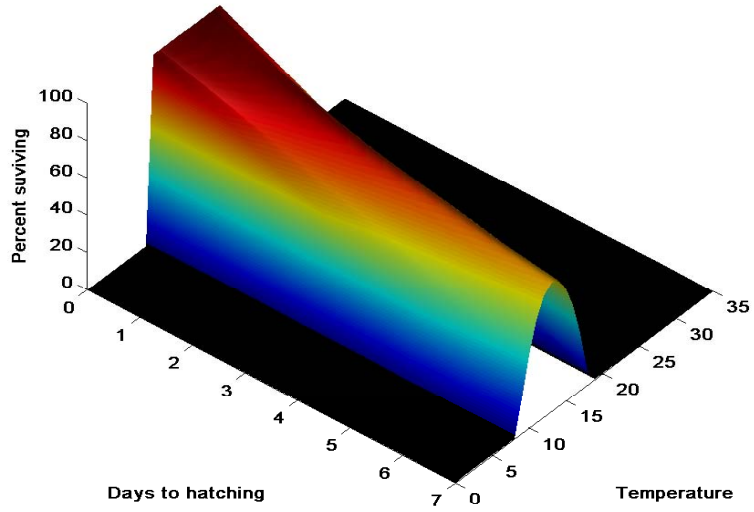

Figure 7.4: Model surface illustrating the percent of eggs surviving on a given day pre-hatch dependant on the ambient temperature experienced. At each temperature the decay decreases linearly to the day of hatching. Note the the embryonic period lasts for seven days

Growth and mortality

Egg mortality was calculated daily, based on a temperature-dependant relationship generated by a suite of hatchery-rearing experiments (Morehead & Hart, 2003):

$$S_{E_t} = 2.7169T_\mu^2 + 78.738T_\mu - 492.92 \tag{7.1}$$

where SE_t is the percent of eggs surviving at time t and T_μ is the sea-surface temperature averaged over the embryonic period (Fig. 7.4).

The length-at-hatching of striped trumpeter larvae was calculated using the 2nd order polynomial temperature-dependant relationship generated by Morehead & Hart (2003):

$$L_H = 0.036T_\mu^2 + 1.013T_\mu - 3.2171 \tag{7.2}$$

where L_H is the length at hatch and T_μ is the sea-surface temperature averaged over the embryonic period. These estimates were used to initiate the growth trajectories at hatching, which occurred after the completion of day seven post-spawning.

A function reflecting the growth of larvae post-hatch was generated by collating data from a range of aquaculture experiments where the effect of temperature on different stages of larval growth were recorded (Battaglene & Cobcroft, In Press). The trajectory of the growth curves were calculated by applying a 2nd order polynomial growth function to the length at age data from an aquaculture trial at 16°C:

$$L_t = 0.0007T^2 + 0.24T - 0.61 \tag{7.3}$$

where L_t is the length at time t and T is the water temperature, in this case held at 16°C. To determine the effect of temperature on growth post-hatch the mean length at day 25 post-hatching was recorded for temperatures ranging from 14 – 18C at 1°C increments, subsequently fitted with a linear relationship, scaled to the slope of Eqn. 7.3 at 16°C:

$$a = 0.015T - 0.135 \tag{7.4}$$

where a is the slope of Eqn. 7.3 and T is the water temperature. This same process was then applied to the curvature of Eqn. 7.3

$$x = 0.00005T - 0.0004 \tag{7.5}$$

where x is the curvature of Eqn.3 and T is the water temperature. The growth model surface is shown in Figure 7.5.

Survival from hatching through to settlement was also based on information from aquaculture trials. A decay equation was fitted to the number of larvae/paperfish surviving at age (t) for larvae reared at 16°C. The effect of temperature (T) was then modelled by fitting a 2^{nd} order polynomial function:

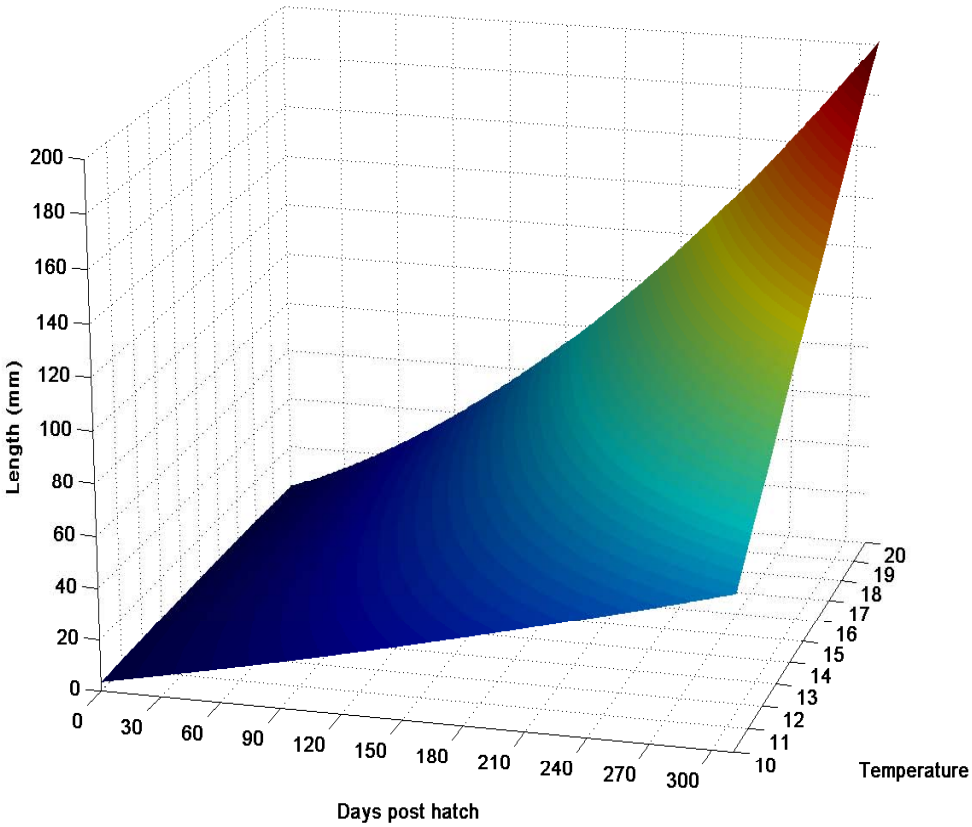

Figure 7.5: Larval growth trajectories of striped trumpeter (*Latris lineata*) predicted for temperatures ranging from 10 – 20°C in the model. Projections are based on hatchery-rearing trials of striped trumpeter larvae.

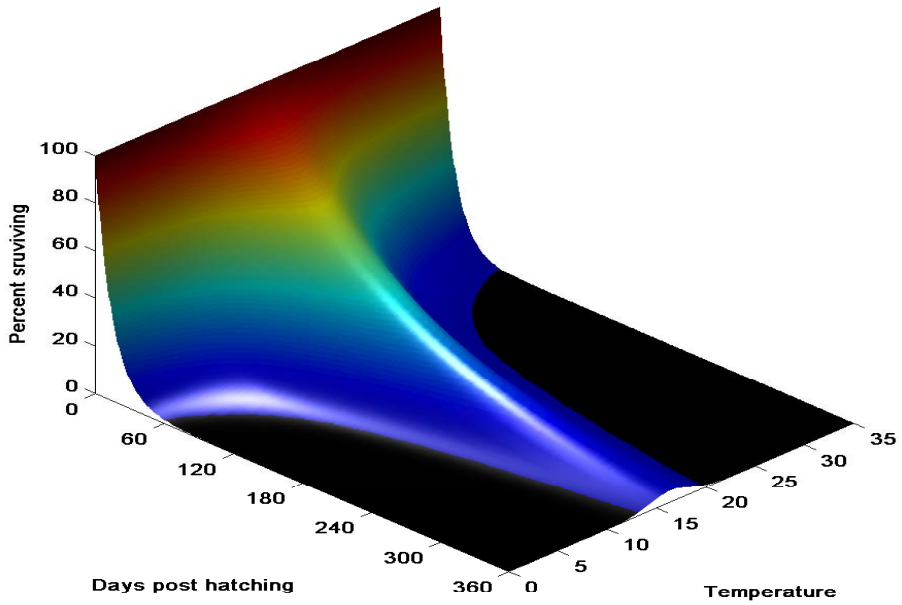

Figure 7.6: Model surface profile for the percent of individuals surviving at a given day post-hatch, assuming a constant temperature profile through time. A 2^{nd} order polynomial function explains the relationship between temperature (T) and decay rate (Z); $Z = 0.0002T^2 - 0.0056T + 0.0557$. The highest survival rate is experienced at 16°C with a decay rate of 0.00825 day^{-1}.

$$M_t = 0.0002T^2 - 0.0056T + 0.0557 \tag{7.6}$$

Where M_t is the percent remaining after time t (Fig.7.6).

7.2.4 Competence and retention

Flexion of the larval notochord and development of the caudal fin in striped trumpeter larvae occurs at approximately day 30 post-hatch (Battagelene et al., *in press*). This event is considered a point of reference for the onset of active behaviour due to increased swimming ability (Fisher et al., 2000). In this model we assume passive migration up until this 'age at competency', beyond this age passive migration continues until a parcel advects into a

coastal zone.

Once a VLP had passed the age at competency (day 30) it was able to actively retain itself if in the proximity of a suitable habitat. A suitable habitat was defined using a depth-stratified function. Striped trumpeter are found in depths ranging from 10 – 300m (Last et al., 1983), with inshore reefs, 10 – 50m in depth, known as nursery habitat for juveniles (Tracey & Lyle, 2005). Hence a logistic function modelled the increase in the probability of active retention as depth decreased:

$$R_{t,j} = \frac{e^{2.182+0.01D_t}}{1+e^{2.182+0.01D_t}} \tag{7.7}$$

where $R_{t,j}$ is the probability of active retention occurring at time t and location j and $D_{t,j}$ is the water depth at location j. Parcels that were retained in a coastal area were not advected at each time step, but were subject to the temporally changing environmental conditions (SST) of that location until settlement.

Settlement length was determined by measuring one hundred and twenty eight 310-day old striped trumpeter reared for aquaculture. The morphological characteristics of these fish ranged from a pre-settlement form (paperfish) to completely transformed adult morphology (Fig. 7.7). Form was categorised into 4 stages: (1) paperfish (Fig. 7.8a), (2) early to mid-transition, (3) mid- to late-transition, and (4) completed transition (Fig. 7.8b). Length to the nearest mm and weight to the nearest gram were also recorded. A cumulative Gaussian distribution fitted to the length distribution of fish that had begun the transformation to adult morphology ($>$ stage 2) was used to determine the probability of a VLP settling.

7.2.5 IBM 'simulated' recruitment index

A VLP contributed to the model recruitment index if it satisfied both the depth-based active retention and larvae length-based settlement before the end of the model run. Such VLP were assigned to pre-defined settlement polygons (Fig. 7.2). The sum of VLPs within each

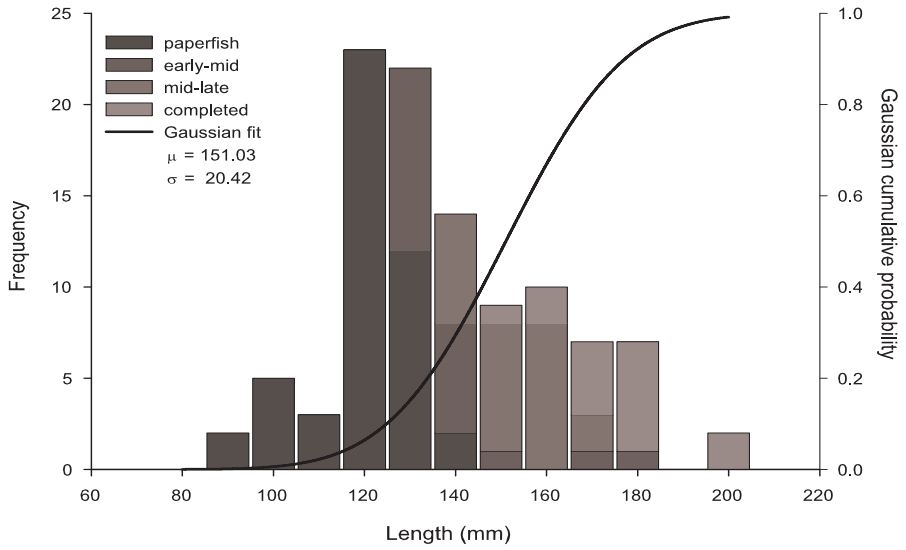

Figure 7.7: Distribution of fork lengths of hatchery-reared striped trumpeter (*Latris lineata*) paperfish, stacked by developmental stage (explained on figure). A cumulative Gaussian distribution is fitted to the length frequency data of those fish that have begun the transformation to an adult morphology. μ = mean, σ = 1 standard deviation.

Table 7.1: The variables involved in each stage of the Individual Based Model (IBM) of *Latris lineata* larval dispersal. Stage 1 uses virtual larvae parcels to simulate dispersal with limited biological parameters. Stage 2 extends on stage 1 by estimating survival of individuals within each virtual larvae parcel.

Variable	Stage 1	Stage 2
Temperature-dependant egg mortality	no	yes
Temperature-dependant larval growth	yes	yes
Temperature-dependant larval mortality	no	yes
Age-based active retention behaviour	yes	yes
Larval length-based settlement behaviour	yes	yes

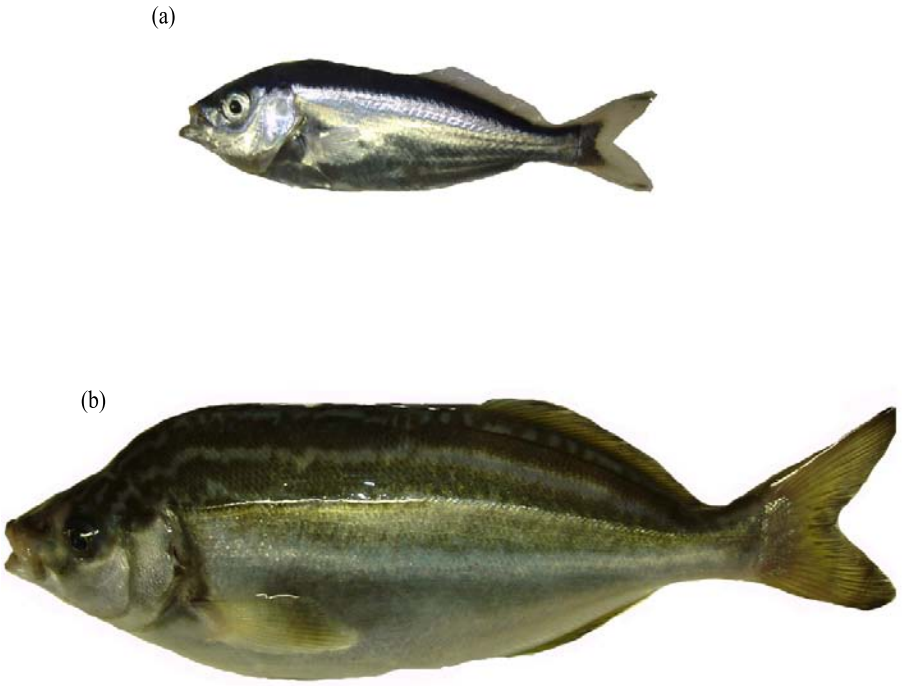

Figure 7.8: (a) A 97 mm striped trumpeter showing paperfish morphology, (b) A 164 mm striped trumpeter showing juvenile morphology.

settlement polygon represented the recruitment index to each area under stage 1 of the IBM, and similarly the sum of the number of individuals surviving from all VLPs settled within an area generated the simulated recruitment index for stage 2 of the IBM.

7.2.6 VPA 'observed' recruitment index

A virtual population analysis (VPA) was applied to ascertain the annual recruitment of striped trumpeter between 1990 and 1998. This model was constrained to only fish caught offshore using hook and line methods, because the variability in inshore gillnet catches was considered too 'patchy' and would bias the annual recruit indices[2]. Based on the age-at-recruitment to the offshore fishery only fish older than four years were included in the model (Tracey & Lyle, 2005). Five hundred and four striped trumpeter from the offshore aggregations have been successfully aged via the ring structure of the sagittal otoliths over this period. This data was converted to a proportion of individuals caught from a year for age classes four to fifteen, with a 'year' assigned as the biological year determined for striped trumpeter (October to September). The data was then subjected to the VPA model using the mortality estimates (F = 0.096 yr^{-1} and M = 0.096 yr^{-1}) determined by Tracey & Lyle (2005). This index was then used to compare the results of both stage 1 and 2 of the IBM recruitment indices.

7.3 Results

7.3.1 VPA 'observed' recruitment index trend

The most prominent feature of the VPA recruitment index is the exceptionally strong recruitment pulse from 1993 and to a lesser extent the pulse from 1994. For the years after these two events recruitment was consistently low with the exception of a small increase from 1996 (Fig. 7.9). The years prior to 1993 are of little consequence to this analysis as

[2]Lyle, personal communication

the IBM could not simulate these years because of temporal limits in the remote-sensed physical data (altimetry), i.e. only available post-1993.

7.3.2 IBM 'simulated' recruitment index trend

The percent of settling VLPs across Australia and New Zealand ranged from 23.5 to 34.3% of those initially released each year (Table 7.2). Using a rule of thirds to categorise recruitment variability to Tasmania, stage 1 of the IBM predicted a strong recruitment pulse in 1999, medium recruitment from 1994, 1995, 2000 and 2001, and poor recruitment from 1993, 1996, 1997 and 1998 (Fig. 7.9). These results are discordant with the observed VPA recruitment index, particularly in reference to the significant recruitment pulses back-calculated to the spawning seasons of 1993 and 1994. Stage 2 of the model produced a similar temporal trend to that generated by stage 1 of the model, with the exception of a decrease in surviving recruits in the years 1993, 1994, and 1995. Again using a rule of thirds, 1994 and 1995 are years of poor recruitment according to the results of stage 2 (Fig. 7.9). The range of temporal variability between years was low for the results of stage 1. When the results were standardized the temporal variability was only 51% of the range of the observed recruitment index. Stage 2 of the model showed an improvement on this result, with 68% of the variability of the recruitment index.

The spatial pattern of settlement was relatively consistent for all years for which simulations were performed. The predominant spatial inter-annual variability of VLP settlement is in the extent of advection west of Victoria into the Great Australian Bight. This occurred in both 1997 and 2000 and to a lesser extent in 1993 (Fig. 7.10–7.13). The extent of dispersal northward along the east coast of Australia also varied from year to year. In 1994 there was increased advection of VLPs to the northern NSW coast, for all other years there were low numbers of parcels dispersed northward of the spawning node located in NSW. The South Island of New Zealand also showed minor inter-annual variability, with 1994 being quite a poor year (Fig. 7.10).

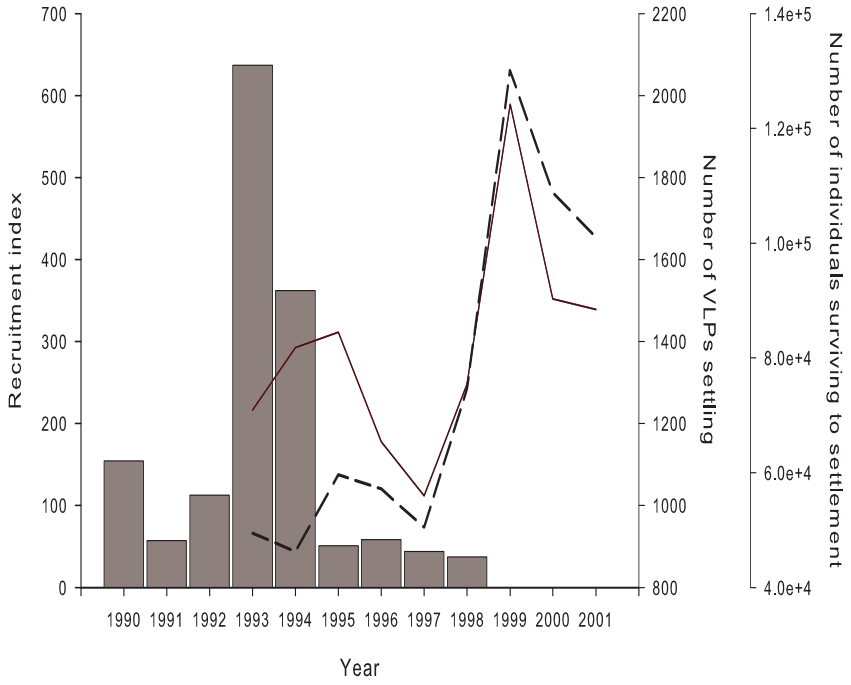

Figure 7.9: Annual trend in the virtual population analysis (VPA) derived 'observed' recruitment index of striped trumpeter (*Latris lineata*) (columns). The solid line represents the simulated recruitment index of settling virtual larval parcels (VLPs) explained by stage 1 of the IBM larval dispersal model and the dashed line represents the simulated recruitment index of individuals surviving explained by stage 2 of the model. All three indices represent the situation for Tasmania only.

Table 7.2: Results of individual-based bio-physical integrated larval dispersal model of *Latris lineata* larval dispersal. Stage 1 and stage 2 report virtual larval parcels (VLP) and 'individual larvae' successfully settling each year respectively.

	Stage 1 (VLPs)			Stage 2 (Individual larvae)		
Year	No. seeded[a]	No./ (%)settling	No./ (%) settling (TAS)	No. seeded[a]	No./ (%)settling	No./ (%) settling (TAS)
1993	11872	2794 (23.5)	1223 (10.4)	1.187×10^6	1.537×10^5 (12.9)	6.758×10^5 (5.7)
1994	11858	3406 (28.7)	1385 (11.7)	1.186×10^6	1.752×10^5 (14.8)	5.893×10^5 (5.0)
1995	11970	3229 (26.9)	1423 (11.9)	1.197×10^6	1.631×10^5 (13.6)	6.851×10^5 (5.5)
1996	11814	3220 (27.3)	1156 (9.8)	1.181×10^6	1.946×10^5 (16.5)	6.482×10^5 (5.5)
1997	12110	3493 (28.8)	1024 (8.5)	1.211×10^6	2.109×10^5 (17.4)	7.894×10^5 (6.5)
1998	11900	2569 (21.6)	1296 (10.9)	1.190×10^6	1.457×10^5 (12.2)	5.002×10^5 (4.2)
1999	11928	4093 (34.3)	1978 (16.6)	1.193×10^6	2.761×10^5 (23.1)	9.711×10^5 (8.1)
2000	11886	3156 (26.5)	1504 (12.6)	1.189×10^6	2.243×10^5 (18.9)	9.789×10^5 (8.2)
2001	12099	3584 (29.6)	1478 (12.2)	1.210×10^6	2.183×10^5 (18.0)	8.380×10^5 (6.9)
Mean	11937	3282 (27.5)	1385 (11.6)	1.194×10^6	1.958×10^5 (16.4)	7.388×10^5 (6.2)

[a] Cumulative of 15 release days each year

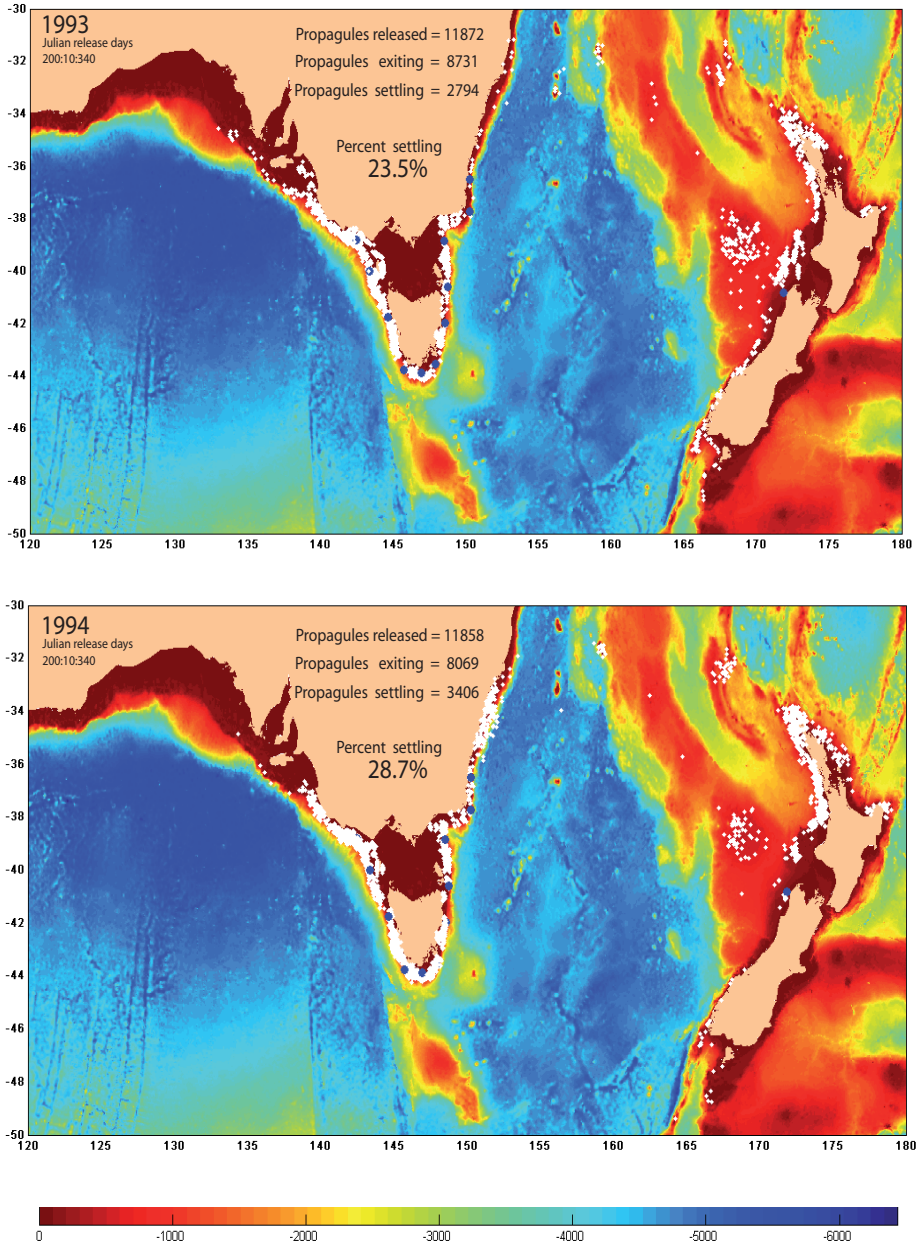

Figure 7.10: Map of study domain, showing parcels successfully settling (white dots) and the spawning nodes that contributed successful propagules (blue dots). The background colour illustrates the bathymetric profile of the region.

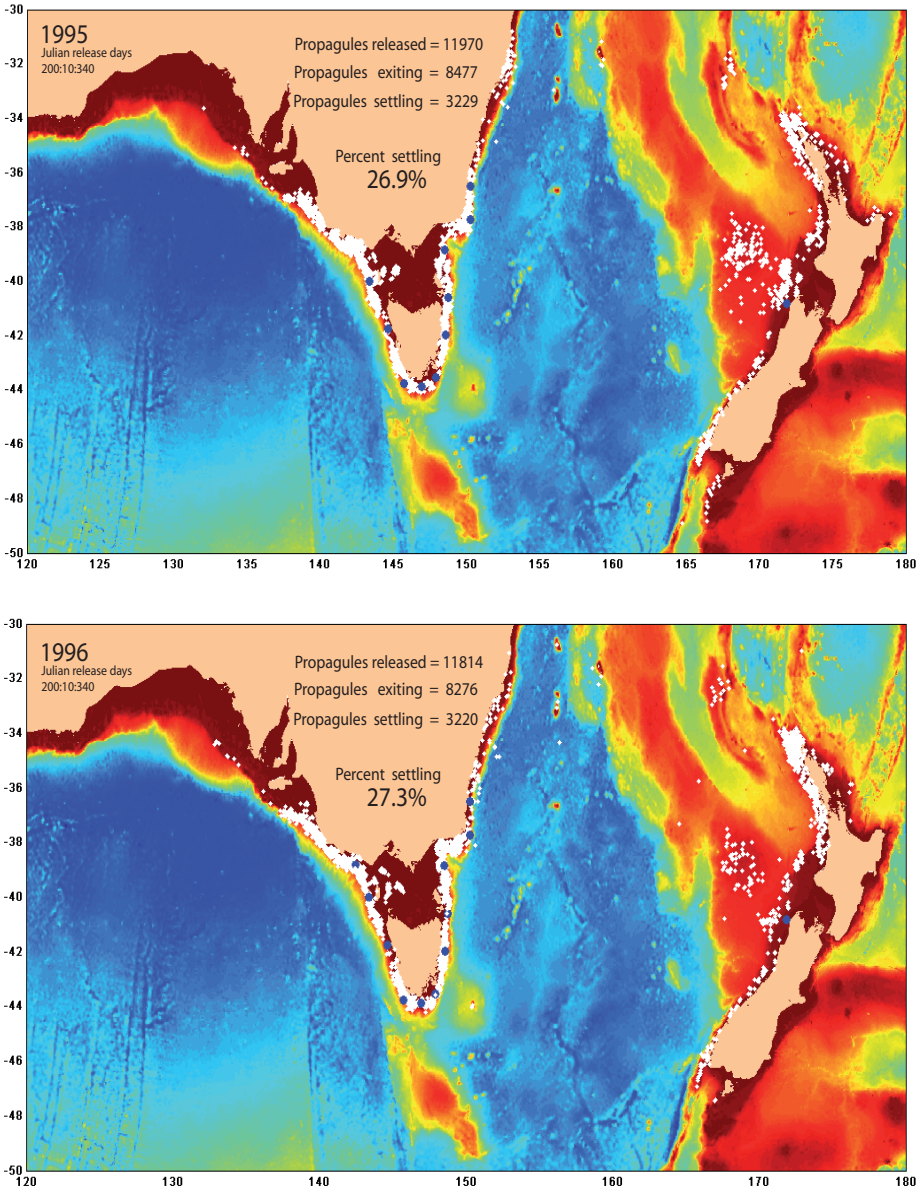

Figure 7.11: Map of study domain, showing parcels successfully settling (white dots) and the spawning nodes that contributed successful propagules (blue dots). The background colour illustrates the bathymetric profile of the region.

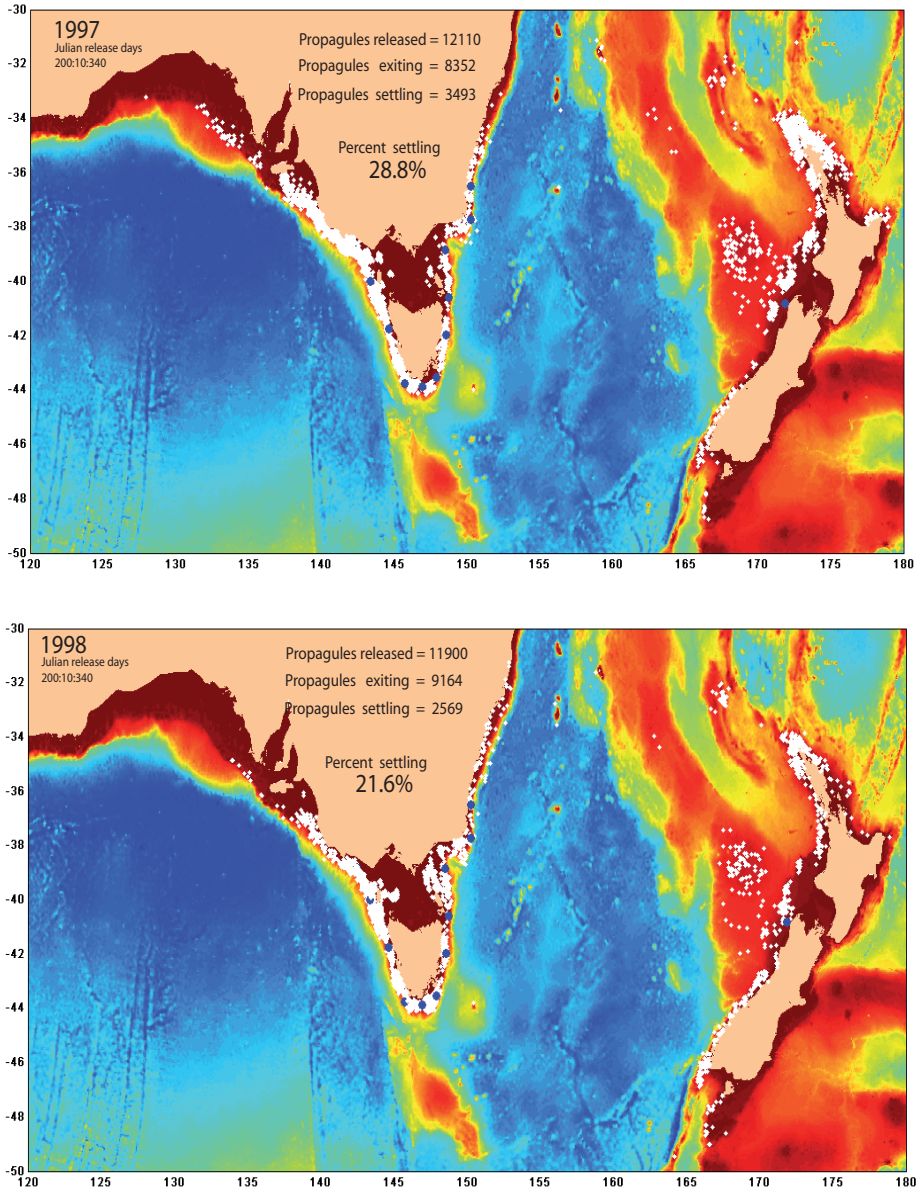

Figure 7.12: Map of study domain, showing parcels successfully settling (white dots) and the spawning nodes that contributed successful propagules (blue dots). The background colour illustrates the bathymetric profile of the region.

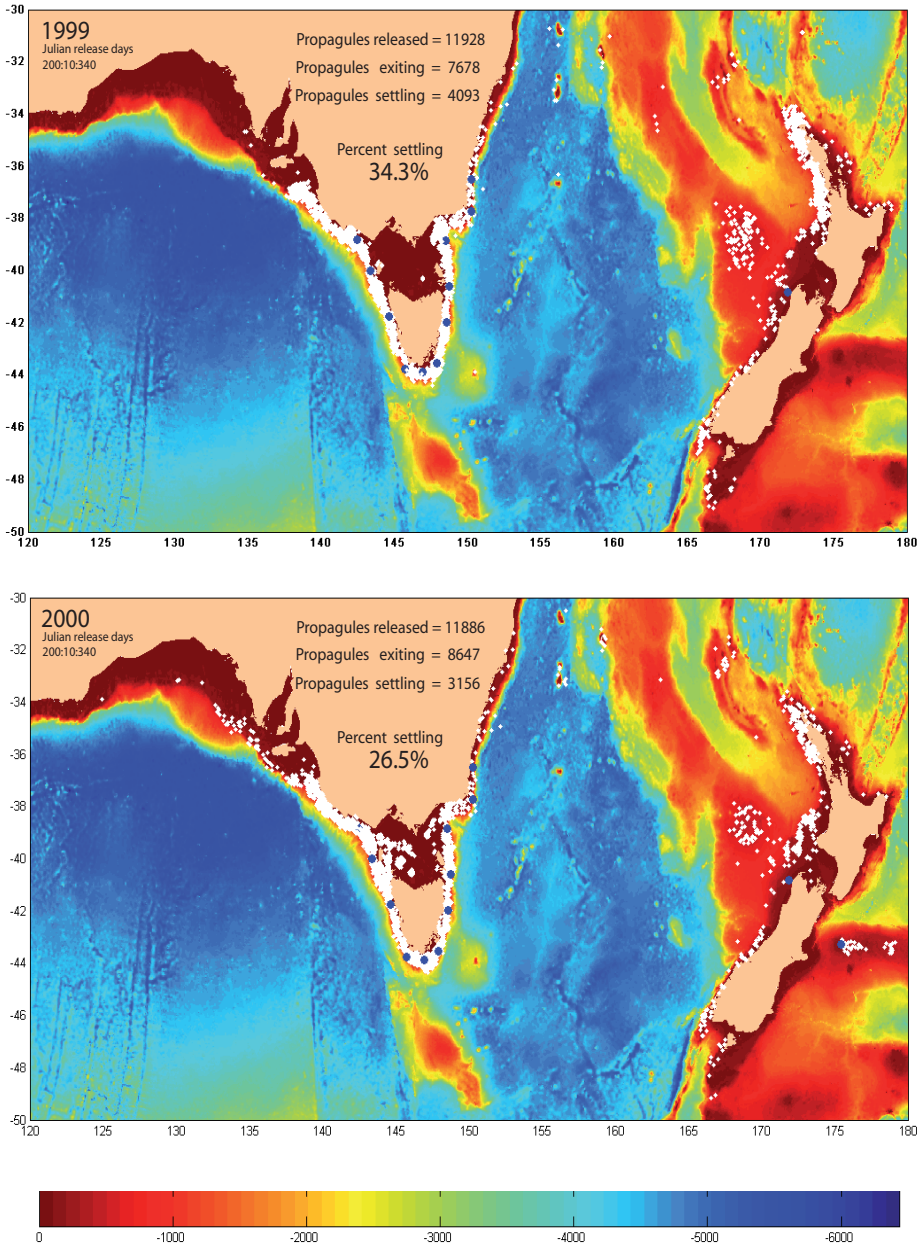

Figure 7.13: Map of study domain, showing parcels successfully settling (white dots) and the spawning nodes that contributed successful propagules (blue dots). The background colour illustrates the bathymetric profile of the region.

7.3.3 Spawning node contribution to recruitment

The comparative contribution of spawning nodes to the model domain predicted that the spawning sites along the west coast of Tasmania contributed the most settling VLPs (36%). The west coast node (TASw) provided the highest number of any location, consistently across all years, contributing a mean of 13.6%. With the exception of the east (TASe) coast of Tasmania which contributed the lowest percent of VLPs (3.8%), the sites off the northeast (TASne), south (TASs) and southeast (TASse) of Tasmania contributed approximately the same percent of settling parcels as the three Victorian locations contributing 22% and 19% respectively over the total period (Fig. 7.14).

The spawning node off the northwest of the South Island of New Zealand contributed 12% of successful VLPs (Fig. 7.14), although there was 100% self-recruitment to New Zealand (Fig. 7.15), equating to 50% of the total contribution of VLP to New Zealand. The percent contribution of VLP from Australia to New Zealand was 17% from Victoria, 12% from NSW and 20% from Tasmania, with 60% of the Tasmania VLP coming from the TASne and TASe nodes.

7.3.4 Settlement 'hotspots'

The model simulations identified Victoria and Tasmania as settlement 'hotspots' for Australia, with 42 and 31% of VLP settling to these states respectively. Within Tasmania the greatest number of VLPs settled to northwest Tasmania (16%), with northeast (8%), southeast (9%) and southwest Tasmania (10%) receiving approximately similar levels (Fig.7.16). New Zealand also had a large proportion of settling VLPs (24%), however, the settlement polygon for New Zealand is significantly larger than any other settlement polygon (Fig. 7.2).

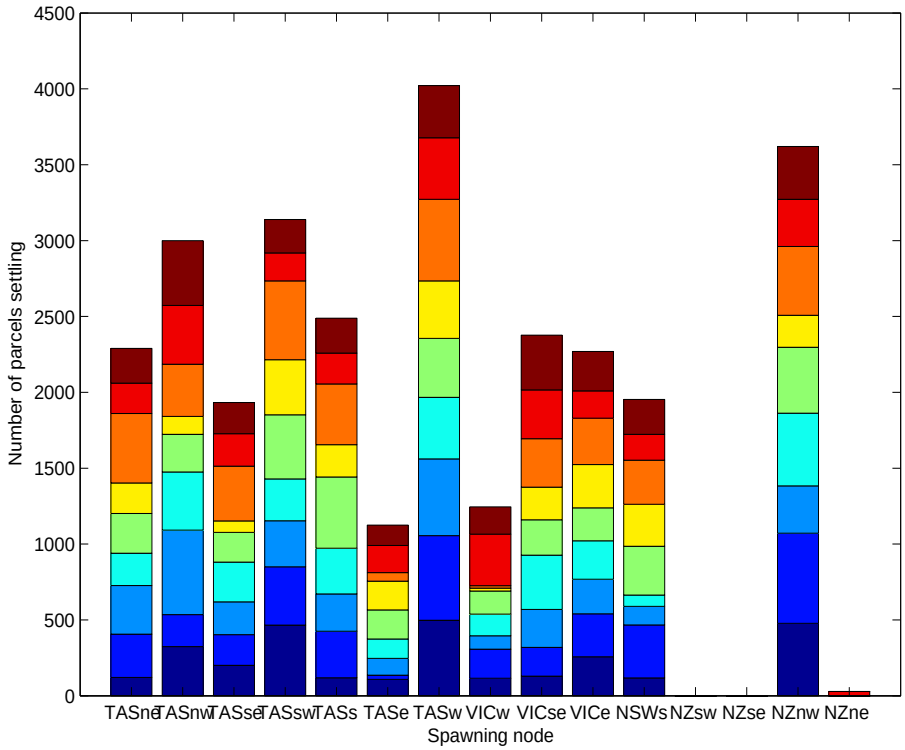

Figure 7.14: The number of virtual larval parcels (VLPs) settling within the model domain from each spawning node. Bars are stacked by spawning year, dark blue represents the year 1993 to red which represents the year 2001.

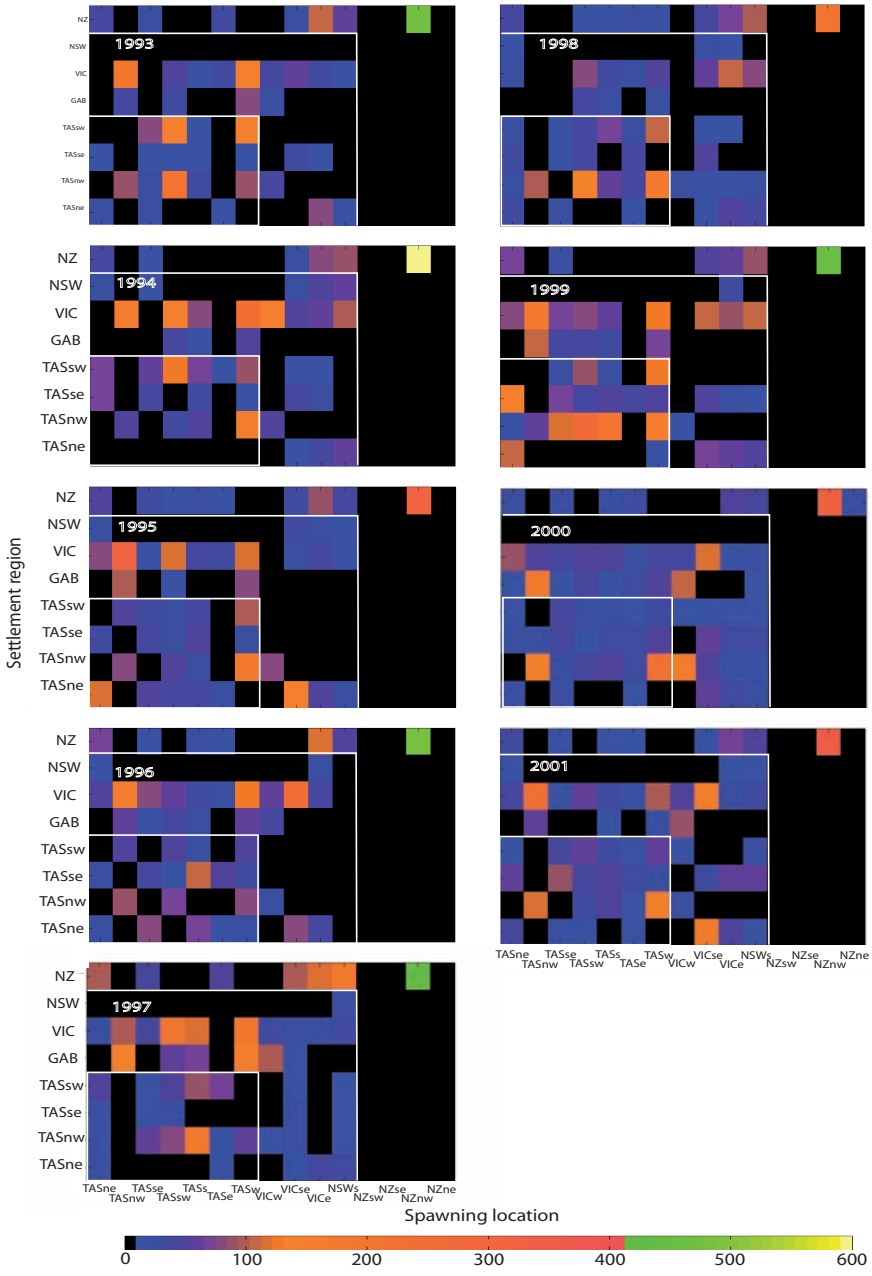

Figure 7.15: Matrix plots for years 1993 – 2002 illustrating the number of Virtual Larval Parcels (VLPs) settling to each predefined settlement polygon (y-axis) (Figure. 7.2) from each spawning node (x-axis). White lines divide Mainland Australia (Victoria and New South Wales), Tasmania and New Zealand.

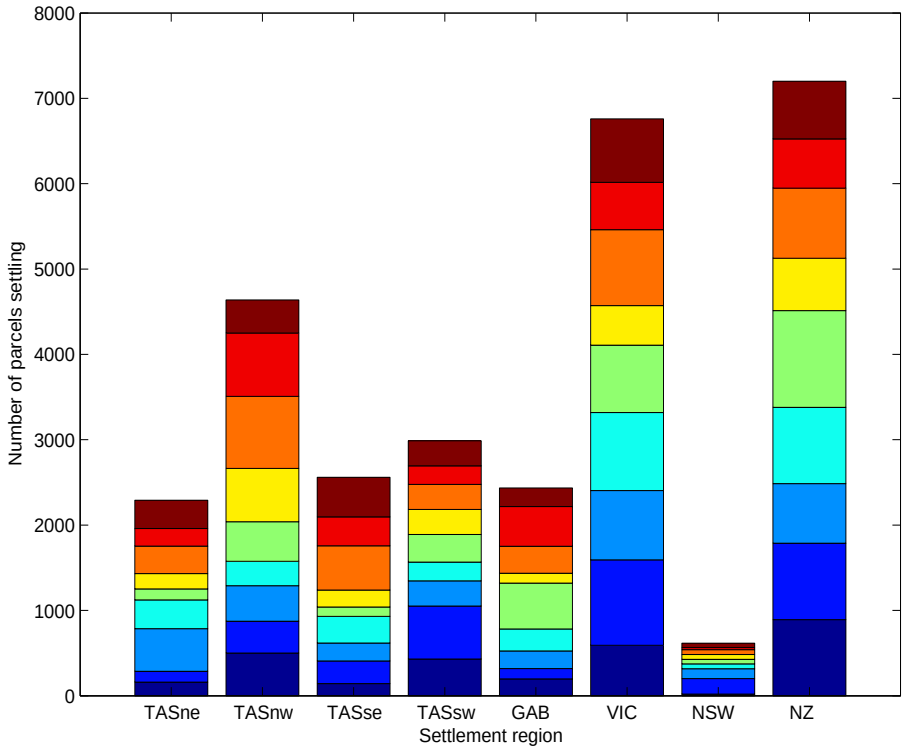

Figure 7.16: The number of *Latris lineata* virtual larval parcels (VLPs) settling to each pre-defined settlement polygon (Figure 7.2). Bars are stacked by spawning year, dark blue represents the year 1993 to red which represents the year 2001.

7.3.5 Influence of release date within a spawning season

The model indicated that the day of release within a season had a significant effect on the number of parcels successfully settling with the exception of a few anomalous events (eg day 230 in 1997) (Fig. 7.17). The general trend was influenced by the Gaussian description of the within-season spawning intensity described by the gonadosomatic index (Fig. 7.3). For most years the model predicted the greatest number of settlers resulted from a day on or close to the peak spawning date. The distribution of successful settlers are less 'peaked' in 1993, 1998 and 2000, suggesting a more even distribution of settlers from release dates within these years. Coincidentally these years provide the least number of successful settlers for all model years, suggesting reduced peak recruitment, rather than increased shoulder recruitment. In the model 1999 had the highest level of recruitment; the distribution of recruits from release days within this year illustrates a high level of success across the five release days spanning the period of peak spawning (75 days) when the greatest number of virtual larvae parcels were released from each spawning node.

7.3.6 Age at retention/settlement

The average day that retention occured (VLPs encountered coastal depths after day 30) ranged from 86 days in 2000 to 124 days post-hatch in 1993 (Fig. 7.19a). Interestingly VLPs in 1993 took significantly longer on average to retain than in any other year (Fig. 7.19a). The average age of settlement was dependant on the sea-surface temperature, with VLPs settling earlier in warmer years as a function of increased temperature-dependant growth. From 1993 to 1996 settlement occurred around 285 – 295 days, while from 1997 to 2001 settlement occurred around 270 – 275 days (Fig. 7.19b).

7.3.7 Growth of virtual larvae

The average length of VLPs at retention reflected the age at retention, with a longer average length for those years in which VLPs took longer to retain (Fig. 7.20). The length at

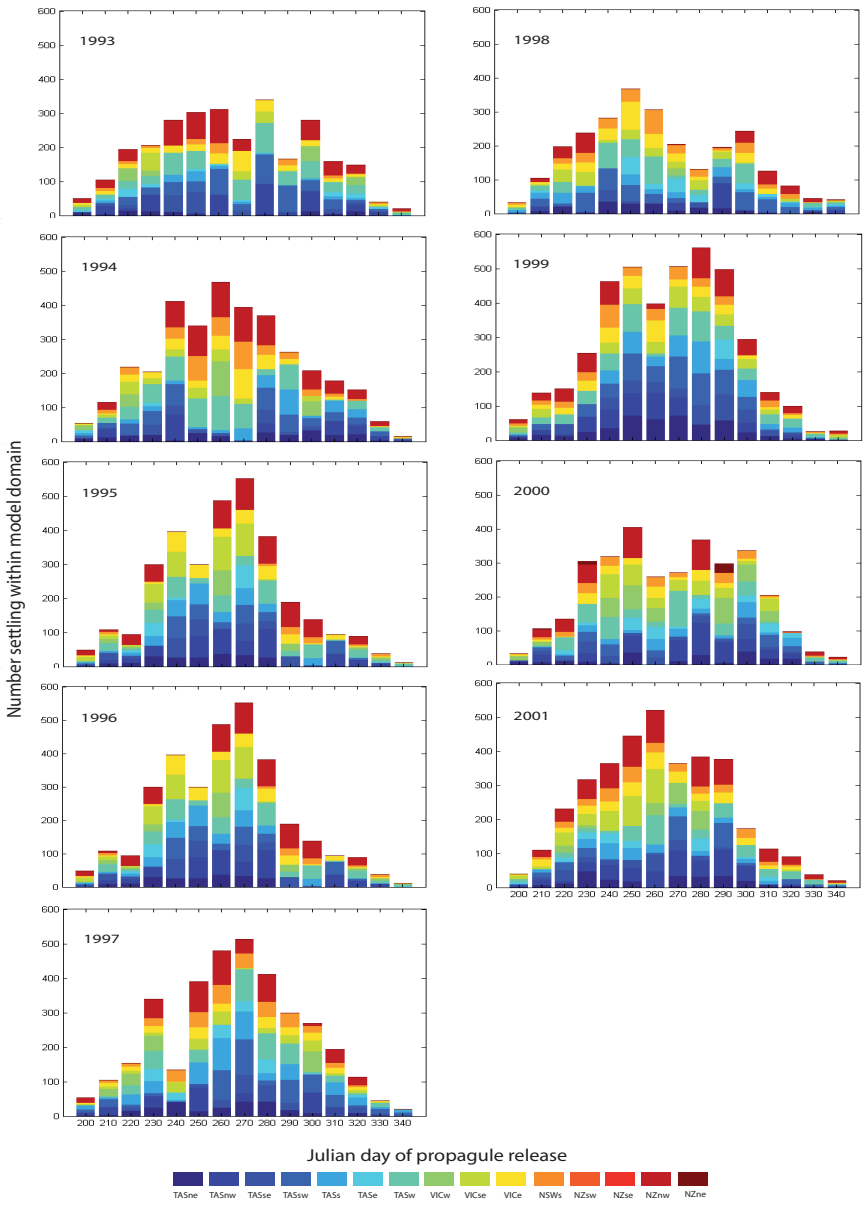

Figure 7.17: The number of virtual larval parcels successfully settling from each release day within a year. The bars are stacked by the contributing spawning nodes defined by the colour bar.

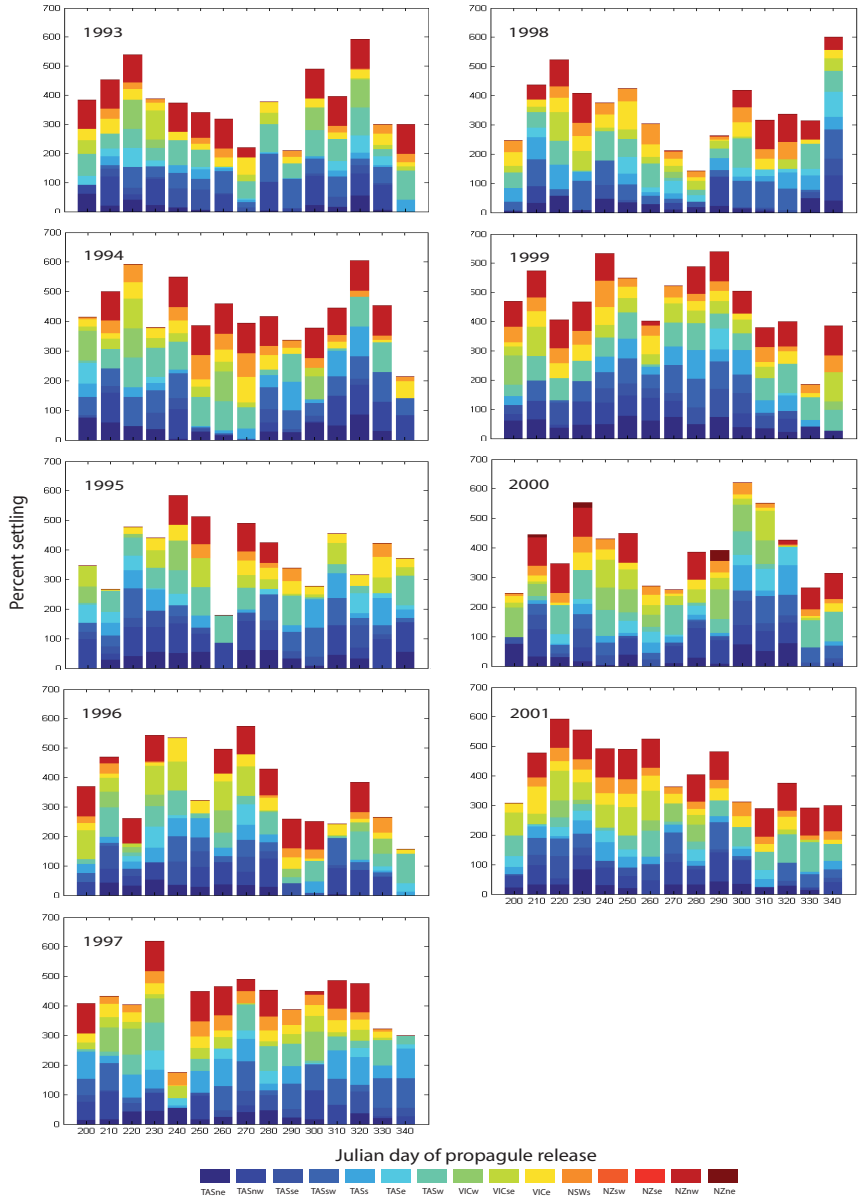

Figure 7.18: The percent of virtual larval parcels successfully settling from the total released each day the model was seeded within a year. The bars are stacked by the contributing spawning nodes defined by the colour bar.

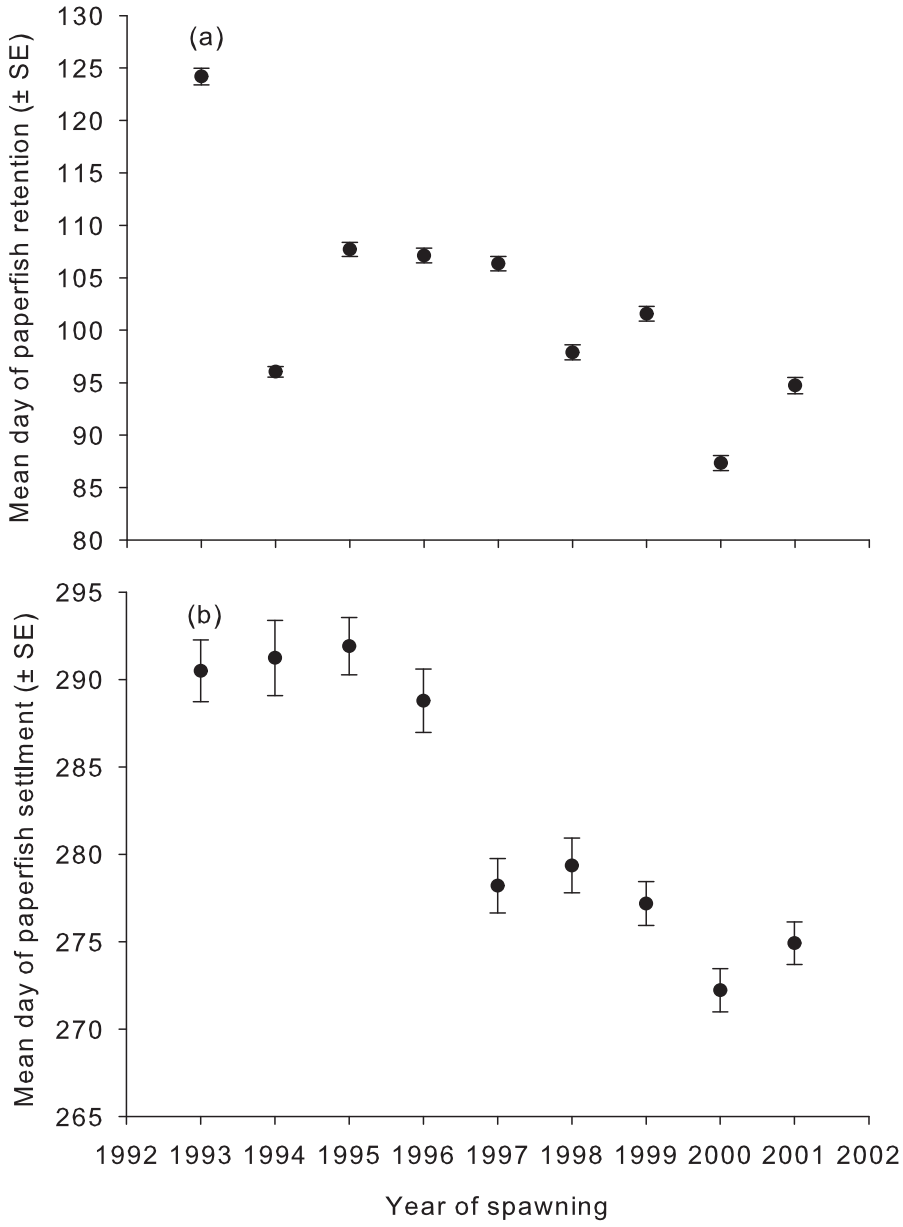

Figure 7.19: (a) The average day post-release that *Latris lineata* virtual larval parcels (VLPs) actively retained in a given year, and (b) the average day that VLPs settled in a given year.

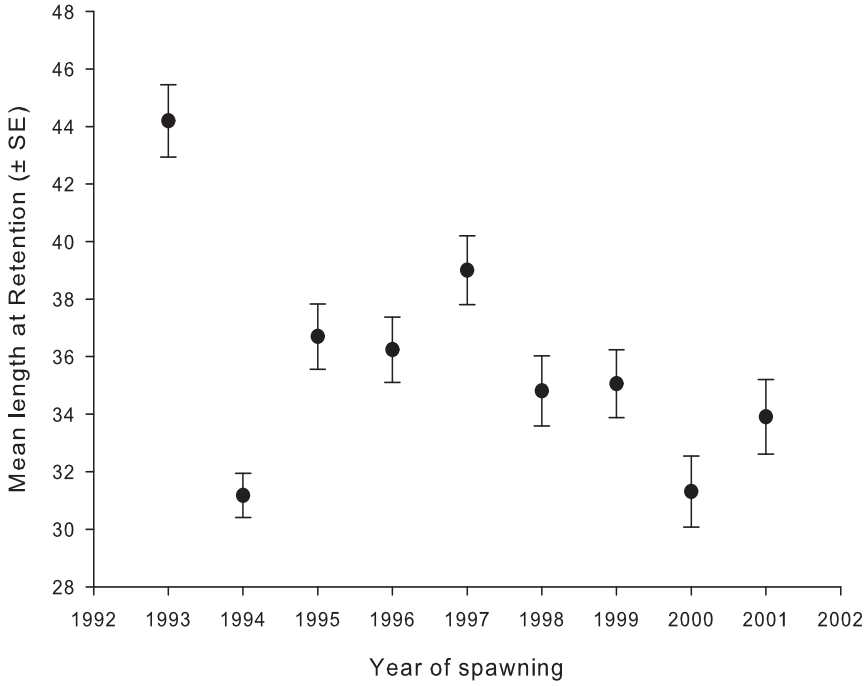

Figure 7.20: The mean length of simulated *Latris lineata* virtual larval parcels (VLPs) at which active retention occured each year.

settlement between years was relatively consistent due to the dependence on the length-at-settlement function.

7.4 Discussion

Gaining an understanding of the processes that lead to temporal variation in successful larval exchange and retention is vital to the study of marine population dynamics, management of fishery stocks, and the design of marine reserves (Cowen et al., 2000). The biophysical model used in this study provided potential insight into the larval transport and settlement of striped trumpeter, although in its current form, the model has not captured

the controlling variables or combination of variables that lead to the observed temporal recruitment variability of juvenile striped trumpeter.

Several informative findings have emerged from the model. The timing of spawning events had a significant impact on the number of VLPs arriving in a location for settlement. Generally, the more VLPs released on a given day the more that would return from that day, following the trend of the Gaussian distribution of spawning intensity imposed as a prior to the model. However, if this positive correlation was interrupted, particularly if there was recruitment failure on peak spawning days due dispersal of larvae to unfavourable locations, this would result in recruitment failure for that year; for example the model year 1998.

The model also indicated a significant degree of self-recruitment of New Zealand striped trumpeter, with a small contribution of VLPs from Australia. The majority were sourced from the more northerly release nodes of New South Wales (NSW), Victoria (VIC) and northeast Tasmania (TASne), and are a consequence of these animals being more likely to get entrained in the relatively fast-moving Tasman Front. In reality, the number sourced from Australia may actually be lower since the model assumes that equal number of VLPs are released from each spawning node. However, based on fishing catch returns the adult populations of striped trumpeter from NSW and Victoria are substantially smaller than the population of Tasmania. This would suggest that less propagules would arise from these spawning nodes. This may explain the genetic divergence reported between the Tasmanian and New Zealand populations of striped trumpeter reported by Tracey, Smolenski & Lyle (2007), although only a very low degree of mixing is required to produce genetic homogeneity (Dudgeon et al., 2000).

For our simulations, we used a relatively coarse set of velocity fields and long time steps (daily) to transport the VLP's to reduce computational load so more focus could be placed on the integration of biological parameters. The model suggests that the dispersal of striped trumpeter larvae in southeast Australian waters and the Tasman Sea appears to be linked to mesoscale oceanographic processes within the region. This result is most ev-

ident in that the model was unable to recreate the exceptionally strong recruitment pulse observed from 1993 and 1994. These years also provided a significant increase in the abundance of 0+ Jackass morwong (*Nemadactylus macropterus*), a temperate reef-associated species, off the east coast of Tasmania , which has a similar early life history but a different spawning season occurring around March (Jordan, 2001).

The model provided surprisingly little temporal or spatial variation in recruitment from runs of both stage 1 and 2. A similar result was reported by Griffin et al. (2001) for the dispersal of the western rock lobster (*Panulirus cygnus*), an invertebrate with a long pelagic larval duration (PLD), off the west coast of Australia. The impacts of the mesoscale anomolies evident in the model flow fields varied temporally and subsequently influenced the movement of the VLPs, however, settled larvae were advected to similar locations each year and with the percent of successful settlers ranging from 21 to 30% in contrast to the observed interannual variability that was several orders of magnitude larger. In this study it was evident from the dispersal of VLPs that the predominant westerly wind stress of the region had a strong influence on where retention occurred with significantly larger numbers settling to the west coasts of Tasmania and Victoria, as well as the western shores of New Zealand. There was less settlement on the east coast of Australia, a phenomenon that is most likely unrealistic, particularly for Tasmania where large numbers of juveniles are seen inshore in years of good recruitment. The westerly wind stress also advected almost all of the VLPs released from the south and east of New Zealand out of the model domain before they had a chance to actively retain.

Although this model contains an unprecedented degree of information relevant to the biology of the study species and the interaction of this biology with the environment there are still many simplifications and assumptions made that may have lead to the model failing to recreate the observed temporal variability. The model domain lies in a dynamic oceanographic region with the convergence of several water masses (Harris et al., 1987; Cresswell et al., 1994), hence increasing the resolution and complexity of the flow fields using a high-resolution hydrodynamic model, and reducing the time-steps to allow for finer

scale movements may increase the model's capacity to reflect observed trends by allowing for the representation of finer scale oceanographic features that were not captured in this current version. In particular the inclusion of wind-driven currents would be advantageous as the model works at the sea-surface and assumes that these surface currents are primarily responsible for larval advection.

There were also two significant biological/behavioural traits that have not been incorporated into the model that, we strongly suspect detracted from the model's ability to reproduce the observed interannual variability. Firstly, it was assumed that the number of propagules released each year was relatively constant, removing any influence of spawning production from the model. The observed trend of significant interannual variability in recruitment would invalidate this assumption as the size of the spawning stock biomass varies significantly as a function of recruitment variability and in addition the strong relationship between size and fecundity for striped trumpeter (Tracey et al., 2007) would lead to significant variations in spawning production as the size structure of the adult biomass changed. Secondly, the model did not incorporate vertical migration, either as a passive/or active response. Vertical migration behaviours by larvae coupled with vertically stratified flows may retain larvae nearshore, some studies have indicated that mesoscale and sub-mesoscale circulations may minimise long-distance dispersal by retaining larvae for a portion of or throughout their pelagic stage (Cowen & Castro, 1994).

The large amount of information regarding their growth and survival as larvae in response to temperature from hatchery rearing trials provided an opportunity to assess the impact of biological traits on the outputs of a bio-physical model. However, the extended nature of the larval-paperfish stage, lasting up to nine months, means that the species has significant capacity for active migration, with a mean length at settlement of approximately 150 mm, as large as many small pelagic fish. This means from post-flexion to settlement (a period of around eight months) striped trumpeter have increased control over their advection. The model is limited as it does not incorporate any active behaviour beyond the assumed desire to retain in a coastal environment if they passively arrive to an area con-

sidered acceptable based only on the depth of that location. Incorporating active behaviour cues is probably one of the greatest challenges facing these modelling studies. There are some studies that deal with active migration suggesting that homing to suitable habitat is possible using chemical cues once a larvae is capable of active migration (Stobutzki & Bellwood, 1994; Leis et al., 1996). Transient pulses of primary production common in the oceanic water column could also underlie the extreme temporal variability of settlement characteristic of many marine organisms (Thresher et al., 1988). The advent of ocean-colour imagery through the SeaWiFs and MODIS satellite programs allows for integrating potential feeding cues, or 'hotspots' into a model and also simulate potential predator-prey interaction, although this data only extends back to 1997. So as a time-series is developed the real possibilities of this data will become evident. However, it will be a significant task to compile a realistic suite of species-specific behaviour traits and cues that will recreate the interannual effects of such behaviour.

Even though we have made considerable effort to integrate realistic biological information into this model, there are a number of simplifying assumptions. For example, in wild populations the seasonal cycle of growth is not always, perhaps never, solely under temperature control (Ricker 1979), as there are concomitant changes in food type and availability, day length and oxygen saturation. The survival module implies only natural mortality with the exception of loss due to predation, a factor assumed to be of considerable consequence for oceanic larvae (Sponaugle & Cowen, 1997). Although sensitivity to these parameters were not tested for this model they may result in differential mortality and will therefore affect the realised outcome of larval transport (Hare et al., 1999).

The usefulness of successfully correlating hydrographic data with biological responses such as growth and recruitment variability cannot be understated as a predictor of stock structure and hence a powerful tool for fisheries management. By enhancing the physical parameters of the model and applying further sensitivity analysis to the parameters of the model, confidence in the model outputs may increase and ultimately define a recruitment index that correlates well with the observed data. As such, the information and framework

provided by this model is a step forward in the science of larval advection modelling.

Chapter 8

General Discussion

Research on small-scale, low value fisheries, such as multi-species scalefish fisheries, is often poorly-funded with limited resources stretched over a range of species. This typically leads to limited and patchy datasets. Developing an understanding of the biology and ecological interactions of a species in such situations is a major challenge to achieving effective management, particularly when seeking to implement an ecosystem approach to fisheries management.

This study applied a suite of approaches to synthesise the available but patchy dataset for striped trumpeter to achieve realistic and representative information about the striped trumpeter population around Tasmania. These methods were predominantly based around otolith analysis, including ageing, geometric morphometrics and trace element analysis, combined with innovative techniques available through technological advancements as well as methods adopted to fisheries science derived from other disciplines including: molecular biology and oceanographic modelling using remote sensing data.

This study has applied the axiom that realistic species-specific biological information is a necessary requirement to support management, and that this information, coupled with knowledge of spatial habitat utilisation, is essential to achieve ecologically sustainable

fisheries development and management.

8.1 Synthesis of biological and ecological traits of striped trumpeter

8.1.1 Biology and life history theories

An understanding of a species life history strategies is a key requirement for managing a data limited fishery. This facilitates generalisations of parameters useful for the development of sustainable management. Life history theories seek to explain the evolution of organism traits as adaptive responses to environmental variation and differential mortality or resource allocation to life stages (Roff, 1992; Stearns, 1992). The most notable example in ecology of a life history strategy continuum is the theory of r- and K-selection (MacArthur & Wilson, 1967; Pianka, 1970). According to this theory a population displaying attributes such as delayed reproduction, low fecundity, large parental investment and a long lifespan would be described as K-selected. An r-selected population would have much shorter life duration, mature early and grow rapidly. The limitation of the r-K continuum is that it only accounts for two extremes and fails to recognise additional aspects of interspecific variation that occur in nature (Winemiller, 2005). As an extension of the r-K theory several researchers have proposed triangular models of fish life history strategies (e.g. Kawasaki, 1980; Winemiller & Rose, 1992). The Winemiller & Rose (1992) life history model proposes the following three life strategy descriptions.

1. Opportunistic - short generation time, high reproductive effort, small body size, low batch fecundity, and low investment per offspring;
2. Periodic - long generation time, moderate reproductive effort, large body size, high batch fecundity, and low investment per offspring, and;
3. Equilibrium - moderate to long generation time, low reproductive effort, variable body size, low batch fecundity, and high investment per offspring.

If we consider the biological traits of striped trumpeter as described through this study, against the model proposed by Winemiller & Rose (1992), the species clearly falls within the category of a 'periodic strategist'. The oldest fish recorded in this study was 43 years of age although the maximum age of striped trumpeter is most likely substantially older than this. The largest fish examined in this study (90 cm) was considerably smaller than the reported maximum size, 125 cm and 25 kg (Gomon et al., 1994) (Chapter 2). The need to use a two-phase growth function illustrated the dramatic decrease in growth rate upon reaching maturity. The standard von Bertalanffy growth function tended to underestimate L_{∞} because it could not accommodate the rapid decrease in growth rate with age (Chapter 2). Batch fecundity of striped trumpeter is exceptionally high with estimates ranging from about 0.2 million for a 2 kg fish (540 mm FL) to 2.3 million for a 9.5 kg fish (800 mm FL) (Chapter 3), and recruitment variability is evident through the strong recruitment pulses back-calculated to 1993 and to a lesser extent 2004 (Chapter 2).

Protracted longevity and high fecundity maximises fitness when environmental variation influencing early life stage survival is periodic and survival of these early life stages is more variable than that of adults (Winemiller & Rose, 1992). The strategy works by bet-hedging, whereby reproductive effort is allocated across multiple years, so that some individuals may achieve reproductive success despite long intervals with environmental conditions that may be unfavourable to early life stage survival.

Even in data-deficient situations some generalizations can be made about species re-

silience to fishing where there is an understanding of the life history strategy the species employs. For example, a periodic strategist such as the striped trumpeter would be expected to have low resilience to over-exploitation. The regenerative capabilities of a striped trumpeter population would be low, possibly requiring several generations before a depleted spawning stock biomass would experience favourable conditions providing for successful recruitment to the stock. This fact has serious implications to the equilibrium view of population dynamics (Fletcher & Deriso, 1988; Hixon et al., 2002). Species displaying this strategy would require complex, data intensive assessment models to accommodate the lack of temporal equilibrium.

Unfortunately for management, large uncertainty is an unavoidable reality for projections involving periodic strategists. However, a knowledge of the biological traits can assist managers in assigning a broad conservative management approach.

8.1.2 Stage-specific traits and habitat utilisation

Another component of life history theory important to fisheries assessment and management is an understanding of the variations in key parameters such as growth and mortality between life-stages. Many fish species, particularly periodic strategists have a complex history compiled of potentially two or more distinct phases. Evidence from this study and earlier research on striped trumpeter both from the wild (Murphy & Lyle, 1999) and aquaculture (Battaglene & Cobcroft, In Press) indicates that striped trumpeter have a tripartite life-cycle: beginning with an extended pelagic larval/post-larval paperfish stage lasting approximately 9 months (Ruwald, 1992); a demersal juvenile phase where energy allocation is focused on somatic growth; and finally the adult phase where there is a significant shift in energy allocation accompanying the onset sexual maturity (Chapter 2).

The two-phase growth model developed in Chapter 2 was able to reflect the biphasic shift from the juvenile to adult phase and provided a more accurate description of observed lifetime growth patterns than a standard von Bertalanffy growth function. The age at transference selected by the growth model was 4.4 years. This is a reasonable approximation of

the age at onset of maturity, given that the age at 50% maturity occurs at around 5 years of age.

Anecdotal evidence has suggested that during the juvenile phase striped trumpeter have a preference to shallower inshore reefs, while as adults they inhabit the deeper offshore reefs associated with the continental shelf break. Using otolith microchemistry this study has supported this hypothesis. With the onset of maturity there is an apparent exodus of juveniles from the inshore habitats to join the adult biomass on the upper edge of the continental slope (Chapter 6). Although this component of the study focused on a single cohort (1993 cohort), this cohort has been very influential to the striped trumpeter population for over a decade. Considering the extent of recruitment variability, protecting juveniles is paramount to maximise the potential for replenishment of the striped trumpeter population.

The nature of the striped trumpeter fishery in Tasmania can be categorised into two distinct sub-fisheries, which has important implications for management of this species. Juveniles are taken predominantly by gillnet in inshore waters, usually at depths $<$50 m, whereas adult fish are taken in deeper offshore waters by hook methods (dropline, handline, bottom longline, trotline) and as by-product in large mesh gillnets (shark nets). This division allows for targeted biological and spatial assessment for the sub-fisheries independently, facilitating unique management strategies depending on the life stages determined to be critical for population growth.

8.1.3 Stock structure, mixing and ‘source – settlement’ hotspots

Defining the scale of connectivity, or exchange, among marine populations is pivotal to our understanding of the population dynamics, genetic structure, and biogeography of many coastal species (Cowen et al., 2006). The common approach to assessment and management of marine scalefish stocks assumes discrete populations, although it has long been recognised that this assumption is complicated by migrations and mixing between management units (Stevenson, 1999). There are two main reasons for understanding the stock

structuring of a species. Firstly, most stock assessments assume a closed population, i.e. no migration to and from the assessment unit, and, secondly, the impact of phenotypic and genotypic diversity within a management area needs to be considered, i.e. variation on a temporal or spatial scale of life history parameter, or the existence of multiple discrete populations within a management unit.

Tasmanian coastal fisheries are managed as discrete units in isolation of fisheries from mainland Australia and neighbouring countries, i.e. New Zealand. A consequence of the extended pelagic larval/paperfish stage displayed by striped trumpeter is that migration to and from discrete management units is possible. This concept of a closed population is further confounded by the possibility that active migration was occurring post-settlement. The discovery of a fish that was tagged as a juvenile off Tasmania and re-captured 5800 km to the west at the St.Paul/Amsterdam Islands in the Indian Ocean confirmed the potential for large scale migrations.

In addressing this issue both otolith morphometrics (Chapter 4) and sequencing of mitochondrial DNA (Chapter 5) indicated that there was little mixing between remote populations such as Tasmania, St. Paul/Amsterdam Islands and New Zealand. The larval dispersal model also supported this with no virtual larvae migrating from New Zealand to Australia and only a small number migrating from Australia to New Zealand (Chapter 7). It is possible that the dispersal model may have also over-estimated this number as the majority of virtual larvae that journeyed across the Tasman were from the spawning nodes off eastern Victoria and southern New South Wales. These locations are assumed to have smaller populations of striped trumpeter compared with Tasmania based on catches (Ziegler et al., 2006).

The larval model not only indicates the importance of the location of spawning but also the timing of spawning in influencing successful larval recruitment. Hence spatio-temporal management could focus on protecting adult habitats (spawning areas) that disproportionately provide successful larval recruitment or alternatively protect fish during the peak spawning period.

This study did not identify discrete sub-units within the Tasmanian population of striped trumpeter. Phylogenetic analysis of mtDNA sequence from samples collected from the southwest, southeast and northeast of Tasmania indicated a single genetic unit. This finding might be expected due to the protracted pelagic dispersal period, allowing ample opportunity for mixing around the Tasmanian coastline. The larval dispersal model also indicated significant advection back to Tasmania as well as evidence of a degree of retention to natal spawning regions.

8.2 Implications for management: implementing policy and legislative principles

8.2.1 Towards ecosystem based management

The future direction of fisheries management needs to be considered when planning sampling and assessment protocols for small-scale fisheries so that the data collected and subsequent analyses fit potential management models. An increased awareness of uncertainty and the subsequent incorporation of the precautionary approach and broader impacts of fishing, have resulted in a worldwide re-evaluation of existing fishery management plans, working towards ecosystem based management (EBM), leading to the development of the term ecosystem based fisheries management (EBFM) (Hilborn et al., 2001; Garcia et al., 2003; Astles et al., 2006).

Ecosystem objectives in management plans usually flow from high-level national policies or strategies and international agreements. Consequently they are often broadly stated and hence are difficult to incorporate directly in management plans (Sainsbury et al., 2000). Whilst a considerable level of thought and effort has taken place around the concept of ecologically sustainable development (ESD) in fisheries and fisheries research in an ecosystem-based context, the complexity of the issues raised often extend beyond an agency's traditional sphere of experience. This has meant that ESD and EBFM have been

elusive concepts to implement effectively and to demonstrate achievement in a practical manner (Fletcher et al., 2005). Like all natural resource management issues, fisheries management involves far more than the implementation of minimum biological limits for the harvested species. The development of effective fishery management arrangements must deal with a highly complex system of environmental, social, economic and political values.

8.2.2 Working with limited data

The fundamental basis for ecosystem based approaches to fisheries management is achieving management strategies that take account of the impacts of harvesting a species on its surrounds and other biota that co-inhabit the ecosystem. In data-poor situations with little or no information about the status of the target species or ecosystem processes, Implementing management strategies may simply involve using life history characteritics and general knowledge to develop precautionary allocations or safety margins, such as reduced catch limits or larger closed areas. In the case of striped trumpeter in Tasmania, recent management action has involved the introduction of reduced trip limits for commercial fishers (250 kg), possession limits for recreational fishers and an increase in the minimum size limit.

In systems with moderate amounts of data (e.g. catch data and abundance trends for key species), EBFM could be characterized by effective single-species management with the addition of precautionary limits allowing for ecosystem allocations. Progression from data-poor to data-rich situations will be facilitated by adaptive management and greater understanding of how ecosystems respond to alternative fishing strategies (Pikitch et al., 2004). At the same time fisheries researchers must not underestimate the data they have at hand. A compilation of fisheries dependent and independent data can provide insights into the intrinsic dynamics of a fspecies but also how that fishery interacts in an ecosystem context. It is extremely important to avoid making complete knowledge of the ecosystem or limitations in the information on species biology the adversary of using available knowledge, including meta-analysis. Hence, uncertainty arising from data limitations should not prevent the development of operational objectives leading towards EBFM.

8.2.3 Implications for management: Life history characteristics

An understanding of reproductive biology is important, however, for species such as striped trumpeter that may experience prolonged periods of poor recruitment, this is especially important and will underpin biologically appropriate management. As the current minimum size limit in Tasmania is still below the estimated size at 50% maturity of females, female striped trumpeter are potentially vulnerable to fishing for at least one to two years prior to reaching maturity. Increasing the size limit to greater than or equal to the size at 50% maturity of females will – in theory – allow each cohort a chance to contribute to the spawning stock biomass before becoming vulnerable to the fishery.

While it is important to allow the juveniles to mature, the fecundity-body size relationship of striped trumpeter illustrates the relative contribution to egg production from the various age groups. Larger females contribute significantly greater numbers of eggs than smaller females, studies of other species have indicated that eggs tend to be larger and that subsequent larvae have greater survival rates than larvae hatched from smaller fish (Kocher et al., 1989; Kamler, 2005). This implies that protection of larger females is important if the production of the spawning stock is to be maximised. With the absence of significant recruitment to the striped trumpeter population over the last decade, the general trend in size composition has been a shift towards larger fish as the strong 1993 cohort continues to grow.

Given this situation it may be prudent to also impose an upper size limit for striped trumpeter. For example a size window of 525 – 750 mm FL would allow for a proportion of females to enter the spawning population before being exposed to fishing and would also provide protection for the larger fish that provide disproportionately more of the overall spawning production of striped trumpeter. Based on the current size structure of striped trumpeter, as reported in the most recent fishery assessment, the opportunity cost of lost catch to the commercial sector would be minimal (Ziegler et al., 2006).

8.2.4 Implications for management: spatial structure

Currently, spatial management represents one of the primary tools of EBFM, including no-take zones or area-based gear restrictions for stock or life stage protection or to manage interactions with predators/prey and threatened, endangered and protected species (Babcock et al., 2005). The implementation of these tools is often complimented through other conservation-based measures, such as marine protected areas. However, before these tools can be effectively applied it is necessary to identify essential fish habitats, nursery areas and reproductive hotspots for a species. Gathering this type of information is typically difficult to justify for small-scale fisheries where the collection of basic biological information is generally the priority. To date, small-scale fisheries have been a minor consideration in the application of MPAs and no-take zones, with a lack of spatial information available for the target species being a major factor (Castilla, 2000). Marine protected areas are well suited for benthic marine species as they consider the pelagic dispersal of propagules and the patchy distribution of benthic habitat (Carr & Raimondi, 1999). Spatial management based on species-specific information (aggregation zones, juvenile habitats, etc), combined with traditional management strategies (size limits, trip limits etc) could potentially be a powerful tool for sustainable management of striped trumpeter.

The larval dispersal model (Chapter 7) showed the importance of the timing of spawning to successful larval recruitment, with the month of peak spawning crucial to the supply of large numbers of propagules available for subsequent recruitment. Exploited fish species may be more vulnerable to capture during spawning as a consequence of their aggregating behaviour (Eklund et al., 2000). This information could be used to implement closures for striped trumpeter during the spawning season, particularly during the peak spawning period (October). This will reduce pressure on the dwindling adult population and protect egg production and hence potential recruitment.

To reduce pressure on those individuals that successfully settle inshore – particularly in years of good recruitment – the most effective method would be to reduce inshore gillnet-

ting effort. Even though the current size limit of 450 mm effectively eliminates targeting striped trumpeter using gillnets, incidental captures do occur and legal size fish are rare in shallow waters. Post-release survival of juvenile striped trumpeter caught in gillnets is appears to be low[1]. Thus, the size limit increase alone may not provide effective protection of juveniles as long as intense gillnetting activity occurs on inshore reefs; noting that greater than 7000 recreational licences have been issued annually in recent years (Ziegler et al., 2006), in addition to commercial netting. An alternative to gear restrictions would be an inshore closure for netting, although this would be politically difficult to effectively implement as large areas of the inshore coastal zone would need to be closed, limiting a range of other fisheries.

8.3 Future directions for striped trumpeter research

This study has revealed biological and ecological traits displayed by striped trumpeter: this information can now be used to refine current management strategies. However, there are other areas that require further research to support sustainable management of striped trumpeter, particularly in an ecosystem context.

Limited sampling over the last five years has resulted in little data to provide insight into trends in population size and age structure. Establishing a system to collect regular samples of striped trumpeter either from active sampling or market measuring is important to ensure ongoing data collection. Samples from the northwest and west coast of Tasmania are particularly sparse. Anecdotal evidence from commercial fishers suggests that particularly large fish are caught in these regions. This information would prove useful as an indicator of size/age structure from an area with low fishing pressure. Further development of the larval dispersal model would be useful to better assess the input of the spawning populations of the west and northwest coasts. In its current form the model suggests that these locations contribute substantial numbers of larvae not only to Tasmania but also the waters

[1] Jeremy Lyle, personal communication

of western Victoria and potentially into South Australian waters.

Perhaps the greatest challenge working against the implementation of a sustainable fishery for striped trumpeter is recruitment variability. The larval advection model was not able to reproduce the observed recruitment pulse. Predicting such recruitment variability could prove useful as a predictive management tool. Allowing prior knowledge of when a recruitment pulse would enter the fishery. In the absense of a model that can accurately predict recruitment variability a sampling protocol monitoring inshore reefs may be an effective measure to facilitate predictive management. As juveniles are now protected trends in abundance of juveniles would provide a relative estimate of future recruitment to the fishery.

The impact of the recreational sector also needs to be quantified. Lyle (2005) indicated that this sector caught an equivalent amount of striped trumpeter to the commercial sector in 2000-01; however, their catch is not accounted for in current fishery assessments. It is expected that the relative impact of the recreational sector has increased as the number of recreational vessels of a size sufficient to travel and fish at locations where striped trumpeter inhabit has increased. Several survey methods are available such as creel surveys and recreational fishing logbooks.

The results of this study considerably broaden the picture as to factors influencing the life-history strategies of striped trumpeter. Considering this information while ensuring a periodic assessment of input parameters to monitor population response to factors such as climate change provides a robust platform for assessing and managing this species. Broadening these objectives to strategically account for the biology, trophic interactions and environmental influences of multiple species that are of commercial and ecological importance is sure to be a cost effective strategy to move single species management forward to multi-species management in an ecosystem based context.

Bibliography

ABARE (2006), Australian fisheries statistics 2005. Australian Bureau of Agricultural and Resouce Economics. Canberra.

Aboim, M., Menezes, G., Schlitt, T. & Rodgers, A. (2005), 'Genetic structure and history of populations of the deep-sea fish *Helicolenus dactylopterus* (Delaroche, 1809) inferred from mtDNA sequence analysis', *Molecular Ecology* **14**, 1343–1354.

Adams, D., Rohlf, F. & Slice, D. (2004), 'Geometric morphometrics: ten years of progress following the 'revolution'', *Italian Journal of Zoology* **71**, 5–16.

Akaike, H. (1974), A new look at the statistical model identification., *in* 'Institute of Electrical and Electronic Engineers Transactions on Automatic Control', Vol. AC-19, IEEE Control Systems Society, pp. 716–723.

Alvarado Bremer, J., Baker, A. & Mejuto, J. (1995), 'Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) populations in the Atlantic Ocean', *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 1720–1732.

Anderson, J. (1988), 'A review of size-dependent survival during pre-recruit stages of fishes in relation to recruitment.', *Journal of Northwest Atlantic Fishery Science* **8**, 55–66.

Anderson, M. (2001), 'A new method for non-parametric multivariate analysis of variance', *Austral Ecology* **26**, 32–46.

Anderson, M. & Willis, T. J. (2003), 'Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology', *Ecology* **84**(2), 511–525.

Andrew, T., Hecht, T., Heemstra, P. & Lutjeharms, J. (1995), 'Fishes of the Tristan da Cunha group and Gough Island, South Atlantic Ocean', *Ichthyological Bulletin of the J.L.B. Smith Institute of Ichthyology* **63**, 1–41.

Astles, K., Holloway, M., Steffe, A., Green, M., Ganassin, C. & Gibbs, P. (2006), 'An ecological method for qualitative risk assessment and its use in the management of fisheries in New South Wales, Australia.', *Fisheries Research* **82**, 290–303.

Avise, J., Alisauskas, R., Nelson, W. & Davison, A. (1992), 'Matriarchal population genetic structure in an avian species with female natal philopatry', *Evolution* **46**, 1084–1096.

Babcock, E., Pikitch, E., M.K, M., Apostolaki, P. & Santora, C. (2005), 'A perspective on the use of spatialized indicators for ecosystem-based fishery management through spatial zoning.', *ICES Journal of Marine Science* **62**, 469–476.

Battaglene, S. & Cobcroft, J. (*In Press*), 'Advances in the culture of striped trumpeter larvae: a review.', *Aquaculture*.

Bayliff, W., Ishizuka, I. & Deriso, R. (1991), 'Growth, movement and attrition of northern bluefin tuna, *Thunnus thynnus*, in the Pacific Ocean, as determned by tagging.', *Inter-American Tropical Tuna Commission Bulletin* **20**(1), 1–94.

Beamish, R. J. & Fournier, D. A. (1981), 'A method for comparing the precision of a set of age determinations', *Canadian Journal of Fisheries and Aquatic Sciences* **38**(8), 982–983.

Beck, M., Heck, K. J., Able, K., Childers, D., Eggleston, D., Gillanders, B., Halpern, B., Hays, C., Hoshino, K., Minello, T., Orth, R., Sheridan, P. & Weinstein, M. (2001), 'The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates.', *Bioscience* **51**, 663–641.

Begg, G., Friedland, K. & Pearce, J. (1999), 'Stock identification and its role in stock assessment and fisheries management: an overview', *Fisheries Research* **43**, 1–8.

Bergenius, M., Meekan, M., Robertson, D. & McCormick, M. (2002), 'Larval growth predicts the recruitment success of a coral reef fish.', *Oecologia (Berlin)* **131**, 521–525.

Beverton, R. & Holt, S. (1957), 'On the dynamics of exploited fish populations'. Fisheries Investigations (series 2). U.K. Ministry of Agriculture and Fisheries, p533.

Bird, J., Eppler, D. & Checkley, Jr., D. (1986), 'Comparisons of herring otoliths using Fourier series shape analysis', *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 1228–1234.

Bobko, S. & Berkeley, S. (2004), 'Maturity, ovarian cycle, fecundity, and age-specific parturition of black rockfish (*Sebastes melanops*)', *Fishery bulletin* **102**, 418–429.

Bolles, K. & Begg, G. (2000), 'Distinction between silver hake (*Merluccius bilinearis*) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometrics', *Fishery bulletin* **98**, 451–462.

Booke, H. (1981), 'The conundrum of the stock concept - are nature and nurture definable in fishery science?', *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1479–1480.

Booth, A. & Buxton, C. (1997), 'Management of the panga *Pterogymnus laniarius* (pisces: Sparidae), on the Agulhas Bank, South Africa using per-recruit models', *Fisheries Research* **32**, 1–11.

Booth, J. (1986), 'Recruitment of packhorse rock lobster *Jasus verreauxi* in New Zealand.', *canadian Journal of Fisheries and Aquatic Sciences* **43**, 2212–2220.

Booth, J. & Phillips, B. (1994), 'Early life history of spiny lobster', *Crustaceana* **66**, 271–294.

Bradford, R., Bruce, B., Stephen, B., Chiswell, M., Booth, J., Jeffs, A. & Wotherspoon, S. (2005), 'Vertical distribution and diurnal migration patterns of *Jasus edwardsii* phyllosomas off the east coast of the North Island, New Zealand', *New Zealand Journal of Marine and Freshwater Research* **39**, 593–604.

Bransden, M., Cobcroft, J., Battaglene, S., Morehead, D. T., Dunstan, G. A., Nichols, P. & Kolkovski, S. (2005), 'Dietary 22:6n - 3 alters gut and liver structure and behaviour in larval striped trumpeter (*Latris lineata*).', *Aquaculture* **248**, 275–285.

Brasher, D., Ovenden, J., Booth, J. & White, R. (1992), 'Genetic subdivision of Australian and New Zealand populations of *Jasus verreauxi* (Decapodia: Palinuridae) - preliminary evidence from the mitochondrial genome.', *New Zealand Journal of Marine and Freshwater Research* **26**, 53–58.

Bruce, B. D., Evans, K., Sutton, C. A., Young, J. W. & Furlani, D. M. (2001), 'Influence of mesoscale oceanographic processes on larval distribution and stock structure in jackass morwong (*Nemadactylus macropterus* : Cheilodactylidae)', *ICES Journal of Marine Science* **58**(5), 1072–1080.

Burridge, C. & White, R. (2000), 'Molecular phylogeny of the antitropical subgenus goniistius (Perciformes: Cheilodactylidae: Cheioldactylus): evidence for multiple transequatorial divergences and non-monophly', *Biological Journal of the Linnean Society* **70**, 435–458.

Cadrin, S. & Friedland, K. (1999), 'The utility of image processing techniques for morphometric analysis and stock identification', *Fisheries Research* **43**, 129–139.

Cai, W., Shi, G., Cowan, T., Bi, D. & Ribbe, J. (2005), 'The response of the southern annular mode, the East Australian Current, and the southern mid-latitude ocean circulation to global warming.', *Geophysical Research Letters* **32**, L23706.1–L23706.4.

Campana, S. (1999), 'Chemistry and composition of fish otoliths: pathways, mechanisms and applications.', *Marine Ecology Progress Series* **188**, 263–297.

Campana, S. (2001), 'Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods', *Journal of fish biology* **59**, 197–242.

Campana, S. & Thorrold, S. (2001), 'Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations?', *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 30–38.

Campana, S., Christina Annand, M. & McMillan, J. (1995), 'Graphical and statistical methods for determining the consistency of age determinations', *Transactions of the American Fisheries Society* **124**, 131–138.

Campana, S. E. & Casselman, J. M. (1993), 'Stock discrimination using otolith shape analysis', *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 1062–1083.

Cardinale, M., Doering-Arjes, P., Kastowsky, M. & Mosegaard, H. (2004), 'Effects of sex, stock, and environment on the shape of known-age atlantic cod (gadus morhua) otoliths', *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 158–167.

Carr, M. (2000), 'Marine protected areas: challenges and opportunites for understanding and conserving coastal marine ecosystems', *Environmental Conservation* **27**(2), 106–109.

Carr, M. & Raimondi, P. (1999), 'Marine protected areas as a precautionary approach to management.', *CalCOFI report* **40**, 71–76.

Castilla, J. (2000), 'Roles of experimental marine ecology in coastal management and conservation.', *Journal of experimental Marine Biology and Ecology* **250**, 3–21.

Castonguay, M., Simard, P. & Gagnon, P. (1991), 'Usefulness of Fourier analysis of otolith shape for atlantic mackerel (*Scomber scombrus*) stock discrimination', *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 296–302.

Cerrato, R. M. (1990), 'Interpretable statistical tests for growth comparisons using parameters in the von bertalanffy equation', *Canadian Journal of Fisheries and Aquatic Sciences* (7), 1416–1426.

Chen, S., Lestrel, P., Kerr, W. & McColl, J. (2000), 'Describing shape changes in the human mandible using elliptical Fourier functions', *European journal of Orthodontics* **22**, 205–216.

Chen, Y., Jackson, A. & Harvey, H. (1992), 'A comparison of von bertalanffy and polynomial functions in modelling fish growth data', *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 1228–1235.

Chiswell, S., Wilkin, J., Booth, J. & Stanton, B. (2003), 'Trans-tasman sea larval transport: Is Australia a source for New Zealand rock lobsters?', *Marine Ecology progress series* **247**, 173–182.

Cobcroft, J., Pankhurst, N., Sadler, J. & Hart, P. (2001), 'Jaw development and malformation in cultured striped trumpeter *Latris lineata*', *Aquaculture* **199**, 267–282.

Collette, B. & Parin, N. (1991), 'Shallow-water fishes of the walters shoals, Madagascar Ridge', *Bulletin of Marine Science* **48**(1), 1–22.

Cowen, R. & Castro, L. (1994), 'Relation of coral reef fish larvae distributions to island scale distribution around Barbados, West Indies.', *Bulletin of Marine Science* **54**, 228–244.

Cowen, R., Lwiza, K., Spongaugle, S., Paris, C. & Olson, D. (2000), 'Connectivity of marine populations: Open or closed?', *Science* **287**, 857–859.

Cowen, R., Paris, C. & Srinivasan, A. (2006), 'Scaling of connectivity in marine populations.', *Science* **311**, 522–527.

Creasey, S. & Rogers, A. (1999), 'Population genetics of bathyal and abyssal organisms', *Advances in Marine Biology* **35**, 1–151.

Cresswell, G., Wells, G. & Petersen, J. (1994), Australian satellite tracked drifters 1991-1994, Report to Fisheries Research and Development Corporation. CSIRO Division of Oceanography: Hobart, Tas. p150.

Cushing, D. & Horwood, J. (1994), 'The growth and death of fish larvae.', *Journal of Plankton Research* **16**, 291–300.

DeMartini, E. & Roberts, D. (1990), 'Effects of giant kelp (Macrocystis) on the density and abundance of fishes in a cobble-bottom kelp forest.', *Bulletin of Marine Science* **46**(2), 287–300.

Deriso, R. B. (1987), 'Optimal f sub(0.1) criteria and their relationship to maximum sustainable yield', *Canadian Journal of Fisheries and Aquatic Sciences* **44**(Suppl. 2), 329–348.

Dudgeon, C., Gust, N. & Blair, D. (2000), 'No apparent genetic basis to demographic differences in scarid fishes across continental shelf of the Great Barrier Reef', *Marine Biology (Berlin)* **137**, 1059–1066.

Duhamel, G. (1989), 'Ichthyofaune des iles Saint Paul et Amsterdam (Ocean Indien Sud)', *Mesogee* **49**, 21–47.

Duhamel, G. (1997), 'L'ichtyofaune des iles Australes Francaises de L'Ocean Indien', *Cybium* **21**(1), 147–168.

Edgar, G., Samson, C. & Barrett, N. (2005), 'Species extinction in the marine environment: Tasmania as a regional example of overlooked losses in biodiversity.', *Conservation Biology Series (Cambridge)* **19**, 1294–1300.

Edyvane, K. (2003), Conservation, monitoring and recovery of threatened giant kelp (*Macrocystis pyrifera*) beds in Tasmania – final report. Department of Primary Industries, Water and Environment, GPO Box 44a, Hobart, TAS 7001, Australia.

Eklund, A., McClellan, D. & Harper, D. (2000), 'Black grouper aggregations in relation to protected areas within the Florida Keys national marine sanctuary.', *Bulletin of Marine Science* **66**, 721–728.

Excoffier, L., Smouse, P. & Quattro, J. (1992), 'Analysis of molecular variance inferred from metric distances and DNA haplotypes; application to human mitochondrial DNA restriction data', *Genetics* **131**, 479–491.

Fisher, R., Bellwood, D. & Job, S. (2000), 'The development of swimming abilities in reef fish larvae.', *Marine Ecology Progress Series* **202**, 163–173.

Fletcher, R. & Deriso, R. (1988), 'Fishing in dangerous waters: remarks on a controversial appeal to spawner-recruit theory for long-term impact assessment.', *American Fisheries Society Monographs* **4**, 232–244.

Fletcher, W., Chesson, J., Sainsbury, K., Hundloe, T. & Fisher, M. (2005), 'A flexible and practical framework for reporting on ecologically sustainable development for wild capture fisheries.', *Fisheries Research* **71**, 175–183.

Forrester, G. & Swearer, S. (2002), 'Trace elements in otoliths indicate the use of open-coast versus bay nursery habitats by juvenile california halibut', *Marine Ecology Progress Series* **241**, 201–213.

Forster, M. (2000), 'Key concepts in model selection: performance and generalizability', *Journal of Mathematical Psychology* **44**, 205–231.

Fowler, A. J. & Doherty, P. (1992), 'Validation of annual growth increments in the otoliths of two species of damsel fish from the southern Great Barrier Reef.', *Marine and Freshwater Research* **43**, 1057–1068.

Francis, M. (1988), *Coastal fishes of New Zealand.*, Heinemann-Reed, Auckland. p63.

Francis, M. (1993), 'Does water temperature determine year class strength in new zealand snapper (*Pagrus auratus, sparidae*)?', *Fisheries Oceanography* **22**, 65–72.

Francis, M., Mulligan, K., Davies, N. & Beentjes, M. (1999), 'Age and growth estimates for New Zealand hapuku, *Polyprion oxygeneios*', *Fishery Bulletin* **97**, 227–242.

Furlani, D. M. & Ruwald, F. (1999), 'Egg and larval development of laboratory-reared striped trumpeter *Latris lineata* (Forster in Bloch and Schneider 1801) (Percoidei: Latridiidae) from Tasmanian waters.', *New Zealand Journal of Marine and Freshwater Research* **33**, 153–162.

Garcia, S., Zerbi, A., Aliaume, C., Do Chi, T. & Lasserre, G. (2003), The ecosystem approach to fisheries. issues, terminology, principles, institutional foundations, implementation and outlook., FAO Fisheries Technical Paper. No. 443. Rome, FAO. p71.

Gauldie, R., Fournier, D. & Dunlop, D. (1986), 'Atomic emission and proton microprobe studies of the ion content of otoliths of chinook salmon aimed at recovering the temperature life history of individuals.', *Comparative Biochemistry and Physiology A Comparative Physiology* **84**, 607–615.

Gillanders, B. (2002), 'Connectivity between juvenile and adult fish populations: Do adults remain near their recruitment estuaries?', *Marine Ecology Progress Series* **240**, 215–223.

Gillanders, B. (2005), 'Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats.', *Estuarine Coastal and Shelf Science* **64**, 47–57.

Gillanders, B. & Kingsford, M. (2000), 'Elemental fingerprints of otoliths of fish may distinguish estuarine 'nursery' habitats.', *Marine Ecology Progress Series* **201**, 273–286.

Gillanders, B., Able, K., Brown, J., Eggleston, D. & Sheridan, P. (2003), 'Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries.', *Marine Ecology Progress Series* **247**, 281–295.

Gomon, M., Glover, J. & Kuiter, R. (1994), *The fishes of AustraliaâE^{TM}s south coast*, The flora and fauna of South Australia Handbooks Committee. Adelaide, State Printers. p992.

Graham, D. (1956), *A treasury of New Zealand Fishes.*, Reed, Wellington. p424.

Grewe, P., Smolenski, A. & Ward, R. (1994), 'Mitochondrial DNA variation in jack-ass morwong, *Nemadactylus macropterus* (Teleostei: Cheilodactylidae) from Australian and New Zealand waters.', *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 1101–1109.

Griffin, D., Wilkin, J., Chubb, C., Pearce, A. & Caputi, N. (2001), 'Ocean currents and the larval phase of australian western rock lobster, *Panulirus cygnus*', *Marine and Freshwater Research* **52**, 1187–1199.

Haddon, M. (2001), *Modelling and quantitative methods in fisheries.*, Chapman and Hall, Boca Raton FL. p406.

Hall, T. (1999), 'Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT', *Nucleic Acids Research* **41**, 95–98.

Hare, S., Mantua, N. & Francis, R. (1999), 'Inverse production regimes: Alaska and west coast pacific salmon.', *Fisheries* **24**, 6–14.

Harris, G., Nilsson, C., Clementson, L. & Thomas, D. (1987), 'The water masses of the east coast of Tasmania: Seasonal and interannual variability and the influence on phytoplankton biomass and productivity', *Marine and Freshwater Research* **38**(5), 569–590.

Harris, G. P., Griffiths, F. B., Clementson, L. A., Lyne, V. & van der Doe, H. (1991), 'Seasonal and interannual variability in physical processes, nutrient cycling and the structure of the food chain in Tasmanian shelf waters', *Journal of Plankton Research* **13**(suppl.), 109–131.

Hearn, W. S. & Polacheck, T. (2003), 'Estimating long-term growth-rate changes of southern bluefin tuna (*Thunnus maccoyii*) from two periods of tag-return data', *Fishery Bulletin* **101**(1), 58–74.

Higgins, D., Thompson, J., Gibson, T., Thompson, J., Higgins, D. & Gibson, T. (1994), 'Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice', *Nucleic Acids Research* **22**, 4673–4680.

Hilborn, R., Maguire, J., Parma, A. & Rosenberg, A. (2001), 'The precautionary approach and risk management: can they increase the probability of successes in fishery management?', *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 99–107.

Hixon, M., Pacala, S. & Sandin, S. (2002), 'Population regulation: historical context and contemporary challenges of open vs. closed systems.', *Ecology (New York)* **83**, 1490–1508.

Hobday, A., Hartmann, K., Hartog, J. & Bestley, S. (2006). SDODE: Spatial dynamics ocean data explorer. User Guide. CSIRO Marine and Atmospheric Research, Hobart.

Hoenig, J. M. (1983), 'Empirical use of longevity data to estimate mortality rates', *Fishery Bulletin* **82**(1), 898–903.

Huberty, C. (1994), *Applied discriminant analysis*, Wiley and Sons. New York. p496.

Hutchinson, W. (1993), The reproductive biology and induced spawning of striped trumpeter, *Latris lineata*, Masters thesis, University of Tasmania.

Ihssen, P., Booke, H., Casselman, J., McGlade, J., Payne, N. & Utter, F. (1981), 'Stock identification: Material and methods', *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1838–1855.

Ikeda, Y., Arai, N., Sakamoto, W., Mitsuhashi, M. & Yoshida, K. (1999), 'Preliminary report on pixe analysis for trace elements of octopus Dofleini statoliths.', *Fisheries Science (Tokyo)* **65**, 161–162.

Iwata, H. & Ukai, Y. (2002), 'Shape: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors', *Journal of Heredity* **93**, 384–385.

Johnson, C., Ling, S., Ross, J., Shepherd, S. & Miller, K. (2005), Establishment of the long-spined sea urchin (*Centrostephanus rodgersii*) in Tasmania: First assessment of potential threats to fisheries. University of Tasmania, Tasmanian Aquaculture and Fisheries Institute, School of Zoology..

Jordan, A. (2001), 'Age, growth and spatial and interannual trends in age composition of jackass morwong, *Nemadactylus macropterus*, in Tasmania', *Marine and Freshwater Research* **52**, 651–660.

Kailola, P., Williams, M., Stewart, P., Reichelt, R., McNee, A. & Grieve, C. (1993), *Australian fisheries resources*, Bureau of Resource Sciences, Department of Primary Industries and Energy, and the Fisheries Research and Development Corporation. Canberra, Australia.

Kamler, E. (2005), 'Parent-egg-progeny relationships in teleost fishes: an energetics perspective.', *Reviews in Fish Biology and Fisheries* **15**, 399–421.

Kawasaki, T. (1980), 'Fundamental relations among the selections of life history in marine teleosts.', *Bulletin of the Japanese Society of Fisheries* **46**, 289–293.

Kimura, D. K. (1980), 'Likelihood methods for the von bertalanffy growth curve', *Fishery Bulletin* **77**(4), 765–766.

Kingsford, M. J., Schiel, D. R. & Battershill, C. (1989), 'Distribution and abundance of fish in a rocky reef environment at the subantarctic Auckland Islands, New Zealand', *Polar Biology* **9**, 179–186.

Knight, W. (1968), 'Asymptotic growth: an example of nonsense disguised as mathematics', *Journal of the Fisheries Research Board of Canada* **25**, 1303–1307.

Kocher, T., Thomas, W., Meyer, A., Edwards, S., PÃo Aobo, S., Villablanca, F. & Wilson, A. (1989), 'Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers', *Proceedings of the National Academy of Science USA* **86**, 6196–6200.

Kuhl, F. & Giardina, C. (1982), 'Elliptic Fourier features of a closed contour', *Computer Graphics and Image Processing* **18**, 236–258.

Kumar, S., Tamura, K., Jakobsen, I. & Nei, M. (2001), 'MEGA2: molecular evolutionary genetics analysis software', *Bioinformatics (Oxford)* **17**(12), 1244–1245.

Lanave, C., Preparata, G., Saccone, C. & Serio, G. (1984), 'A new method for calculating evolutionary substitution rates', *Journal of Molecular Evolution* **20**, 86–93.

Last, P., Scott, E. & Talbot, F. (1983), *Fishes of Tasmania*, Tasmanian Fisheries Development Authority. p563.

Leis, J., Sweatman, H. & Reader, S. (1996), 'What the pelagic stages of coral reef fishes are doing out in blue water: daytime observations of larval behavioural capabilities.', *Marine and Freshwater Research* **47**, 401–411.

Lestrel, P. (1997), Fourier descriptors and their application in biology, Cambridge University Press, UK. p466.

Longerich, H., Jackson, S. & Gunther, D. (1996), 'Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation.', *Journal of Analytical Atomic Spectrometry* **11**, 613–904.

Lou, D., Mapstone, B., Russ, G. R., Davies, C. & Begg, G. (2005), 'Using otolith weight-age relationships to predict age based metrics of coral reef fish populations of different spatial scales.', *Fisheries Research* **71**, 279–294.

Ludsin, S. A., Fryer, B. J., Yang, Z., Melancon, S. & Markham, J. L. (2004), Exploration of the existence of natural reproduction in Lake Erie lake trout using otolith microchemistry. 2004 project completion report. Great Lakes fishery commission, Ann arbor, MI. p45.

Lyle, J. (2005), 2000/01 survey of recreational fishing in tasmania. TAFI Technical Report. Tasmanian Aquaculture and Fisheries Institute. p24.

Lyle, J. & Murphy, R. (2002), 'Long distance migration of striped trumpeter', *Fishing today* **14**(6), 6–7.

MacArthur, R. & Wilson, E. (1967), The theory of island biogeography. Monographs in population biology 1. Princeton University Press. Princeton, NJ. p224.

Mace, P. (1994), 'Relationships between common biological reference points used as thresholds and targets of fisheries management strategies', *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 110–122.

Macewicz, B. & Hunter, J. (1993), 'Spawning frequency and batch fecundity of jack mackerel, *Trachurus symmetricus*, off California during 1991', *CalCOFI report* **34**, 112–121.

MacLennan, D. (1993), Gear selectivity and the variation of yield, *in* 'Council meeting of the International Council for the exploration of the sea'. Dublin, Eire.

Masuda, F. (1976), 'Can the elemental contents in skeletal carbonates be used as paleothermometers?', *Journal of Geological Society Japan* **82**, 565–572.

McGarvey, R. & Fowler, A. J. (2002), 'Seasonal growth of king george whiting (*Sillaginodes punctata*) estimated from length-at-age samples of the legal-size harvest', *Fishery Bulletin* **100**(3), 545–558.

Mertz, G. & Myers, R. A. (1996), 'Influence of fecundity on recruitment variability of marine fish', *Canadian Journal of fisheries and Aquatic Sciences* **53**, 1618–1625.

Messieh, S. (1975), 'Growth of otoliths in young herring in the Bay of Fundy', *Transactions of the American Fisheries Society* **104**, 770–774.

Miller, T., Crowder, L., Rice, J. & Marschall, E. (1988), 'Larval size and recruitment mechanisms in fishes: toward a conceptual framework.', *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 1657–1670.

Morehead, D. T. (1998), 'Effect of capture, confinement and repeated sampling on plasma steroid concentrations and oocyte size in female striped trumpeter *Latris lineata*', *Marine and Freshwater Research* **49**, 373–377.

Morehead, D. T. & Hart, P. R. (2003), 'Effect of temperature on hatching success and size of striped trumpeter (*Latris lineata*) larvae', *Aquaculture* **220**(1-4), 595–606.

Muoneke, M. & Childress, W. (1994), 'Hooking mortality: a review for recreational fisheries', *Reviews in Fisheries Science* **2**, 123–156.

Murphy, R. & Lyle, J. (1999), Impact of gillnet fishing on inshore temperate reef fishes, with particular reference to banded morwong. Final report to FRDC. Project No. 95/145. Tasmanian Aquaculture and Fisheries Institute. p135.

Nei, M. (1987), Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M. & Tajima, F. (1981), 'DNA polymorphism detectable by restriction endonucleases', *Genetics* **97**, 145–163.

Nylander, J. (2004). MrModeltest 2.1. Evolutionary Biology Centre, Uppsala University.

Ovenden, J., Brasher, D. & White, R. (1992), 'Mitochondrial DNA analyses of the red rock lobster *Jasus edwardsii* supports an apparent absence of population subdivision throughout Australasia.', *Marine Biology* **112**, 319–326.

Paris, C. & Cowen, R. (2004), 'Direct evidence of a biophysical retention mechanism for coral reef fish larvae', *Limnology and Oceanography* **49**(6), 1964–1979.

Pauly, D. (1980), 'On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks', *J. Cons. CIEM* **39**(2), 175–192.

Pauly, D. (1983), 'Length-converted catch curves: A powerful tool for fisheries research in the tropics. part 1', *FISHBYTE* **1**(2), 9–13.

Pianka, E. (1970), 'On r- and k-selection.', *American Naturalist* **104**, 592–597.

Pikitch, E., Santora, C., Babcock, E., Bakun, A., Bonfil, R., Conover, D., Dayton, P., Doukakis, P., Fluharty, D., Heneman, B., Houde, E. D., Link, J., Livingston, P., Mangel, M., McAllister, M., Pope, J. & Sainsbury, K. (2004), 'Ecosystem-based fishery management.', *Science* **305**, 346–347.

Pitcher, T. & MacDonald, P. (1973), 'Two models for seasonal growth in fishes', *Journal of applied ecology* **10**(2), 599–606.

Quinn, T. J., I. & Deriso, R. B. (1999). Quantitative fish dynamics. Oxford University Press. p542.

Reynolds, R., Rayner, N., Smith, T., Stokes, D. & Wang, W. (2002), 'An improved in-situ satellite sst analysis for climate', *Journal of Climate* **15**, 1609–1625.

Ridgway, K., Dunn, J. & Wilkin, J. (2002), 'Ocean interpolation by four-dimensional weighted least squares -application to the waters around australasia. journal of atmospheric and oceanic technology', *Journal of Atmospheric and Oceanic Technology* **19**(9), 1357–1375.

Roberts, C. (2003), 'A new species of trumpeter (Teleostei: Percomorpha; Latridae) from the central South Pacific Ocean, with a taxonomic review of the striped trumpeter *Latris lineata*', *Journal of the Royal Society of New Zealand* **33**(4), 731–754.

Roff, D. (1980), 'A motion for the retirement of the von bertalanffy function', *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 127–129.

Roff, D. (1992). The evolution of life histories: theory and analysis. Chapman and Hall. p548.

Rohlf, F. (1998), 'On applications of geometric morphometrics to studies on ontogeny and phylogeny', *Systematic Biology* **47**, 147–158.

Rothschild, B. (1986). Dynamics of marine fish populations. Harvard University Press. Cambridge, Mass.

Roughgarden, J., Gaines, S. & Possingham, H. (1988), 'Recruitment dynamics in complex life cycles.', *Science* **241**, 1460–1466.

Ruwald, F. (1992), Larval feeding trials with striped trumpeter, *Latris lineata*., *in* D. Hancock, ed., 'In Larval biology. Australian Society for Fish Biology Workshop, Hobart, 20 August 1991.', Bureau of Rural Resources Proceedings No. 15, AGPS, Canberra.

Sainsbury, K. (1979), 'Effect of individual variability on the von bertalanffy growth equation', *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 241–247.

Sainsbury, K., Punt, A. & Smith, A. (2000), 'Design of operational management strategies for achieving fishery ecosystem objectives.', *ICES Journal of Marine Science* **57**, 731–741.

Saitou, N. & Nei, M. (1987), 'The neighbor-joining method; a new method for reconstructing phylogenetic trees', *Molecular Biology and Evolution* **4**, 406–425.

Schneider, S., Roessli, D. & Excoffier, L. (2000). Arlequin: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneve. Switzerland.

Schnute, J. (1981), 'A versatile growth model with statistically stable parameters', *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1128–1140.

Sissenwine, M. & Shepherd, J. (1987), 'An alternative perspective on recruitment overfishing and biological reference points', *Canadian journal of fisheries and Aquatic Sciences* **44**, 913–918.

Smith, S., Buddermeier, R., Redalje, R. & Houck, J. (1979), 'Strontium-calcium thermometry in coral skeletons.', *Science* **204**, 404–407.

Sponaugle, S. & Cowen, R. (1997), 'Early life history traits and recruitment patterns of caribbean wrasses (Labridae).', *Ecological Monographs* **67**, 177–202.

St John, J. & Syers, C. (2005), 'Mortality of the demersal West Australian dhufish, *Glaucosoma hebraicum* (Richardson, 1845) following catch and release: the influence of capture depth, venting and hooking type', *Fisheries Research* **76**, 106–116.

Stearns, S. (1992). The evolution of life histories., Oxford University Press. p264.

Stevenson, R. (1999), 'Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units', *Fisheries Research* **43**, 247–249.

Stobutzki, I. & Bellwood, D. (1994), 'An analysis of the sustained swimming abilities of pre- and post-settlement coral reef fishes.', *Journal of experimental Marine Biology and Ecology* **175**, 275–296.

Swofford, D. (2002). PAUP* ver 4.0b10. Phylogenetic analysis using parsimony and other methods. Sinauer Associates. Sunderland, MA.

Tamura, K. & Nei, M. (1993), 'Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees', *Molecular Biology and Evolution* **10**, 512–526.

Tasmania Royal Commission into Fisheries of Tasmania (1882), Tasmanian edible fishes & laws relating to the preservation of fish. Journals and Printed Papers of the Legislative Council XXXIII (92).

Thompson, W. & Bell, F. (1934), 'Biological statistics of the pacific halibut fishery. 2. effect of changes in intensity upon total yield and yield per unit of gear', *Reports of the internatioanl fisheries (Pacific Halibut) commision* **8**, 49.

Thresher, R. (2004), 'Long-term trends in the latitude of the sub-tropical ridges over south-east australia: climate correlations and conseqeunces.', *Proc. 7th Internat. Conf. Southern hemisphere Meteoral. Oceanogr.* pp. 166–168.

Thresher, R., Bruce, B., Furlani, D. & J.S., G. (1988), 'Distribution, advection and growth of larvae of the southern temperate gadoid *Macruronus novaezelandiae* in Australian coastal waters.', *Fishery Bulletin* **87**, 29–48.

Thresher, R. E., Proctor, C. H., Gunn, J. S. & Harrowfield, I. R. (1994), 'An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae).', *Fishery Bulletin* **92**, 817–840.

Torres, G., Lombarte, A. & Morales-Nin, B. (2000), 'Sagittal otolith size and shape variability to identify geographical differences in three species of the genus Merluccius', *Journal of the Marine Biological Association of the United Kingdom* **80**, 333–342.

Tracey, S. & Lyle, J. (2005), 'Age validation, growth modeling, and mortality estimates of striped trumpeter (*Latris lineata*) from southeastern Australia: making the most of patchy data', *Fishery bulletin* **103**(1), 169–182.

Tracey, S., Lyle, J. & Duhamel, G. (2006), 'Application of elliptical Fourier analysis of otolith form as a tool for stock identification', *Fisheries Research* **77**, 138–147.

Tracey, S., Lyle, J. & Haddon, M. (2007), 'Reproductive biology and per-recruit analyses of striped trumpeter (*Latris lineata*) from Tasmania, Australia: Implications for management', *Fisheries Research* **84**, 358–367.

Tracey, S., Smolenski, A. & Lyle, J. (2007), 'Genetic structuring of *Latris lineata* at localized and transoceanic scales', *Marine Biology* **152**, 119–128.

Trotter, A. J., Pankhurst, P. & Battaglene, S. (2005), 'A finite interval of initial swimbladder inflation in *Latris lineata* revealed by sequential removal of water-surface films', *Journal of Fish Biology* **67**, 730–741.

Utter, F. (1991), 'Biochemical genetics and fisheries management: an historical perspective', *Journal of Fish Biology* **39**, 1–20.

Wakeley, J. (1993), 'Substitution rate variation among sites in hypervariable region 1 of human mitochondrial DNA', *Journal of Molecular Evolution* **37**, 613–623.

Waples, R. (1998), 'Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species', *journal of Heredity* **89**, 438–450.

Wenink, P., Baker, A. & Tilanus, M. (1994), 'Mitochondrial control region sequences in two shorebird species, the turnstone and the dunlin, and their utility in population genetic studies', *Molecular Biology and Evolution* **22**, 22–31.

West, G. (1990), 'Methods of assessing ovarian development in fishes: a review', *Marine and Freshwater Research* **41**, 199–222.

Willis, T. J. & Anderson, M. (2003), 'Structure of cryptic reef fish assemblages: relationships with habitat characteristics and predator density', *Marine Ecology progress series* **257**, 209–221.

Winemiller, K. (2005), 'Life history strategies, population regulation, and implications for fisheries management.', *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 872–885.

Winemiller, K. & Rose, K. (1992), 'Patterns of life-history diversification in North American fishes: implications for population regulation', *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 2196–2218.

Xia, X. & Xie, Z. (2001), 'DAMBE: data analysis in molecular biology and evolution', *Journal of Heredity* **92**, 371–373.

Young, J. W., Jordan, A. R., Bobbi, C., Johannes, R. E., Haskard, K. & Pullen, G. (1993), 'Seasonal and interannual variability in krill (*Nyctiphanes australis*) stocks and their relationship to the fishery for jack mackerel (*Trachurus declivis*) off eastern Tasmania, Australia', *Marine Biology* **116**(1), 9–18.

Ziegler, P., Lyle, J., Haddon, M., Moltschaniwskyj, N. & Tracey, S. (2006), Tasmanian scalefish fishery assessment - 2005. TAFI Fishery Assessment Report. Tasmanian Aquaculture and Fisheries Institute. p136.