FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Spawning sources, movement patterns, and nursery area replenishment of spawning populations of King George Whiting in south-eastern Australia — closing the life history loop

Gregory P. Jenkins, Paul A. Hamer, Julia A. Kent, Jodie Kemp, Craig Sherman, and Anthony J. Fowler

April 2016

FRDC Project No **2011/017**

FRDC

THE UNIVERSITY OF MELBOURNE

Your fishing licence fees at work

VICTORIA State Government

Version 1.0 1 July 2013

ISBN 978 0 7340 5270 4

Spawning sources, movement patterns, and nursery area replenishment of spawning populations of King George Whiting in south-eastern Australia — closing the life history loop

2011/017

2016

Researcher Contact Details

Name:	Professor Gregory Jenkins
Address:	School of BioSciences, University of Melbourne Vic 3010
Phone:	03 90359973
Fax:	03 83447909
Email:	gjenkins@unimelb.edu.au

FRDC Contact Details

Address:	25 Geils Court Deakin ACT 2600
Phone:	02 6285 0400
Fax:	02 6285 0499
Email:	frdc@frdc.com.au
Web:	www.frdc.com.au

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

Tables

Figures

Acknowledgments

We thank David Hatton, Bruce Jackson, Andrew Brown, Peter Woodruff, Michael Hayley, Rob Harrison, Neville Clark and Nick Scerri for assistance with field sampling. We especially thank Craig Garland for his unflinching efforts in catching large King George Whiting for us in north-west Tasmania. We thank Dr Kim Smith (WA), and Grant Pullen and Andrew Pender (Tas) for assistance in obtaining samples from those States. Dr Hannah Murphy assisted with laboratory analyses of post-larvae.

We greatly appreciate the funding provided for this project by the Fisheries Research and Development Corporation, the Victorian Recreational Fishing Licence Large Grants Scheme and the Victorian Government through Fisheries Victoria.

Abbreviations

ANOVA	Analysis of variance
CI	Corner Inlet
GBS	Genotype-by-sequencing
GSI	Gonadosomatic index
GSt	Gulf St Vincent
HWE	Hardy-Weinberg Equilibrium
KGW	King George Whiting
KI	Kangaroo Island
LA-ICPMS	Laser ablation – inductively coupled plasma mass spectrometry
LOD	Limit of detection
MANOVA	Multivariate analysis of variance
NGS	Next-generation sequencing
NWTas	North-western Tasmania
PLD	Pelagic larval duration
PPB	Port Phillip Bay
QDFA	Quadratic discriminate function analysis
SA	South Australia
SG	Spencer Gulf
SNP	Single nucleotide polymorphism
WestVic	Western Victoria
WP	Western Port

Executive Summary

Overview

This was a collaborative project amongst scientists from the University of Melbourne, Fisheries Victoria, Deakin University and the South Australian Research and Development Institute. The project led to major advances in our understanding of the biology and population structure of King George Whiting in southern Australia. The project was able to demonstrate that Whiting in the Victorian and South Australian fisheries come from different spawning areas, and that adult Whiting from Victoria do not migrate to the known Whiting spawning area in South Australia. The project also identified a previously unknown spawning area for King George Whiting in north-west Tasmania. King George Whiting in Tasmania (2 populations) and Western Australia were found to be genetically distinct from Whiting in Victoria and South Australia.

The study, conducted over 4 years, was designed to determine whether single (State) jurisdictional management of the King George Whiting fishery was appropriate in relation to the population (stock) structure of the species. Innovative methods were used to determine population structure including otolith chemistry and advanced genetic analyses. The results support the current State (Jurisdictional) based management of the fisheries, however further work is recommended to completely clarify the relationship between the Victorian and South Australian King George Whiting populations.

Background

King George Whiting support commercial and recreational fisheries in all southern States and, along with snapper, are the most important finfish fisheries in Victoria and South Australia. The stock structure and population connectivity of the species is uncertain, and there is particular uncertainty about the relationship between Victorian and South Australian stocks. This is because studies modelling the dispersal of King George Whiting larvae to Victorian nursery areas suggests that the spawning area ranges from far west Victoria into south-eastern South Australia, and almost as far west as the known spawning ground in the Investigator Strait region of central South Australia. Thus, there is the possibility that known spawning area in South Australia is also a spawning source for Whiting in Victoria. This would call into question the current State-based (jurisdictional) management of the species and support the need for cross-jurisdictional management.

In the course of this project the researcher became aware of a small fishery for large King George Whiting in north-west Tasmania. This opened the possibility that a previously unidentified spawning ground occurred in this area and a potential spawning source of Whiting recruits to Tasmania and the mainland. Thus, understanding more about this potential source and its relationship to Whiting in other States was a further key question in relation to population connectivity and the need for cross-jurisdictional management.

Aims/objectives

1. To determine whether King George Whiting in juvenile nursery areas of Victoria and South Australia are derived from the same or different spawning sources
2. To understand the movement patterns of juvenile King George Whiting between different nursery areas
3. To determine the relative importance of juvenile King George Whiting from different nursery areas to the replenishment of the known spawning populations in South Australia
4. Based on a full understanding of the life history and stock structure of the species to provide information informing decision making by managers in both States relating to the need for cross-jurisdictional management
5. To determine if the large King George Whiting captured in the North-west Tasmanian commercial fishery represent a previously unknown spawning population and a possible larval source for mainland populations

Methodology

Spawning sources of Whiting populations in South Australia and Victoria were analysed using otolith chemistry and microstructure (daily increment widths) in the core of otoliths from post-larvae sampled from three nursery areas in each State in the spring of 2011 and 2012. Movement of juvenile Whiting between bays in Victoria was analysed for 2+ age juveniles using chemistry of the region of the otolith formed 2-3 months after settlement in bays. Potential movement of adult Victorian Whiting to the known South Australian spawning ground was analysed by establishing baseline chemical and stable isotope signatures for 3+ age juveniles from the 3 major nursery areas in each of Victoria and South Australia and then back-classifying adults from the kangaroo Island spawning area back to these nurseries. The region of the otolith between the first and second annual increments was analysed for chemistry and stable isotopes in both juveniles and adults. Otolith chemistry was also used to analyse life history transects across otoliths of adults from South Australia, Victoria and Tasmania as a method of population/stock discrimination.

Genetic analyses for population structure were undertaken using Microsatellite and SNPs (single nucleotide polymorphisms). Microsatellites were analysed for post-larvae collected from Victoria and South Australia in 2011. SNP analyses were conducted on post-larvae from the same sites in Victoria and South Australia in 2012, and additionally included samples from Western Australia (post-larvae and fin clips) and Tasmania (fin clips).

The age structure and reproductive status of a population of large King George Whiting from north-west Tasmania was analysed using annual increments on otoliths together with macroscopic and microscopic staging of gonad development. Samples were collected by a commercial fisher from September 2013 to June 2015.

Results/key findings

Chemical and microstructure analyses for the otolith cores of post-larvae in Victoria and South Australia indicated that spawning sources for the two States were different. Early larval growth rates were higher for post-larvae in South Australia and physiologically controlled elements such as magnesium showed significant differences. Post-settlement chemical signatures in otoliths from 2+ age Whiting in Victorian bays were significantly different, showing that juveniles up to that age were not mixing between nursery bays. Distinct chemical and isotopic signatures were identified in otoliths of juvenile Whiting from nursery bays in Victoria and South Australia. Signatures from the same region of otoliths from adult Whiting from the Investigator Strait region overlapped with the South Australian but not the Victorian juvenile baselines, indicating that Victorian Whiting were not moving to the known South Australian spawning area.

Genetic studies with microsatellites and SNPs confirmed the results of previous studies indicating that Whiting in Victoria and South Australia were not genetically separated, indicating that at least a small amount of mixing must occur between the two populations. SNP analysis, however, showed that these Whiting were genetically distinct from those in Western Australia and Tasmania, and there was further subdivision of genetic stocks between north-west and north-east Tasmania.

Age and reproductive analysis of the population of large Whiting off north-west Tasmania showed that this population included individuals up to 18 years of age, and had a similar age structure the known spawning area in South Australia. Macroscopic and microscopic analysis of gonad development indicated that Whiting were spawning in the area and that the seasonality of spawning was similar to South Australia, with spawning occurring in the autumn months.

Implications for relevant stakeholders

The implications for management and Industry are firstly that that weight of evidence from this study supports the contention that fisheries in the different States are based on separate populations or stocks, and therefore the current State (jurisdictional) based management is appropriate. Indeed that the results that management may be required at a finer geographical scale than the State level in Tasmania where two genetically distinct populations were identified, and in Victoria where juvenile Whiting that are the primary target of fishing do not mix between bays and therefore each bay should potentially be managed independently. Management on the basis of sub-stocks is already occurring in South Australia.

In terms of the Whiting fishery in Tasmania, managers will in future need to take into account that the north-west coast of the State is a spawning area for the species, and some level of protection for fish in this area, such as closed areas, closed seasons, or maximum size limits may be needed in the future as the fishery develops.

Recommendations

Although our otolith chemistry and microstructure results provide compelling evidence that the Victorian and South Australian stocks are separate, one caveat is the genetic homogeneity across the two stocks indicating that at least a small amount of mixing is occurring. Such mixing would most likely be the result of adult fish from the South Australian population moving south-east and mixing with the spawning population that is the source of Victorian Whiting recruits. Further studies on movement of Whiting based on conventional or acoustic tagging could help resolve the level of mixing between the stocks and whether it is sufficient to be relevant to cross-jurisdictional management.

Keywords

King George Whiting; *Sillaginodes punctatus*; connectivity; population; stock structure; cross-jurisdictional management; otolith chemistry; genetics

Introduction

Background

King George Whiting support one of the most important recreational and commercial fisheries in Victoria and South Australia (SA) (DPI 2008; Fowler et al. 2008; Kemp et al., 2012b; Brown et al., 2013; Fowler et al., 2014). King George Whiting remains the most valuable finfish species in Victoria and is now the second most valuable in SA (Knight and Tsolos 2011; DPI 2010). King George Whiting is also an iconic recreational fishing species and supports major recreational fisheries, with the recreational catch being similar to the commercial catch in SA, but exceeding the commercial catch in Victoria (DPI 2008; Jones 2009). Despite the major importance of this species to recreational and commercial fishing, there are still major gaps in our knowledge of the life cycle, and particularly our understanding of how the stock is distributed across the two States, and the movement patterns, if any, between States. This lack of knowledge of the life cycle is a significant impediment to determining whether single jurisdictional management is suitable for this species.

In both States there is a settlement of post-larvae (approximately 2 cm in length) into shallow seagrass beds within bays and gulfs in winter/spring. These larvae have been drifting in the water column for three to four months before settlement. These juveniles then grow for 3 to 4 years within the sheltered bays and gulfs until they move offshore at about the time of reaching sexual maturity. The majority of the King George Whiting catch is taken in the bays and gulfs between the times of reaching minimum legal length and the point where offshore migration occurs.

The only known spawning area for King George Whiting across the two States, based on collection of running ripe adults and eggs and young larvae, is near the mouth of Spencer Gulf and Gulf St Vincent, as well as Investigator Strait north of Kangaroo Island (Fowler et al. 2008). Extensive sampling of adult King George Whiting along the coast of Victoria did not find any evidence of significant spawning of Whiting, and there was a trend for Whiting to be larger and older in the west of the State, indicating a possible movement of fish to the west (Hamer et al. 2004).

Daily rings on otoliths (ear bones) of post-larvae showed that juvenile Whiting in both States come from spawning in autumn to early winter. A computer model of current patterns and larval drift confirmed that in SA the spawning was likely to be relatively local (Fowler et al. 2000). In contrast, the spawning area for King George Whiting in Port Phillip Bay and Western Port was predicted to be 100s of kilometres to the west, including south-eastern SA, while for Corner Inlet a possible spawning area in eastern Victoria was identified (Jenkins et al. 2000). The results for Port Phillip Bay and Western Port, and to some extent Corner Inlet, suggested that Victorian King George Whiting may come from the known SA spawning grounds.

Notwithstanding this previous research, there are still significant gaps in our knowledge of the life cycle of King George Whiting that limits our ability to successfully manage this important fishery across the two States. We know that Whiting in the Victorian bays come from spawning offshore, but we don't know the locations, with some evidence suggesting they come from spawning in South Australia. We also don't know whether juvenile Whiting (less than 4 years old) move between bays in Victoria, although there is thought to be only limited movement of juvenile King George Whiting between gulfs and west coast bays in SA. Finally, we don't know where mature King George Whiting from Victorian bays are spawning offshore, although there is some evidence that they may migrate west towards the SA spawning grounds.

There are a number of implications of these knowledge gaps for the Whiting fishery. For example, if Whiting in Victorian bays are shown to have been spawned in South Australia, where the only significant fishery on adult Whiting occurs, then the Victorian fishery will need to be taken into account when managing the adult fishery in SA. If it is shown that adult fish from Victorian bays are migrating to South Australia for spawning, then the effect of fishing in Victoria on the spawning stock in SA will have to be considered by management. Overall, the management of the fishery would need to be a joint effort across the two States, rather than being managed separately as is presently the case. In relation to juvenile Whiting caught in Victorian bays, if juveniles do not move between bays then there is a case for the Whiting fishery to be managed separately in each bay. However, if they do move between bays in significant numbers then management should be more broadly focussed across bays.

This project will use the otolith chemistry technique to fill these life history knowledge gaps. Unique chemical signatures laid down in otoliths (ear bones) can be used as a natural tag to determine the origin and movement patterns of fish. This technique has already been used successfully in Victoria for snapper, showing that Port Phillip Bay is the primary spawning and nursery area (FRDC 1999/134). This project will use otolith chemistry to determine whether Whiting in Victoria are spawned in the area of the SA adult fishery or elsewhere. It will also determine whether adult Whiting from Victorian bays are migrating to the known South Australian spawning grounds. Finally, the technique will be used to determine whether juveniles move between bays in Victoria.

Modern genetics techniques will also be used in conjunction with the otolith chemistry results to provide crucial information about the level of connectivity between and within regions, and identify if Victorian populations are derived from South Australian stocks. Genetic studies have undergone a revolution in the past decade, with next-generation sequencing (NGS) methods becoming more readily available and cost effective. The recent improvement in NGS and bioinformatics has prompted a shift from analyses of microsatellite markers to direct sequence variation including single nucleotide polymorphisms (SNPs); single base pair variations that occur widely throughout the genome. This allows for wider, more detailed genome coverage than other types of markers, and as a result, datasets containing thousands of loci can now be examined, resulting in increases in power and accuracy (Allendorf et al., 2010). Previous studies of King George Whiting population structure using microsatellite markers had poor sample coverage of Victoria and used a limited number of genetic markers. Genetic studies will be invaluable as an alternative line of evidence to support the otolith chemistry results.

In the course of this project the investigators became aware that a small mesh-net fishery had recently developed for large King George Whiting on the north-west coast of Tasmania. In terms of understanding the population structure and connectivity of King George Whiting in south-eastern Australia, a key question was whether this recently-identified population represented a spawning population. Previous studies using reverse hydrodynamic modelling (Jenkins et al., 2000) had indicated that dispersal of larvae from north-west Tasmania to Victorian nursery areas was possible. An extension to the project was granted to study the age structure and reproductive characteristics of the population, together with otolith chemistry and genetic analyses.

Need

Fisheries that have populations/stocks straddling cross-jurisdictional boundaries face particular challenges in terms of sustainable management. This is particularly the case when aspects of the species' life history are poorly understood. Fisheries managers require a clear understanding of stock-structure and life history of key species for sustainable management, particularly where the fishery is cross-jurisdictional and requires co-operation between different management agencies.

The King George Whiting fishery is an extremely important recreational and commercial fishery in southern Australia, especially in bays and inlets of Victoria and South Australia. There is some evidence that much of the Victorian fishery for King George Whiting is dependent on spawning in South Australia and extensive larval drift through Bass Strait. The degree to which adult Whiting from Victorian juvenile nursery areas migrate to South Australia for spawning is unknown, with previous research suggesting a possible migration of adult fish from central Victoria to the west. The degree to which juveniles move between nursery areas is also unknown; with no previous research in Victoria. There is a need to understand the present relationship between Victorian and South Australian King George Whiting stocks; are they strongly dependent or are they independent? The management of this species would be greatly improved if we understood the stock structure. For example, the species is presently managed independently by Victoria and South Australia but we do not understand how the populations in the two States relate to each other. Recent reports of a population of large King George Whiting off the coast of north-west Tasmania leads to the possibility of a previously unidentified spawning area and potential connectivity with Whiting populations in mainland jurisdictions.

Objectives

1. To determine whether King George Whiting in juvenile nursery areas of Victoria and South Australia are derived from the same or different spawning sources
2. To understand the movement patterns of juvenile King George Whiting between different nursery areas
3. To determine the relative importance of juvenile King George Whiting from different nursery areas to the replenishment of the known spawning populations in South Australia
4. Based on a full understanding of the life history and stock structure of the species to provide information informing decision making by managers in both States relating to the need for cross-jurisdictional management
5. To determine if the large King George Whiting captured in the North-west Tasmanian commercial fishery represent a previously unknown spawning population and a possible larval source for mainland populations

General Methodology

Field collection of 0+ age post-larvae

Post-larvae of King George Whiting (Figure 1) were collected from sites within three nursery areas in Victoria (Port Phillip Bay, Western Port and Corner Inlet) and South Australia (Gulf St Vincent, Spencer Gulf and Kangaroo Island) (Figure 2). Samples from Port Phillip Bay came from the annual monitoring program of eight sites (Jenkins and King, 2006).

Samples were collected over two sampling years, 2011 and 2012, in the spring, early summer period. Two rounds of sampling were conducted in each State in each year. In 2011, samples from Victoria were collected from all sites from October 12 to October 26 and repeated from November 15 to December 7. In South Australia in 2011, all sites were sampled from October 10 to October 26, and sampling was repeated for Gulf St Vincent sites from November 11 to November 14. In 2012, samples from Victoria from all sites were collected from October 16 to October 31 and repeated from November 13 to November 27. In South Australia in 2012, all sites were sampled from October 15 to October 30, and sampling was repeated for Gulf St Vincent sites from November 15 to November 16.

Samples were collected from shallow, subtidal (0.5 m below Mean Low Water Spring tide) seagrass beds with a small (10 m x 2 m), fine-mesh (1 mm) research seine. Samples were collected within 3 hours of low tide. Hauls of the net were taken until a sample of 30 post-larvae was collected.

In 2011, individual post larvae were placed in separate vials and frozen for later analysis. Because of some cracking of otoliths collected in 2011, a different approach was taken 2012 where post-larvae were preserved in absolute (95%) ethanol.

Figure 1. Post-larvae of King George Whiting

Figure 2. Sites sampled for 0+ age post-larvae of King George Whiting in Victoria and South Australia

Chapter 1 Inferences on spawning sources of King George Whiting post-larvae in Victoria and South Australia from otolith microstructure and chemistry

Introduction

Marine populations can be connected on large spatial scales as a result of significant larval dispersal and also movement and migration of juveniles and adults. The long duration of the pelagic larval phase of many marine fish (weeks to months) can potentially lead to dispersal over significant distances with currents (Simpson et al., 2014). Long larval duration, however, may not always lead to significant dispersal distances where currents are recirculating or larval behaviour and swimming ability can lead to modified dispersal trajectories and high rates of self-recruitment (Jones et al., 2009). For fishery species where the scale of larval dispersal is large there is potential for the scale of the population to exceed the jurisdictional boundaries in place for fisheries management. Therefore, understanding the spawning sources and scale of larval dispersal of exploited fish populations is a key requirement for the rational management of the fishery, particularly in relation to the need for cross-jurisdictional management.

In terms of understanding the larval dispersal and population connectivity of marine fish, otoliths from larvae and juveniles provide significant information in relation to larval duration and growth rates based on daily increments (Sponaugle, 2010), as well as the chemistry information that is incorporated into otoliths (Elsdon et al., 2008). Information on larval duration together with hydrodynamics and larval behaviour can be combined into biophysical models that can be used to predict spawning sources. Information on growth rates and otolith chemistry from the early larval stage contained in otoliths can be used to help determine whether different groups of juveniles or adults are derived from the same or different spawning sources.

Daily increment widths in the core region of otoliths acting as a proxy for larval growth are likely to be strongly influenced by water temperature and also food availability (Jenkins and King, 2006). Therefore differences in water temperature and planktonic food production between spawning areas may be reflected in daily increment widths near the core of the otolith. Larval growth reflected by increment widths may also be influenced by parental effects (Green and McCormick, 2005; Nikolaus Probst et al., 2006).

Differences in the chemistry of the core region of otoliths from larvae and juveniles may reflect differences in water chemistry and environmental conditions such as temperature and salinity associated with different spawning areas (Elsdon et al., 2008). Alternatively, differences could reflect physiologically driven variation in chemical incorporation due to differing metabolic and growth rates (Martin and Thorrold, 2005; DiMaria et al., 2010; Woodcock et al., 2012). Some elements are known to be more strongly associated with ambient water concentrations, modified by environmental conditions such as temperature and salinity (e.g. Ba and Sr) while others may be predominantly related to physiological regulation (e.g. Mg) (Martin and Thorrold, 2005; DiMaria et al., 2010; Woodcock et al., 2012; Barnes and Gillanders, 2013). Whatever the mechanism, differences in chemical composition of the core region of otoliths can provide evidence for separation of spawning sources. Results are less informative, however, when no differences are found because this

could mean fish come from the same spawning source, or alternatively that they come from different spawning sources with similar ambient water chemistry and environmental conditions (Kerr and Campana, 2013).

Studies of otolith daily increments from King George Whiting post-larvae settling in coastal nursery areas revealed that the larval duration was long (3 – 5 months) allowing the possibility of significant dispersal distances (Jenkins and May, 1994; Fowler and Short, 1996). Until the current study (Chapter 4) the only known spawning area for King George Whiting in south-eastern Australia was the Investigator Strait/Kangaroo Island area in South Australia (Figure 2) (Fowler et al., 1999; Fowler et al., 2000b). Although King George Whiting support an important coastal fishery in central Victoria, there is little evidence that fish spawn in that State (Hamer et al., 2004). Reverse hydrodynamic modelling of larval dispersal to Victorian and South Australian nursery areas indicated that while Whiting in the main fishery area in South Australia are likely to spawn relatively locally in the Investigator Strait area (Fowler et al., 2000a) post-larvae settling in central Victorian bays are likely to have been transported hundreds of kilometres from the west from a source area straddling the Victorian / South Australian border (Jenkins et al., 2000). Modelling was based on a 2-dimensional grid and did not include larval behaviour, so the possibility that Whiting settling in central Victorian bays originated from the known South Australian spawning area could be ruled out.

At present, the King George Whiting fisheries in Victoria and South Australia are managed separately by each jurisdiction. However, the possibility that King George Whiting in the Victorian fishery come from a spawning area in South Australia, and potentially the same spawning area that is the source of recruits for the South Australian fishery, raises the possible need for cross-jurisdictional management. In this chapter we address the question of whether King George Whiting in juvenile nursery areas of Victoria and South Australia are derived from the same or different spawning sources, using a combination of larval otolith microstructure and chemistry (Objective 1 of the project). This information is critical in determining whether the current arrangement of separate management by individual jurisdictions is appropriate.

Methodology

Laboratory analysis

Otolith preparation

Port-larval King George Whiting (Figure 1) were measured and their sagittal otoliths were removed under a dissecting microscope with stainless steel needles. Dissected otoliths were triple rinsed in Milli-Q water in an Eppendorf tube, and then placed in clean 0.2 ml Eppendorf PCR tubes to dry. One of the dried otoliths from each fish was embedded in a small disc of epoxy resin (Struers Epofix) set within a cut section of drinking straw. Once cured, the disks were removed from the straw and then mounted on to glass microscope slides with crystal bond, and polished in the sagittal plane to the primordium (Figure 3) from the proximal surface down with lapping films (3M^{TM} diamond films) lubricated with Milli-Q water. The polished sections were then moved onto a new slide for analysis, each of which had up to 24 samples fixed. The analysis slide was then sonicated in Milli-Q water for 3 minutes, liberally rinsed in Milli-Q water, dried and stored in a plastic container. Preparation order of individual otoliths was randomised and the 24 samples fixed to each slide consisted of 4 randomly selected otoliths from each of the 6 regions. This meant that samples analysed within any particular block of ICP-MS time were a random assortment from all regions.

Otolith chemical analysis

Otoliths were analysed for elemental chemistry using laser ablation ICP-MS (New Wave Research UP-213 Nd:YAG ultraviolet laser microprobe coupled to a ThermoFinnigan Element 2 high resolution inductively coupled plasma mass spectrometer). For this chapter each otolith section was ablated in two zones using a 40 µm diameter point sample. The two zones were: 1) centred on the primordium – termed 'core', and 2) approximately 100 µm out from the primordium along the longest axis, termed 'mid', a third zone adjacent to otolith margin was sampled for use in chapter 2 (Figure 3). The sample over the primordium incorporated the primordium and the first 15 days of larval life, which is assumed to have been insufficient time for larvae from a common spawning source to disperse into different environments. The sample at 100 µm out from the primordium represents the time period from approximately 50–80 days post-hatch. By this point in the larval life, if fish had dispersed along different routes they would be expected to be widely enough separated to be potentially subject to different oceanographic/environmental conditions.

The laser settings were 40 µm beam diameter, repetition rate of 5 Hz and fluence of 11 J cm^{-2}. Otolith surfaces were briefly pre-ablated (3 seconds with above settings) to remove any residual surface contamination. Each sample point was ablated for approximately 30 seconds to obtain 30 measurements of each isotope, with an additional 20 measurement of the blank sample gases taken prior to each ablation. Data were collected by the ICP-MS for the isotopes ^{25}Mg (magnesium), ^{55}Mn (manganese), ^{65}Cu (copper), ^{66}Zn (zinc), ^{88}Sr (strontium), ^{85}Rb (rubidium), ^{138}Ba (barium), and ^{208}Pb (lead), along with ^{43}Ca (calcium) which was used as the internal standard with a concentration in otolith of 388,000 µg g^{-1} (Yoshinaga et al., 2000). The sample ablation data were blank subtracted before being integrated for the calculation of element:calcium ratios. Calibration was achieved with the National Institute of Standards (NIST) 612 glass wafer using calculation methods described in (Ludden et al., 1995; Lahaye et al., 1997; Hamer et al., 2003) and expressed as ratios to Ca (µmol mol^{-1}). Replicate calibration standards were analysed after every sequence of 12 otolith ablations and each sequence consisted of a random selection of otoliths from the sampling regions. Detection limits (LOD) were calculated for each sample based on three standard deviations of blank gas samples taken at the beginning and end of each analysis day and were adjusted for ablation yield of each sample (Lahaye et al., 1997). Mean LODs were (ppm): Mg^{25} = 7.17, Mn^{55} = 0.44, Cu^{65} = 1.03, Sr^{88} = 1.72, Ba^{138} = 0.17. Precision and recovery for the NIST 612 standard were: recovery %, mean/S.D., Mg = 103/8, Mn = 100/4, Cu = 103/7, Zn = 100/6, Sr = 100/6, Ba = 100/6, precision RSD (%): Mg = 5, Mn = 4, Cu = 7, Zn = 6, Sr = 6, Ba = 6. Cu:Ca was not used in data analyses due to many data being below LOD, and over 20% of sample data registering negative values after blank subtractions.

Otolith microstructure

Otoliths (sagittae and lapilli) were dissected from *S. punctatus* post-larvae using tungsten needles under a dissecting microscope with a polarising light source. Sagittae were mounted on glass microscope slides in crystal bond and polished with 30 micron and 5 micron lapping film until increments from the primordium could be clearly seen. Lapilli were left to dry on slides and then placed in immersion oil. Otolith increment counts and measurements were made using a compound microscope under a magnification of 1000x .

Increment counts

Otoliths were randomly selected from *S. punctatus* collected in 2012. Sagittae were used for analysis of pre-transition/settlement increments and lapilli were used for analysis of post transition/settlement increments. Increments were counted from the primordium of the sagittae, where clear increments could be seen, until the transition/settlement point where increment width increased rapidly and structure became hard to interpret. Increments at the

primordium of the lapilli are too narrow to be counted accurately, although post-transition increments are distinct. This method is consistent with previous studies which have looked at pelagic larval duration of *S. punctatus* post larvae (Jenkins and May, 1994; Jenkins et al., 2000). Replicate counts were made on each otolith. Increment counts were conducted for forty one and thirty *S. punctatus* otoliths from South Australia and Victoria respectively, which included a minimum of 10 samples from each major nursery area in each state.

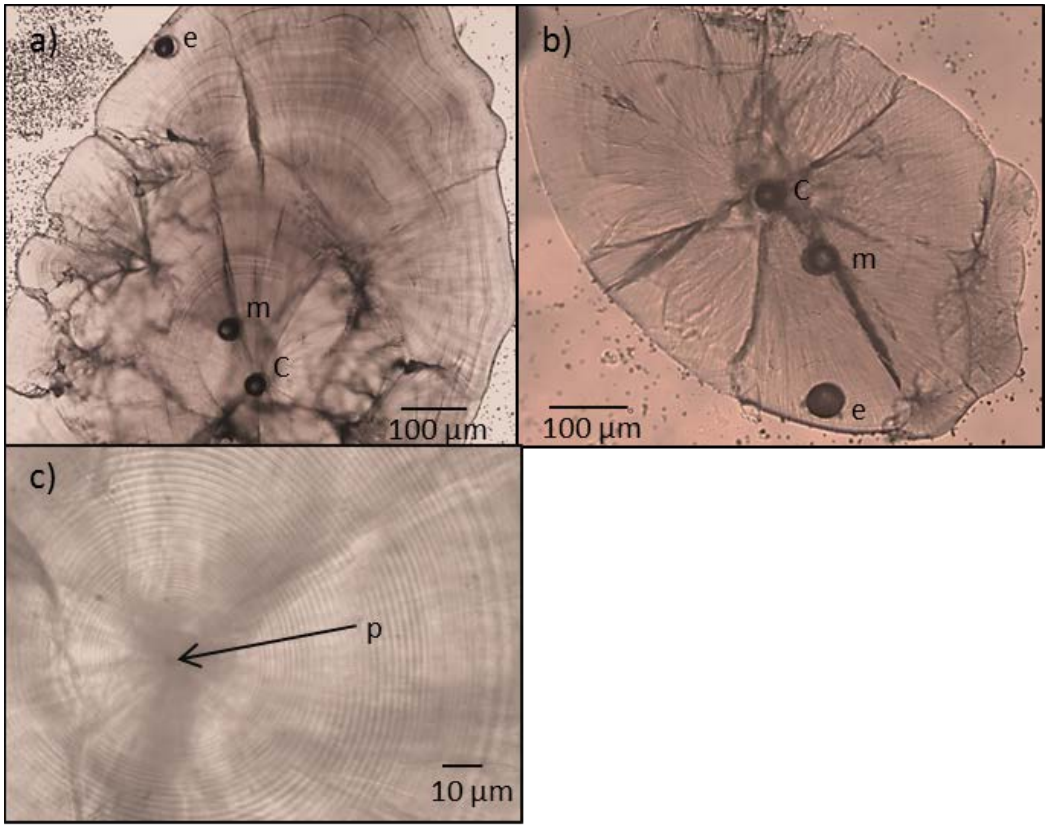


Figure 3 Images of sagittal otolith thin sections from post-larval King George Whiting showing laser ablation sampling pits (a, b) and the core region of the otolith used in increment analysis (c). c=core ablation (early-larval zone), m = mid-larval ablation zone, e=edge ablation zone (refer chapter2), p=primordium.

Increment measurement

Sagittae were used for increment measurements. Post-larval *S. punctatus* otoliths collected from South Australian and Victorian nursery areas during 2011 and 2012 were polished until increments from the primordium could be clearly seen. Samples used for increment measurement are detailed in Table 1. Any otoliths for which the increments were unclear were discarded and not used in analyses. Otolith increment measurements were made using a compound microscope under a magnification of 1000x. A video camera was attached to the microscope, which enabled the otolith to be viewed on a computer monitor. The measurement modules for Leica Application Suite image software were used to measure increments.

Otoliths were examined blindly with respect to year of sampling and sampling location. Measurements of otolith micro-increments were made along the longest axis on either the clearest sagittal otolith (where 2 otoliths were available), or the otolith which had not been used for chemistry analysis where the sister otolith had been ablated. It has been found that results do not differ significantly between sagittae from the same fish (Jenkins and May 1994). Increments were measured from the first increment (at approximately 10µm from the core) to 30µm from the core. The widths of micro-increments in the otoliths of larval fish are

often used to represent somatic growth. Daily formation of micro-increments in larval *S. punctatus* has been validated by both larval rearing (B. D. Bruce and D. A. Short, unpublished manuscript), and field evidence (Jenkins and May 1994). A strong linear relationship between otolith diameter and body length has also been recorded (B. D. Bruce and D. A. Short, unpublished manuscript).

Table 1. Number of *S. punctatus* otoliths used for increment analysis from locations across South Australia and Victoria.

Location	2011	2012
South Australia		
Spencer Gulf	**32**	**58**
Gulf St Vincent	**41**	**26**
Kangaroo Island	**30**	**22**
Victoria		
Port Phillip Bay	**31**	**58**
Western Port	**30**	**31**
Corner Inlet	**33**	**35**

Data Analysis

Otolith chemistry

Variation in the individual element:calcium ratios was analysed by univariate analysis of variance (ANOVA). Separate analyses were conducted for each cohort and otolith sampling zone. Regions (i.e. bays/gulfs) were treated as fixed factors with sites nested within regions and individual fish as replicates. The region term was tested against the nested terms MS and df. Tukey's HSD *post hoc* tests were used to determine the sources of any significant differences among regions, again using the nested terms MS and df from the main analysis.

Otolith element:ca ratios with p-values < 0.1 for the region main effect were retained for multivariate analysis. Multivariate analysis of variance (MANOVA, Pillai's Trace) and pairwise comparisons (Hotelling's T-square) were used to analyse variation in the multi-elemental:Ca ratios among regions and States for each otolith zone within each cohort (significant p-value ≤ 0.01 to account for five tests involving an individual region). Quadratic discriminate function analysis (QDFA) with a cross validation 'leave-one-out' jackknife classification procedure, was used to assess how accurately individual fish could be assigned to their collection regions and State based on the elemental composition of each otolith zone. Priors were chosen as equal across groups. Assessing accuracy of the classification rules for group membership needs to account for chance agreement. The Kappa Index is used to indicate the proportion of agreement between the actual sample group memberships and those predicted by the otolith chemistry beyond that expected by chance (Sim and Wright, 2005). The Kappa Index ranges from 0-1, 0 = no agreement between actual and predicted beyond

random chance, 1 = perfect agreement between actual and predicted beyond random chance). The Kappa Index is also unbiased when sample sizes differ between sample groups (Fielding and Bell, 1997). For ANOVA, MANOVA and QDFA; Mg:Ca, Mn:Ca, and Ba:Ca data were ln(x+1) transformed, raw Sr:Ca was used, and Zn:Ca data were fourth root transformed to meet assumptions of normality and homogeneity of variances (clear departures from assumptions assessed using histograms, Q-Q normal probability plots, box and residual plots). QDFA was chosen over linear discriminant function analysis due to some minor inequality of covariance matrices indicated from qualitative comparisons of within-group scatterplot matrices among the 6 regional groupings (Quinn and Keough, 2002). The Pillai trace statistic was used to test for significance of MANOVA as it is the most robust to any deviations from multivariate normality (Quinn and Keough, 2002). F-to-remove statistics were used to rate the order at which elements contributed to discrimination (Wilkinson et al., 1996). Canonical discriminant function plots of the 95% confidence ellipses around the centroids for each group were used to display variation in the multi-elemental otolith chemistry among regions. For the State grouping, where only one canonical variate was calculated (i.e. 2 x groups) data overlap/separation was displayed by plotting the canonical scores for each State as density functions.

Otolith microstructure

The width of the first 10 daily increments in the core of post-larval King George Whiting was compared amongst nursery areas and sampling years using 2-factor Analysis of Variance (ANOVA) followed by post-hoc Tukey's tests to compare the significance of differences between individual nursery areas. The estimated date of first increment formation based on the pelagic larval duration (PLD) of a sub-set of post-larvae was compared between States using 1-Factor ANOVA.

Results

Otolith Chemistry

Individual element:Ca ratios

Cores

2011

Regional variation in otolith element:Ca ratios otolith cores was most notable for the 2011 cohort and was significant for Ln Mg:Ca, and 4throot Zn:Ca (Figure 4ac, Table 2a). For Ln Mg:Ca, Spencer Gulf (SG) showed significantly higher levels than all Victorian regions, and also Gulf St Vincent (GSt) and Kangaroo Island (KI) (Figure 4a, Table 2ab). Corner Inlet (CI) and Western Port (WP) were also significantly higher than Port Phillip Bay (PPB) (Table 2a), but these differences were much less than those detected between the Victorian and the South Australian locations (Figure 4a). 4throot Zn:Ca was significantly higher in samples from SG than KI, CI, WP and PPB, and GSt was significantly higher than WP and PPB (Figure 4c, Table 2a). KI samples also had significantly higher 4throot Zn:Ca than PPB (Figure 4c, Table 2a).

2012

For the otolith cores in the 2012 cohort, significant regional variation was detected for Ln Mg:Ca, Ln Sr:Ca and Ln Ba:Ca, but not 4throot Zn:Ca (Figure 4acde, Table 2b). Ln Mg:Ca was much lower across all locations except PPB in the 2012 than 2011 cohort (Figure 4a). Ln Mg:Ca for SG and GSt was significantly higher than WP, and KI was significantly higher than WP and CI (Figure 4a, Table 2b). Sr:Ca and Ln Ba:Ca were significantly higher for KI than WP and PPB, and for Sr:Ca also higher than SG (Figure 4de, Table 2b). CI samples were significantly higher in Sr:Ca than SG, PPB and WP (Figure 4e, Table 2b), and GSt was significantly higher in Ln Ba:Ca than WP (Figure 4e, Table 2b).

Mid

2011

Similar to the otolith cores, at the mid-point of the larval phase in the 2011 cohort, SG samples showed higher Ln Mg:Ca than all the other regions, but the difference was not significant for the GSt comparison (Figure 4a, Table 2a). GSt was also significantly higher than WP, CI and PPB, and KI was significantly higher than PPB (Figure 4a, Table 2a). 4throot Zn:Ca was significantly higher in samples from SG than KI, CI, WP and PPB, and SG was also significantly higher than GSt (Figure 4c, Table 2a). GSt was significantly higher for 4throot Zn:Ca than CI, and PPB (Figure 4c, Table 2a), and KI samples were significantly higher than CI, PPB and WP (Figure 4c, Table 2a). SG, GST and KI all had significantly higher Ln Mn:Ca than all the Victorian regions (Figure 4b, Table 2a). Sr:Ca was significantly higher for all the Victorian regions than SG, and WP was also significantly higher than GSt (Figure 4d, Table 2a).

2012

Unlike 2011, there was negligible regional variation in Ln Mg:Ca for the mid-point in the 2012 cohort (Figure 4a, Table 2b). For the 2012 cohort, regional variation for the otolith larval mid-point was, however, significant for Ln Mn:Ca and Sr:Ca (Figure 4bd, Table 2b). For Ln Mn:Ca, SG and GSt were significantly higher than CI. For Sr:Ca, PPB was significantly higher than all the South Australian regions (GSt, SG, KI), and WP and CI were higher than GSt (Figure 4f, Table 2b).

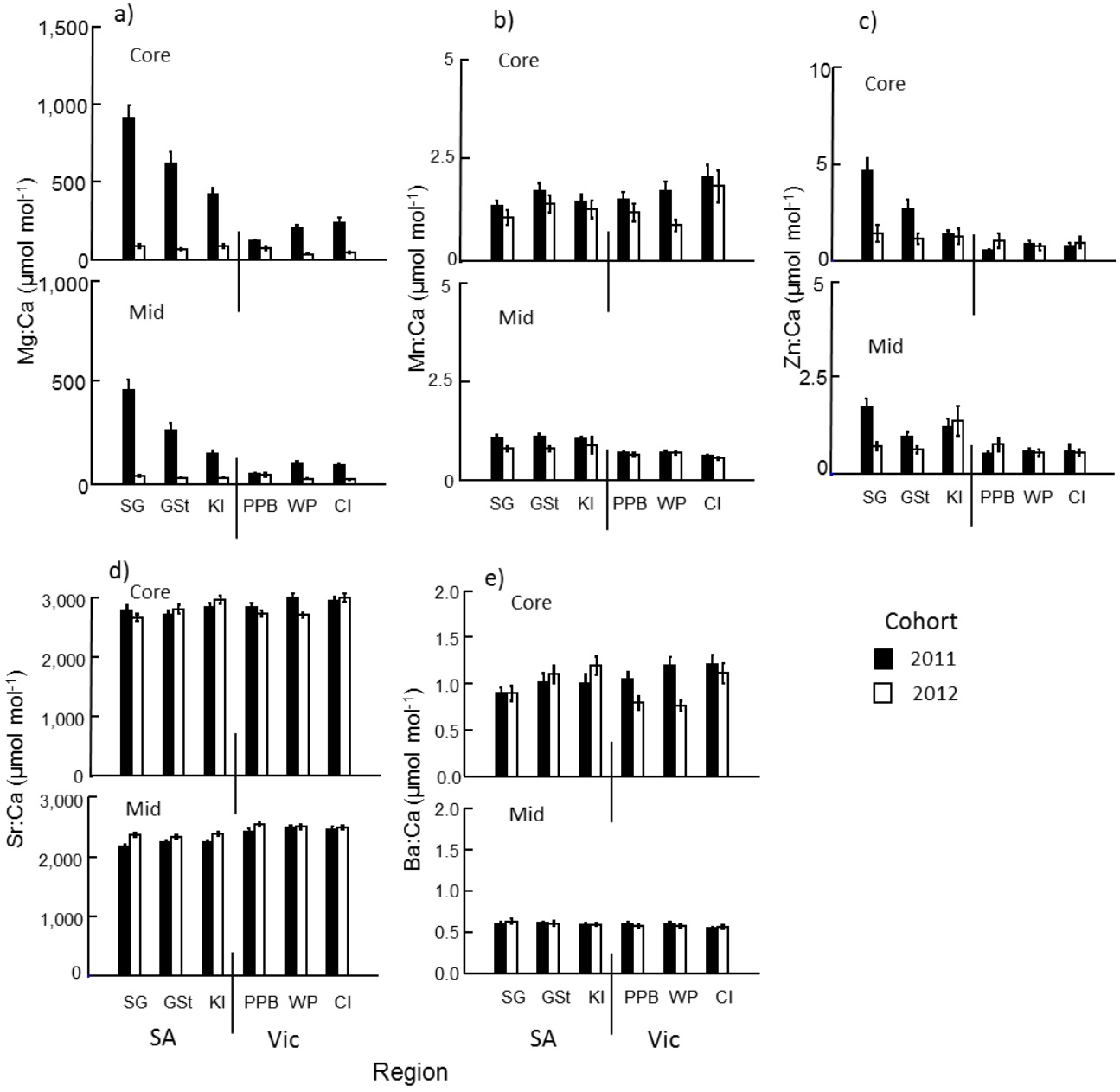


Figure 4. Comparison of mean (±SE) otolith element:Ca ratios among post-larvae sampled from six nursery area (regions) across South Australia (SA) and Victoria (Vic), for two cohorts and for the otolith core and mid zones. SG = Spencer Gulf, GSt = Gulf StVincent, KI=Kangaroo Island, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet.

Table 2. Results summary of ANOVA and post-hoc Tukey's pairwise tests for individual element:ca ratios for the otolith core and mid zones of post-larvae sampled from six nursery area (regions) across South Australia and Victoria, and for two cohorts: a) 2011, b) 2012. SG = Spencer Gulf, GSt = Gulf StVincent, KI=Kangaroo Island, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet.

a) 2011 cohort

Element/Ca	Otolith Zone	p – value: regions	Significant Tukey's pairwise comparisons between regions *p≤0.05, **p≤0.01, ***p≤0.001
Ln Mg:Ca	core	**<0.001**	SG>GSt*KI***CI***WP***PPB***, GSt>WP***PPB***, KI>WP***PPB***, CI>PPB*, WP>PPB*
	Mid	**<0.001**	SG>KI***CI***WP***PPB***, GSt>WP*CI*PPB***, KI>PPB***
Ln Mn:Ca	core	Ns	ns
	Mid	**<0.001**	SG>PPB***WP***CI***, GST>PPB***WP***CI***, KI>PPB***WP***CI***,
4throot Zn:Ca	core	**<0.001**	SG>KI** CI** WP*** PPB***, GSt>WP**PPB***, KI>PPB*
	Mid	**<0.001**	SG>KI**GSt**CI***PPB***WP***, GSt>CI**PPB*, KI> CI***PPB***WP**,
Sr:Ca	core	Ns	
	Mid	**p=0.001**	WP>SG**GSt*, PPB>SG*, CI>SG**
Ln Ba:Ca	core	Ns	ns
	Mid	Ns	ns

b) 2012 cohort

Element/Ca	Otolith Zone	p – value: regions	Significant Tukey's pairwise comparisons between regions *p≤0.05, **p≤0.01, ***p≤0.001
Ln Mg:Ca	core	**<0.001**	KI>WP***CI*, SG>WP**, GSt>WP**
	Mid	ns	ns
Ln Mn:Ca	core	ns	ns
	Mid	**<0.05**	SG>CI*, GSt>CI*
4throot Zn:Ca	core	ns	ns
	Mid	ns	ns
Sr:Ca	core	**<0.001**	KI>WP** SG**PPB*, CI>SG***PPB**WP**
	Mid	**<0.001**	PPB>GSt***SG**KI**, WP>GSt**, CI>GSt*
Ln Ba:Ca	core	**<0.01**	KI>WP**PPB*, GSt>WP*,
	Mid	ns	ns

Multivariate elemental chemistry

Cores

2011 cohort

For the 2011 cohort, MANOVA of the otolith core data was highly significant for both regional and State groupings (Pillai's Trace, $p<0.001$). Discrimination amongst the Victorian and South Australian sampling regions was high (Figure 5a). Discrimination among regions (individual bays/gulfs) was driven mostly by variation in Ln Mg:Ca (F-to-remove = 22.9) and Sr:Ca (F-to-remove = 10.9), with 4th root Zn:Ca adding only minor additional discrimination power (F-to-remove = 1.7). The pairwise regional comparisons indicated that all the South Australian regions differed significantly from all the Victorian regions (Table 3a).

The jackknife cross-validation classification accuracy was high when the data were grouped at the State level (81%), but was lower at the regional level (39%) (Table 3a). Misclassifications at the regional level were mostly among regions within each State. Misclassification of Victorian region samples to South Australian regions were mostly to the KI region, and South Australian regions to Victorian regions, were mostly to PPB and CI (Table 3a). The Kappa Index indicated that the classification rules created from the multi-element otolith chemistry data performed better than random allocation, particularly at the State grouping (region grouping = 0.26, State grouping = 0.62) (Table 3a).

2012 cohort

Discrimination among Victorian and South Australian regions based on otolith core chemistry was less clear for the 2012 cohort than the 2011 cohort (Figure 5ab). Similar to the 2011 cohort, Ln Mg:Ca contributed most to discrimination (F-to-remove = 7.2), followed by Sr:Ca (F-to-remove = 3.2), with minor additional discrimination power provided by Ln Ba:Ca and Ln Mn:Ca (F-to-remove = 2.7 and 2.4 respectively). While for both the regional and State-based groupings the MANOVA was highly significant (Pillai's Trace, $p<0.001$), there was major overlap among the sample distributions from the two States for canonical variate 1 (Figure 5b). Significant regional pairwise differences occurred between SG and WP, CI, between GSt and WP, CI, between KI and PPB, and between WP and CI. PPB was also significantly different to CI (Table 3b).

Jackknife cross-validation classification accuracy was higher for the South Australian samples, with more of the Victorian samples being misclassified to South Australian regions, than South Australia to Victoria (Table 3b). At the regional level, most of the misclassified samples from South Australian regions to Victorian regions were to WP. PPB and CI region samples misclassified mostly to WP, but also to GSt. WP samples misclassified mostly to SG and KI (Table 3b). The Kappa Index for the 2012 cohort were considerably lower than for 2011 cohort, indicating only minor improvement over random classification (region = 0.15, State = 0.30) (Table 3b).

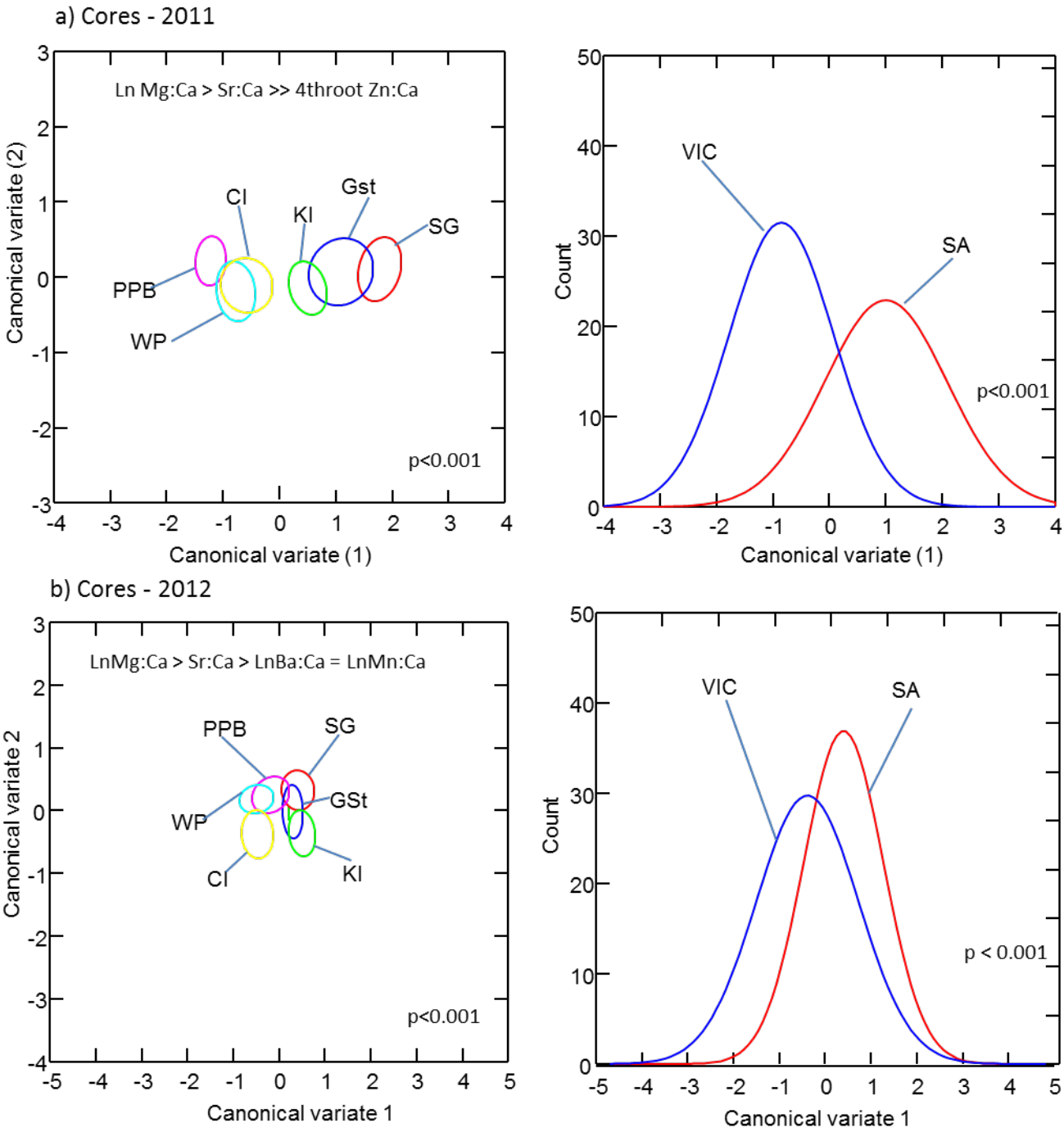


Figure 5. Canonical variate plots from quadratic discriminant function analysis of otolith core chemistry for two cohorts, a) 2011, b) 2012, of post-larvae sampled from six bays across Victoria and South Australia. SG = Spencer Gulf, GSt = Gulf St Vincent, KI=Kangaroo Island, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet. Left: plots with data grouped according to each bay. Right: plot with data grouped according to State (because only two groups, only discriminant function possible).

Table 3. Left tables: Results of post-hoc multivariate pairwise comparisons among regions for the otolith core chemistry of post-larvae sampled from six nursery bays across South Australia and Victoria (only the significant comparison included). Right tables: results of jackknife cross-validation classifications at the regional and State levels of sample grouping, along with Kappa values.

a) Cores - 2011

Significant pairwise comparisons (p≤0.01)			
Regions		**Hotelling's T-Square**	**p-Value**
SG	KI	31.941	<0.001
SG	PPB	195.508	<0.001
SG	WP	126.690	<0.001
SG	CI	101.586	<0.001
GSt	PPB	117.046	<0.001
GSt	WP	70.067	<0.001
GSt	CI	57.388	<0.001
KI	PPB	84.645	<0.001
KI	WP	40.256	<0.001
KI	CI	31.732	<0.001

	% samples classified to each region					
Region collected	SG	GSt	KI	PPB	WP	CI
SG n=39	59	15	21	0	3	3
GSt n=36	39	8	33	14	0	6
KI n=49	15	4	55	12	2	12
PB n=62	0	0	8	52	13	27
WP n=47	0	2	13	28	19	38
CI n=37	5	0	19	32	11	33
State collected	SA	VIC				
SA n=120	80	20				
VIC n=144	18	82				
Grouping	**Overall classification accuracy %**					**Kappa**
Region	39					0.26
State	81					0.62

b) Cores - 2012

Significant pairwise comparisons (p≤0.01)			
Regions		**Hotelling's T-Square**	**p-Value**
SG	WP	24.389	<0.001
SG	CI	35.604	<0.001
GSt	WP	21.763	<0.01
GSt	CI	21.183	<0.01
KI	PPB	24.277	<0.001
KI	WP	36.233	<0.001
KI	CI	28.060	<0.001
PPB	CI	14.311	0.010

	% samples classified to each region					
Region collected	SG	GSt	KI	PPB	WP	CI
SG n=53	21	21	15	7	28	8
GSt n=56	20	21	18	7	25	9
KI n=54	17	18	41	4	15	5
PPB n=55	7	13	7	13	49	11
WP n=56	11	7	12	9	52	9
CI n=57	5	17	4	5	44	25
State collected	SA	VIC				
SA n=163	77	23				
VIC n=168	47	54				
Grouping	**Overall classification accuracy %**					**Kappa**
Region	29					0.15
State	65					0.30

Mid

2011

Similar to the core, for the mid-larval otolith phase MANOVA for both regional and State groupings were highly significant (Pillai's Trace, $p<0.001$). Discrimination between Victorian and South Australian region samples was high (Figure 6a). Ln Mg:Ca contributed the most to discrimination among regions (F-to-remove = 25.6) followed by Ln Mn:Ca, 4throot Zn:Ca, and Sr:Ca (F-to-remove = 5.5, 3.5, 2.3 respectively). Pairwise comparisons indicated that all the South Australian regions were significantly different to all the Victoria regions (Table 4a). The SG region was also significantly different to the other South Australian regions (KI and GSt) and PPB was also significantly different to the other Victorian regions (Table 4a).

Similar to the otolith core, at the State level, overall Jackknife classification accuracy was high (81%) for the mid-larval phase, and lower at the region grouping (45%). Most of the misclassifications from South Australian regions to Victorian regions were to the PPB region. Misclassifications from Victorian regions to South Australian regions were mostly to GSt and KI (Table 4a). The Kappa Index for the mid-larval otolith phase were similar to the those for the otolith core (0.33 for region grouping and 0.62 for State grouping) indicating a clear improvement over random classification (Table 4a)

2012

Despite significant MANOVAs (Pillia's Trace, $p<0.001$), overall discrimination among regions and States for the larval mid-point was low in the 2012 cohort (Figure 6b). Sr:Ca contributed most to discrimination followed by Ln Mn:Ca (F-to-remove = 5.1, 3.4 respectively). Pairwise comparisons indicated that all the GSt region was significantly different to all the Victorian regions, SG was significantly different to PPB and CI, and KI was significantly different to PPB (Table 4b).

Jackknife classification accuracy was moderate at the State grouping (58% for South Australia, 67% for Victoria). At the regional grouping most of the Victorian samples that were misclassified to South Australian regions were misclassified to KI. Most of the South Australian samples that were misclassified to Victorian regions were misclassified to WP and CI (Table 4b). Similar to the otolith core, the Kappa Index for 2011 were lower than 2012, and indicated limited improvement over random classification (region = 0.10, State = 0.26) (Table 4b).

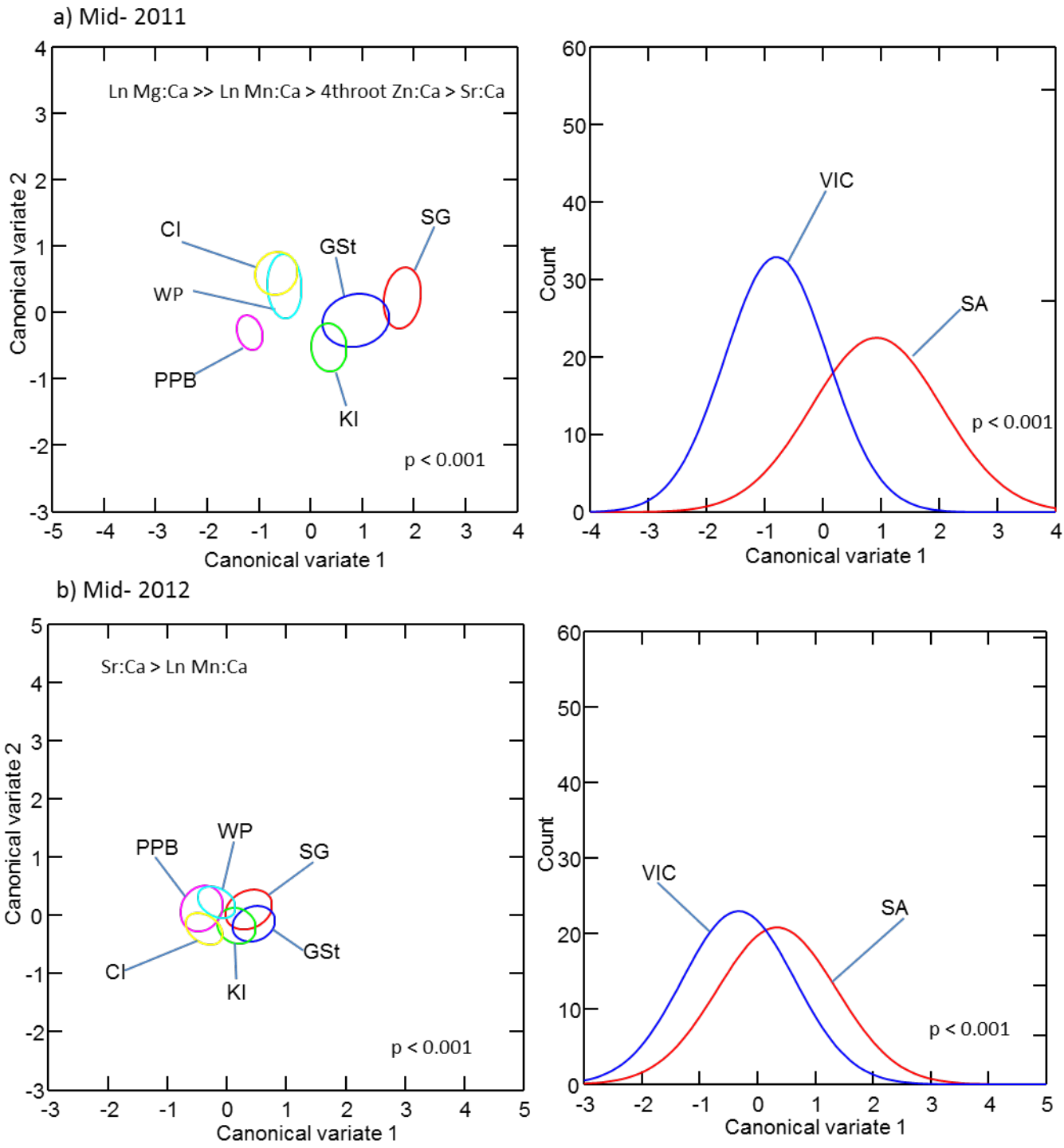


Figure 6. Canonical variate plots from quadratic discriminant function analysis of the otolith mid-larval zone chemistry for two cohorts, a) 2011, b) 2012, of post-larvae sampled from six bays across Victoria and South Australia. SG = Spencer Gulf, GSt = Gulf St Vincent, KI=Kangaroo Island, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet. Left: plots with data grouped according to each bay. Right: plot with data grouped according to State (because only two groups, only discriminant function possible).

Table 4. Left tables: Results of post-hoc multivariate pairwise comparisons among regions for the otolith mid-larval zone chemistry of post-larvae sampled from six nursery bays across South Australia and Victoria (only the significant comparison included). Right tables: results of jackknife cross-validation classifications at the regional and State levels of sample grouping, along with Kappa values. SG = Spencer Gulf, GSt = Gulf StVincent, KI=Kangaroo Island, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet.

a) Mid- 2011

Significant pairwise comparisons (p≤0.01)			
Regions		**Hotelling's T-Square**	**p-Value**
SG	GSt	29.785	<0.001
SG	KI	62.327	<0.001
SG	PPB	222.159	<0.001
SG	5WP	118.617	<0.001
SG	CI	125.366	<0.001
GSt	PPB	107.498	<0.001
GSt	WP	49.427	<0.001
GSt	CI	58.559	<0.001
KI	PPB	66.936	<0.001
KI	WP	38.335	<0.001
KI	CI	49.791	<0.001
PPB	WP	26.695	<0.001
PPB	CI	26.276	<0.001

	% samples classified to each region					
Region collected	SG	GSt	KI	PPB	WP	CI
SG n=40	63	20	10	2	3	2
GSt n=37	32	41	5	11	3	8
KI n=49	10	27	27	20	10	6
PPB n=62	0	6	8	69	10	7
WP n=46	4	9	11	28	37	11
CI n=38	3	13	8	26	26	24
State collected	SA	VIC				
SA n=126	80	20				
VIC n=146	18	82				
Grouping	**Overall classification accuracy %**					**Kappa**
Region	45					0.33
State	81					0.62

b) Mid- 2012

Significant pairwise comparisons (p≤0.01)			
Regions		**Hotelling's T-Square**	**p-Value**
SG	PPB	17.103	<0.001
SG	CI	18.624	<0.001
GSt	PPB	21.963	<0.001
GSt	WP	12.745	<0.01
GSt	CI	20.971	<0.001
KI	PPB	11.568	<0.01

	% samples classified to each region					
Region collected	SG	GSt	KI	PPB	WP	CI
SG n=53	11	10	28	8	26	17
GSt n=56	18	9	30	4	16	23
KI n=52	9	10	29	4	21	27
PPB n=55	9	5	13	11	27	35
WP n=56	5	4	21	4	41	25
CI n=57	5	5	11	5	25	49
State collected	SA	VIC				
SA n=161	58	42				
VIC n=168	33	67				
Grouping	**Overall classification accuracy %**					**Kappa**
Region	25					0.10
State	63					0.26

Daily increment analysis

The width of the inner 10 daily increments in otoliths was significantly higher for post-larvae from South Australian than Victorian nursery areas (Table 5, Figure 7). There was no significant difference between settlement years (i.e. cohorts) and nor was there an interaction between settlement bay and year (Table 5, Figure 7). Tukey's post-hoc comparisons showed that increment widths in otoliths of post-larvae in all South Australian nursery areas were higher than all Victorian nursery areas. Within States, however, the only significant difference was that increment widths were higher in SG than GSt (Figure 7).

Table 5. Two Factor ANOVA results for the effect of nursery bay and settlement year on the width of the first 10 otolith increments for post-larval *Sillaginodes punctatus*.

Source	df	Mean Squares	F-Ratio	p-Value
Bay	5	57.795	16.913	0.000
Year	1	3.211	0.940	0.333
Bay*Year	5	4.066	1.190	0.313
Error	415	3.417		

The pelagic larval duration (PLD) was estimated for a sub-set of post-larvae from each State collected in 2012. Estimated dates of first increment formation ranged from May to mid-July for both States with considerable overlap (Figure 8). There was no significant difference in the estimated date of first increment formation for the two States (ANOVA, $F_{1,69} = 0.527$, $P = 0.47$).

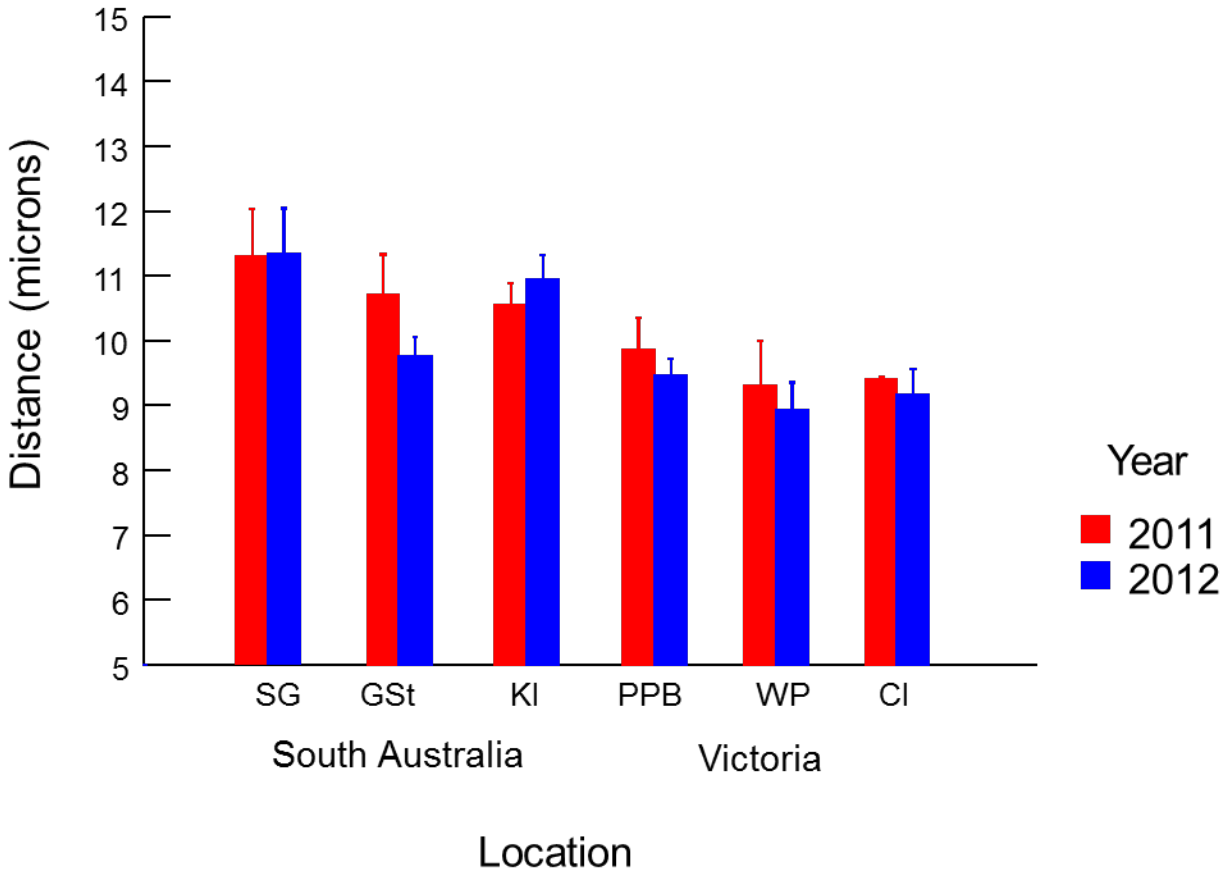


Figure 7. Mean (+/- Standard Error) distance from the core to the tenth daily increment from otoliths of *Sillaginodes punctatus* post-larvae collected from six nursery areas in South Australia and Victoria. SG = Spencer Gulf, GSt = Gulf St Vincent, KI=Kangaroo Island, PPB=Port Phillip Bay, WP=Western Port, CI=Corner Inlet.

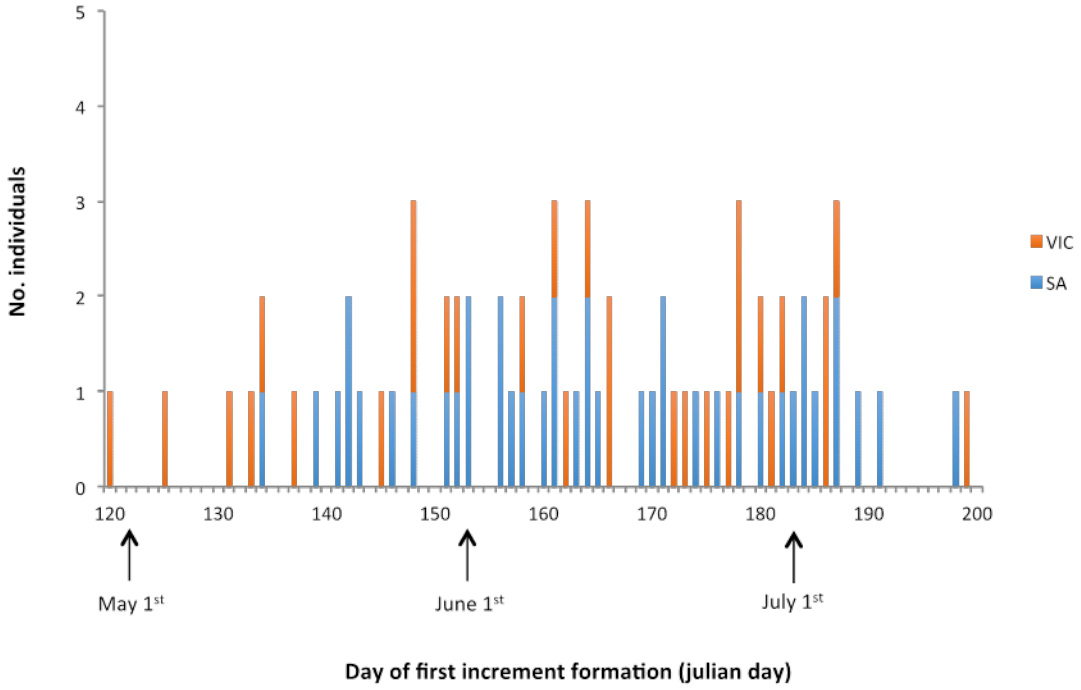


Figure 8. Day of first increment formation for *Sillaginodes punctatus* post-larvae from Victoria (n = 30) and South Australia (n = 41) collected in 2012.

Discussion

Otolith chemistry

The results from the otolith core chemistry are consistent with the hypothesis that post-larval King George Whiting recruiting into bay/gulf nurseries in Victoria and South Australia are not sourced from one common spawning area. Although the differences in otolith core chemistry between the South Australian and Victorian post-larvae were much clearer in 2011, this may reflect less pronounced environmental or physiological influences on otolith chemistry as opposed to greater overlap in spawning area sources for the 2012 cohort. This interpretation is supported by the increment width analysis (discussed below) which showed consistent differences between early larval growth of post-larvae from Victoria and South Australia for both cohorts.

In 2011, the differences in the otolith mid-larval stage (50-80 days age) were consistent with the cores in that the South Australian samples showed clear differences to the Victorian samples, and that the most notable variation was for Mg:Ca, and Zn:Ca. This indicates that they were not only spawned in different environments than the Victorian samples but also dispersed under different conditions. Further, differences in the mid-larval zone between South Australian and Victorian samples also occurred for Mn:Ca and Sr:Ca , which was not evident in the otolith cores. Overall, the main differences between Victorian and South Australian samples in otolith core and mid-larval stage chemistry were due to higher Mg:Ca, Zn:Ca and Mn:Ca in South Australian samples, and higher Sr:Ca in the Victorian samples, with the exception of the KI region cores in 2012.

These results are consistent with reverse hydrodynamic modelling that predicted spawning areas for post-larvae from Victorian and South Australian nursery areas based on larval durations and regional hydrodynamics (Fowler et al., 2000a; Jenkins et al., 2000). Post-larvae in Victorian nursery areas were predicted to have come from spawning 100's of km to the west in far west Victoria along the coast into south-eastern South Australia, but not as far west as the known South Australian spawning grounds (Jenkins et al., 2000). For post-larvae from South Australian nursery areas, spawning was predicted to be much more local, and overlapped the known spawning areas in the Investigator Strait region (Fowler et al., 2000a).

Interestingly, the most notable differences between post-larvae from the two States were for magnesium (Mg) in 2011. Magnesium is an element that is abundant in the environment and there is typically no relationship between concentrations in the water and the otolith (Martin and Thorrold, 2005). Mg concentrations in the blood plasma are higher than in the endolymph (Melancon et al., 2009), indicating significant physiological regulation (Woodcock et al., 2012; Barnes and Gillanders, 2013). Physiological regulation implies that Mg uptake may be affected by variables such as temperature, otolith precipitation rates and somatic growth rates (Martin and Thorrold, 2005). Determining the most important mechanism is difficult given that these variables often strongly co-vary (Martin and Thorrold, 2005). Results of studies have varied, with Mg:Ca showing positive (Barnes and Gillanders, 2013; Stanley et al., 2015), negative (Fowler et al., 1995a, b) or no (Elsdon and Gillanders, 2002; Martin and Thorrold, 2005; DiMaria et al., 2010) relationship with temperature. A positive relationship has been shown between Mg:Ca and otolith precipitation and somatic growth for larval and early juvenile spot *Leiostomus xanthurus* (Martin and Thorrold, 2005) and precipitation rate for snapper (*Pagrus auratus*) (Hamer and Jenkins, 2007) but no relationships with either of these factors were found for larval Pacific cod, *Gadus microcephalus* (DiMaria et al., 2010).

Elements such as Ba and Sr are commonly found to drive discrimination in otolith chemistry studies, often in relation to variation in ambient concentrations relative to calcium (Elsdon et al., 2008). For the early oceanic larval stage of King George Whiting, these elements were less important for discrimination in our study. This may be due to the oceanic spawning and larval phase of the species where variation in the ambient levels of these elements in the water is likely to be low (Hamer et al., 2006). The elements that showed most regional variation during the early and mid-larval phase: Mg, Zn, Mn and Sr are all known to be influenced by physiological processes independent of water chemistry, with Sr:Ca, Mn:Ca and Zn:Ca influenced by a combination of physiological processes, food composition and ambient concentrations (Sadovy and Severin, 1992; Campana, 1999; Miller et al., 2006; Ranaldi and Gagnon, 2008; Sturrock et al., 2015). It is likely that the variation observed in the otolith composition for these elements was related to a combination of physiological and environmental (water chemistry, temperature) factors. The lower growth rate of the Victorian post-larvae is clearly indicative of a physiological difference. There is evidence that otolith Sr:Ca levels are negatively correlated with growth, and Mg:Ca and Mn:Ca are positively related to growth (Sadovy and Severin, 1992; Hamer and Jenkins, 2007; Sturrock et al., 2015). These patterns are consistent with our results, particularly for the mid–larval stage, where Mn:Ca and Mg:Ca were higher in the South Australia samples (faster early larval growth), and Sr:Ca was higher in samples from the Victorian bays (slower post-larval growth).

Otolith microstructure

Otolith microstructure of King George Whiting post-larvae from Victorian and South Australian nursery areas showed significant differences in increment widths, adding to evidence that post-larvae in each State came from different spawning areas. Otolith increment widths correspond to growth rates in the larval stage of King George Whiting (Jenkins and

King, 2006). The primary determinants of larval growth rate are thought to be water temperature and food availability (Anderson, 1988), although water temperature may be the more dominant variable (Meekan et al., 2003). Growth rates in the larval stage of King George Whiting post-larvae sampled in Port Phillip Bay were strongly influenced by inter-annual variation in water temperature in Bass Strait (Jenkins and King, 2006). Our results suggest that the spawning area for post-larvae from South Australia occurs in an area with significantly higher water temperature and/or productivity than the spawning area for post-larvae sampled in Victoria.

One possible alternative explanation to this would be that post-larvae from both States were spawned in the same area, but those settling in South Australian nursery areas were spawned earlier in the spawning season when the water temperature was higher. This can be ruled out, however, as the estimated hatching dates for post-larvae from the two States showed significant over-lap and were not significantly different for spawning in 2012.

Conclusions

It is clear from the results of both the otolith chemistry and otolith microstructure analyses that post-larvae recruiting to Victorian nursery areas are not spawned in the same spawning area for Whiting in central South Australia, that is north of Kangaroo Island and in the southern areas of Spencer Gulf and Gulf St Vincent (Fowler et al., 2000a; Fowler et al., 2000b). This is consistent with the modelling studies based on hydrodynamics and pelagic larval durations that predicted the spawning area for Victorian Whiting would range from west Victoria across to south-eastern coast of South Australia, but not as far west as the known spawning area in South Australia (Jenkins et al., 2000).

What these results mean for the question of cross-jurisdictional management depends on the degree of mixing of adults between the spawning areas sourcing recruits for each State. For example, if adults do not mix between these spawning areas, then the fishery stocks for each State are essentially independent and management by individual jurisdictions is justified. However, if there is mixing, for example if adults are present in a broadly mixed population from western Victoria to central South Australia, and recruitment to each State depends on which end of the distribution an individual spawns, then there is essentially only one stock fished by both States, and cross-jurisdictional management would be recommended. The independence or otherwise of the fisheries in the two States is further explored using otolith chemistry nursery signatures and life history profiles in adults, and genetic analyses, in later chapters.

Chapter 2 Origins of King George Whiting in Victorian nursery areas and the known adult spawning area in South Australia

Introduction

A major source of population connectivity for fish and invertebrate fishery species is the process of larval dispersal from spawning in adult habitat, settlement into different juvenile habitats, and then movement back to the adult habitat with growth (Fowler and Short, 1996; Verweij et al., 2008; Haywood and Kenyon, 2009; Nagelkerken, 2009a; Ford et al., 2010). The larvae of fish settling in coastal nursery habitats are often derived from adult sources (sometimes distant) that occur in different habitats (Hyndes et al., 1998; Jenkins et al., 2000; Nagelkerken, 2009a; Ford et al., 2010). In many cases the connectivity is between spawning in coastal and offshore habitats (e.g. reef, sedimentary) and larval settlement in protected embayment habitats (e.g. seagrass, mangroves) (Nagelkerken, 2009a; Ford et al., 2010), and after a period of residency in nursery habitat, juvenile and sub-adults may then show ontogenetic migration and recruitment back to adult habitat (Hyndes et al., 1998; Gillanders et al., 2003; Nagelkerken, 2009a).

With growth, juvenile fish in estuarine and coastal nursery habitats may gradually move into deeper water before making the migration offshore (Hyndes et al., 1998). Fish and invertebrates generally move kilometres to 100's of kilometres from juvenile to adult habitats. The timing of the ontogenetic migration from the coastal nursery habitat to adult habitat may be a trade-off between higher survival in the juvenile habitat versus higher growth rates in the adult habitat (Nagelkerken, 2009b). The timing of ontogenetic migration for some species coincides with reproductive maturity and the onset of spawning (Hyndes et al., 1998; Fowler et al., 2000b). Where ontogenetic migration from nursery habitats to spawning habitats occurs on a scale that crosses jurisdictional boundaries, there are significant implications for sustainability where management traditionally occurs on an individual jurisdiction basis.

The development of otolith chemistry techniques has led to considerable advances in studying the migration and nursery area origins of adult fish populations (Gillanders and Kingsford, 1996; Hamer et al., 2005; Elsdon et al., 2008; Kerr and Campana, 2013). When otoliths of juveniles in different nursery areas have distinct chemical signatures in their otoliths, older juveniles and adults that have migrated from nursery areas can be retrospectively assigned to their source nursery area based on the chemistry near the core of the otolith (Gillanders, 2002; Hamer et al., 2003, 2005). Unlike traditional tagging methods using artificial tags where only a small sample of the population is tagged, natural tags based on otolith chemistry apply to the entire population of juvenile fish in a nursery area.

This chapter describes research to address objectives 2 and 3 of the project. Firstly, we use otolith chemical signatures formed soon after post-larval settlement to determine whether juvenile King George Whiting move between juvenile nursery embayments in Victoria before finally moving out to the coast. This question has arisen from perceptions of fishers that, in particular, the Western Port fishery was exhibiting higher production than the Port Phillip Bay fishery and, also that larger fish were being captured more regularly in Western Port. One explanation for this is that juvenile fish were moving from Port Phillip or even Corner Inlet into Western Port for a period of time before taking up residence in oceanic waters. Most of the commercial and recreation catch of Whiting in Victoria comes from nursery bays, and the question of movement of juveniles between nursery bays has significant implications for the

management of these fisheries. In particular, the question of whether individual bays should be managed as individual fisheries.

Secondly, we assess whether juvenile (3+ age) *S. punctatus* have distinct elemental signatures for different State-based nursery areas, and then use this information to retrospectively classify adult fish from a known spawning area in South Australia back to their nursery areas of origin. The results are used to improve our understanding of the connectivity between Victorian and South Australian nursery areas and ontogenetic migration to the known South Australian spawning area. Results are interpreted in the context of whether cross-jurisdictional management would be justified given knowledge of population connectivity. For example, if there was evidence that emigrants from Victorian nursery areas were important to replenishment of the known spawning populations in South Australia, then management of the Whiting fisheries in Victorian bays would need to consider this important influence on the South Australian fishery.

Methodology

Part 1: Mixing of juveniles among Victorian nursery bays

Field sampling and laboratory processing

Juvenile, notionally 2+ age King George Whiting were sampled from Port Phillip Bay, Western Port and Corner Inlet in Victoria (Figure 9) between January and May, 2014. Samples from Corner Inlet were collected by haul seine net from a commercial fishing vessel while samples from Port Phillip Bay and Western Port were collected using rod and line fishing. Samples were placed on ice in the field and transferred to the laboratory where they were stored frozen. Juvenile Whiting were thawed and the otoliths (sagittae) were dissected and examined whole under a dissecting microscope with transmitted light to check the age based on annual increments (Fowler and Short, 1998; Fowler et al., 2000b). Whiting of this age can be reliably aged based on examination of whole otoliths (Fowler and Short, 1998; Fowler et al., 2000b). Otoliths from 30, confirmed 2+ age fish were then randomly selected from each bay for otolith chemistry analysis.

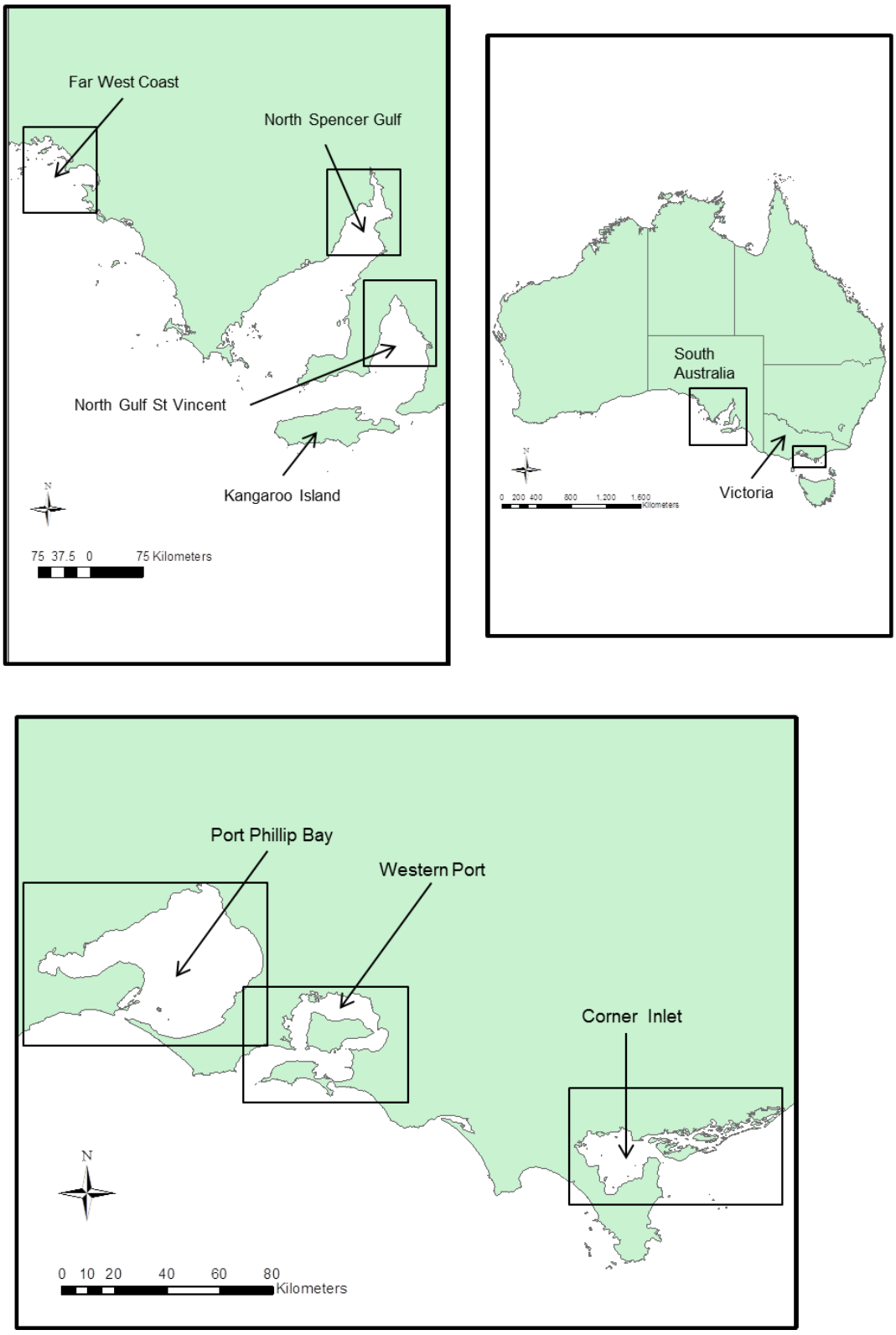


Figure 9. Areas sampled for 2+ age juvenile King George Whiting in Victoria and 3+/4+ age Whiting from Victoria and South Australia

Otolith chemistry

Otoliths were mounted in epoxy resin (Struers Epofix) and sectioned in the transverse plane using a slow speed diamond saw lubricated with Milli-Q water, followed by polishing with 12 µm 3M diamond film lubricated in Milli-Q water. The prepared sections were liberally rinsed with Milli-Q water then dried in a laminar flow cabinet, before being mounted on glass microscope slides (4 samples from each bay on each slide) with a thin film of epoxy. The cured slides were then sonicated for 3 mins in Milli-Q water, allowed to dry in a laminar flow hood and then stored in plastic containers for analysis.

Otoliths were analysed for elemental chemistry using laser ablation ICP-MS (New Wave Research UP-213 Nd:YAG ultraviolet laser microprobe coupled to a ThermoFinnigan Element 2 high resolution ICP-MS). Each otolith section was ablated using a 40 µm diameter point sample located haphazardly within a zone between 350-500 µm from the otolith core towards the ventral tip (Figure 10). ICP-MS methods and laser settings were the same as for the analysis of 0+ age otoliths described in chapter 1. This zone was chosen to represent the otolith growth phase when the fish were from about 30-40 mm total length and approximately 2-3 months post-settlement into seagrass beds within the bays (Jenkins et al. 1996). This was assumed to be sufficient time for their otolith chemistry to have fully incorporated any local environment effects (Elsdon and Gillanders 2005) and was slightly later in the post-settlement phase than the edge zones sampled on the post-larval otoliths in Chapter 1. We deliberately sampled sightly further from the core on the 2+ age samples (i.e. advanced post-larval stage), because the post-larvae analysed in chapter 1 were mostly collected at sizes between 22-30 mm (early post-larval stage), and despite showing variation among bays at the otolith edges, many of the samples may not have fully incorporated the local environmental effects. Because we did not sample in exactly the same ontogenetic zone in the age 2+ otoliths as in the post-larval otoliths in chapter 1, it was inappropriate to back-classify individuals from the age 2+ samples to baselines derived from the post-larval otolith edge samples for the three Victorian bays in chapter 1. Rather, we used the interpretation of variation among the bays in the post-larval chemistry of the age 2+ samples to infer sustained separation of the bay populations through the juvenile nursery period (at least up until age 2+ years). If discrimination among bays was high it would support the hypothesis of limited movement/mixing of fish among the three bays during their juvenile nursery phase. If the bays were poorly discriminated and showed a high degree of overlap in their post-larval stage otolith chemistry it would support the hypothesis that movement/mixing of fish among bays during their juvenile nursery phase was high.

Data analysis

The was no significant variation among the Victorian bays for Mg:Ca (raw) and Zn:Ca (4throot transform) (ANOVA, $p>0.05$) in the advanced post-larval otolith stage of the age 2+ fish (Figure 12, Table 6). Multivariate analysis focussed on Mn:Ca, Ba:Ca and Sr:Ca, which all showed significant variation among bays (Figure 12, Table 6). Quadratic discriminant function analysis with a jackknife cross-validation classification procedure was used to assess discrimination among the three bays for the advanced post-larval stage of the age 2+ fish. Sr:Ca required no transformation, Mn:Ca was Ln(x+1) transformed and Ba:Ca transformed using a Box-Cox power transform ($\lambda = -0.7$) to meet assumptions of normality and homogeneity of variances. Canonical variate plots were used to display the among bay variation for the individual samples.

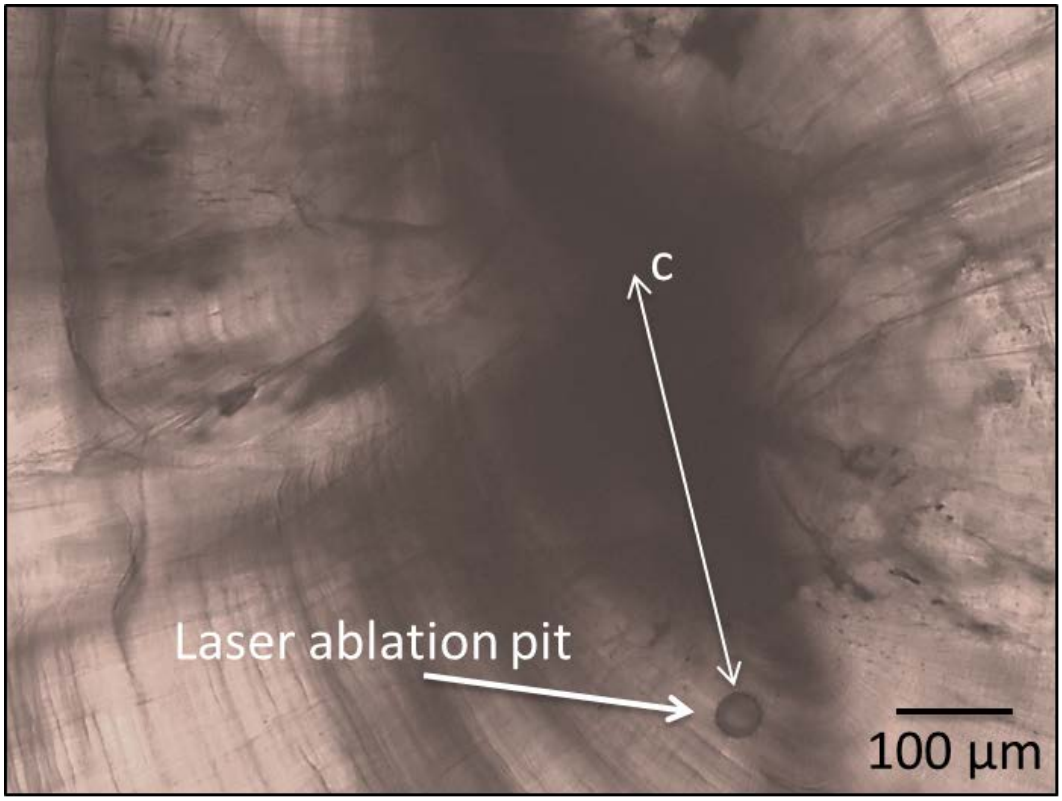


Figure 10 Image of the core region of an age 2+ years King George whiting otolith transverse section showing location of the 40 µm laser ablation sampling pit in the advanced post-larval stage used in the study of connectivity among Victorian nursery bays. C=core

Part 2: Determining contributions of Victorian and South Australian nursery source to replenishment of a known King George Whiting spawning area in South Australia

Field sampling and laboratory processing

Juvenile baselines

Otoliths used to determine juvenile baseline signatures came from archived collections of otoliths of fish obtained from market sampling or angling competitions. The 3+/4+ year old juveniles were collected from the major south-east Australian King George Whiting nursery areas of Port Phillip Bay, Western Port, and Corner Inlet in Victoria, and North Gulf St Vincent, North Spencer Gulf, and the far west coast of South Australia (Figure 9). They were collected in 2008 and 2009 and were dissected from fresh or frozen specimens and stored in paper envelopes. For this study Whiting were aged from annual increments (Fowler and Short, 1998; Fowler et al., 2000b) to ensure those used in otolith chemical analyses were all of the same year classes (birth years of 2005 and 2006). A total of 10 to 30 otoliths were analysed from each nursery area for each of the two cohorts.

Otolith chemistry baselines (elements and stable isotopes $\delta^{13}C$, $\delta^{18}O$) for juvenile source/nursery areas (i.e. bays and gulfs) in Victoria and South Australia where developed by ablating transects and micro-milling (see below) between the outer edge of the first and inner edge of the second opaque increment zones on transverse sections of otoliths of 3-4 + year old King George Whiting (Figure 11). King George Whiting of this age are juveniles and would

have deposited the analysed otolith material within the nursery area (bay/gulf) of capture (i.e. further supported by Part 1 results). By sampling a transect across an entire year of life an integrated signature is created that is less prone to seasonal effects on chemistry or mismatching between sampling points (time/ontogeny) among fish as occurs with fixed point ablation methods.

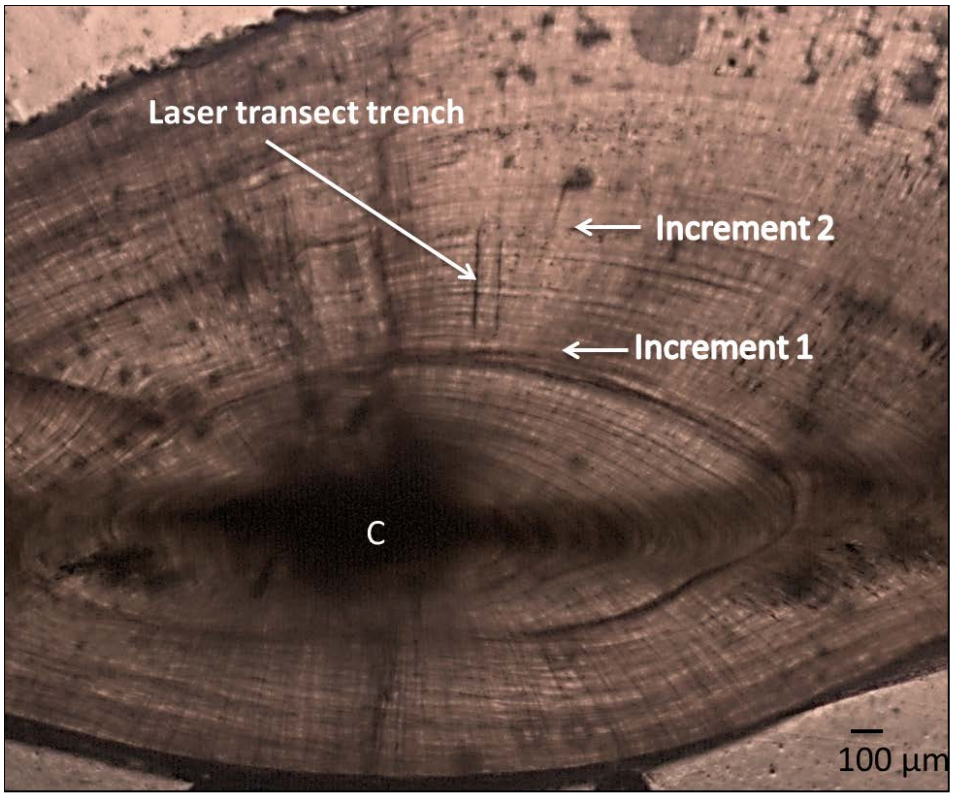


Figure 11 Image of 4+ year old King George Whiting showing the transect used for determining the juvenile baseline otolith chemistry for analysis of adult nursery area origin. C=core

Adults

Adult samples (aged up to 17+ years) for back-classification to the juvenile baselines were collected in 2011-2013 as part of a market sampling program from known spawning areas in South Australia (Kangaroo Island/Investigator Strait /southern Gulf St Vincent) (Figure 9). The adult samples were aged and only adults from the same birth years as the juvenile baselines (i.e. 2005, 2006) were used for otolith chemistry analyses.

Otoliths from adults used for back-classification to juvenile nursery areas were analysed by ablating transects and micro-milling between the first and second opaque increment zones on transverse sections for direct comparison with juvenile baseline signatures.

Elemental Analysis

All otolith samples were prepared for analysis by LA-ICP-MS and stable isotope analysis by transverse sectioning (approximately 400 µm thick) as described in part 1 above. For the analysis of element:ca ratios, the otolith sections were ablated using an 80 µm beam diameter, fluence of 10-11 J cm^{-2} , repetition rate of 5 Hz, and stage movement of 2 µm s^{-1}. Each

transect ran from the start of the first to the start of the second opaque increment zones (Figure 11). The transect paths were pre-ablated prior to sampling to remove any residual surface contamination. Each transect was 300-350 µm in length and involved approximately 150 individual measures of the selected isotopes; ^{25}Mg (magnesium), ^{55}Mn (manganese), ^{65}Cu (copper), ^{66}Zn (zinc), ^{88}Sr (strontium), ^{138}Ba (barium), along with ^{43}Ca (calcium) which was used as the internal standard with a concentration in otolith of 388,000 µg g^{-1} (Yoshinaga et al., 2000). The sample ablation data were blank subtracted (i.e. 40 blank measures prior to laser being fired) before being integrated across the transect for the calculation of element:calcium ratios. Calibration was achieved with the National Institute of Standards (NIST) 612 glass wafer using calculation methods described in (Ludden et al., 1995; Lahaye et al., 1997; Hamer et al., 2003) and expressed as ratios to Ca (µmol mol^{-1}). Replicate calibration standards were analysed after every sequence of 12 otolith ablations and each sequence consisted of a random selection of otoliths from the different sampling regions.

Stable Isotope analyses

A New Wave Research Micromill was used to mill a rasta (i.e. series of adjacent drill passes) adjacent to the laser transect path between the first and second increment zones. The milled otolith material was approximately 1-2 mg per sample (mean = 1.4 mg) and was stored in 0.2 ml Eppendorf PCR tubes. Analyses of stable isotopes; $\delta^{13}C$, $\delta^{18}O$, were conducted by Iso-Analytical (UK). For analysis, the sample powder (>1 mg) was placed in clean glass septum capped vials that were then placed in a drying oven for 24 hours prior to the caps being fitted to ensure no moisture was present. The vials then had their headspaces flushed with pure helium (99.995%). After flushing, ~0.5 ml of pure phosphoric acid was injected into the vials and mixed with the sample powder. The samples were left to react with the acid for 24 hours at ambient temperatures then heated for 40 minutes to 80° C to ensure complete conversion to carbon dioxide. Phosphoric acid suitable for isotopic analysis of carbonate samples was prepared according to the procedure published by (Coplen et al., 1983). The CO_2 gas was then analysed by continuous flow, isotope ratio mass spectrometry (IRMS). In brief, the CO_2 is flushed from the septum vial using a double holed needle and resolved on a packed column gas chromatograph. The carbon dioxide then enters the ion source of a Europa Scientific 20-20 IRMS and is ionised and accelerated. Here, gas species of different mass are separated in a magnetic field and then simultaneously measured using a Faraday cup collector array at m/z 44, 45, and 46. The reference material used for this analysis was calcium carbonate standard IA-R022 ($\delta^{13}C_{V\text{-}PDB}$ -28.63 ‰ and $\delta^{18}O_{V\text{-}PDB}$ -22.69 ‰), which is traceable to NBS-19 (Limestone, $\delta^{13}C_{V\text{-}PDB}$ +1.95 ‰ and $\delta^{18}O_{V\text{-}PDB}$ -2.2 ‰). During analysis, NBS-19, IA-R022 and NBS-18 ($\delta^{13}C_{V\text{-}PDB}$ -5.01 ‰ and $\delta^{18}O_{V\text{-}PDB}$ -23.20 ‰) were analysed as check samples for assessment of accuracy. The International Atomic Energy Agency, Vienna, distributes NBS-18 and NBS-19 as international reference standards.

Data analysis

Juvenile baseline and adult otolith chemistry were compared qualitatively by univariate graphs of the individual element:Ca and stable isotope ratio data. Because our objective was to construct multivariate baseline otolith chemistry signatures, it was unnecessary to conduct univariate ANOVA. MANOVA with post-hoc pairwise comparisons, quadratic discriminant function analysis and jackknifed cross-validation classification at the bay and State levels were used to analyse the discriminatory power of the baseline signatures for both cohorts. Based on these analyses, (i.e. only moderate discrimination among bays in each State, but strong discrimination at State level) we used a Maximum Likelihood Estimation (MLA) (Millar, 1987, 1990a; Millar, 1990b) approach to estimate the proportion of the adults (i.e. the unknowns) derived from Victorian and South Australian nursery areas for both the 2005 and 2006 cohorts. The HISEA program described in (Millar, 1990b) (http://www.stat.auckland.ac.nz/~millar/) was used to conduct the MLA analyses. For each cohort the simulation mode with 1000 simulations was initially used to estimate the

variability of the estimator (i.e. baseline data). Bootstrapping (1000 re-samplings of sample sizes the same as the original sample sizes) (Quinn and Keough, 2002) of the baseline and mixed sample data was used to estimate the mean and standard deviation of the proportions of adult spawning area fish originating from the Victorian and South Australian nursery area baselines. For all statistical analysis, data for Mg:Ca, Sr:Ca and $\delta^{13}C$ were analysed in raw form, and the Box-Cox transform was used for Mn:Ca (λ=-0.1), Zn:Ca (λ=-0.2), Ba:Ca (λ=-0.4), and $\delta^{18}O$ (λ= 1.4) to meet normality and homogeneity of variance assumptions.

Results

Part 1: Mixing of juveniles among Victorian nursery bays

As discussed in the Methods, we sampled the age 2+ fish slightly further out from the otolith core than the majority of the post-larval edge samples from chapter 1. For comparative purposes, we have included the edge data from the post-larval otoliths for both the 2011 and 2012 cohorts with the advanced post-larval stage data for the 2011 cohort age 2+ fish in Figure 12. The comparisons show some clear differences in overall levels of Mg:Ca, Mn:Ca, Zn:Ca and Ba:Ca between the post-larval and age 2+ otoliths, which may indicate ontogenetic effects or the lag effects between settlement into the bays and incorporation of local otolith chemistry effects. Although the variation was consistent among bays for Ba:Ca, and consistent with prior knowledge of elevated Ba levels in Port Phillip Bay compared to the other two Victorian bays (Hamer et al. 2006), it was not appropriate to use the otolith edge data from the post-larvae in chapter 1 to retrospectively classify the age 2+ samples.

Element:Ca ratios of the advanced post-larval otolith stages of age 2+ year juveniles varied significantly among Victorian bays for Ln Mn:Ca, Box-Cox Ba:Ca and Sr:Ca (Figure 12, Table 6). For Mn:Ca, CIN showed significantly higher levels of Mn:Ca than both PPB and WP, which were not different to each other (Figure 12, Table 6). For Ba:Ca, PPB showed significantly higher levels than both CI and WP, and CIN was significantly higher than WP (Figure 12, Table 6). Finally, for Sr:Ca, PPB showed significantly higher levels than both CI and WP, which were not significantly different from each other (Figure 12, Table 6).

Canonical variate plots from QDFA (Ln Mn:Ca, Box-Cox Ba:Ca, Sr:Ca) showed clear separation among the sample groups from the three bays (Figure 13). MANOVA indicated that all bays differed significantly from each other (Table 7). Jackknife cross-validation classification accuracy was high with an overall accuracy of 71% (Kappa 0.57), and was highest for CI at 80%, with PPB and WP at 67% accuracy (Table 7). Most misclassified samples from PPB were to CI, whereas misclassified samples from WP and CI were evenly spread between the two other bays (Table 7).

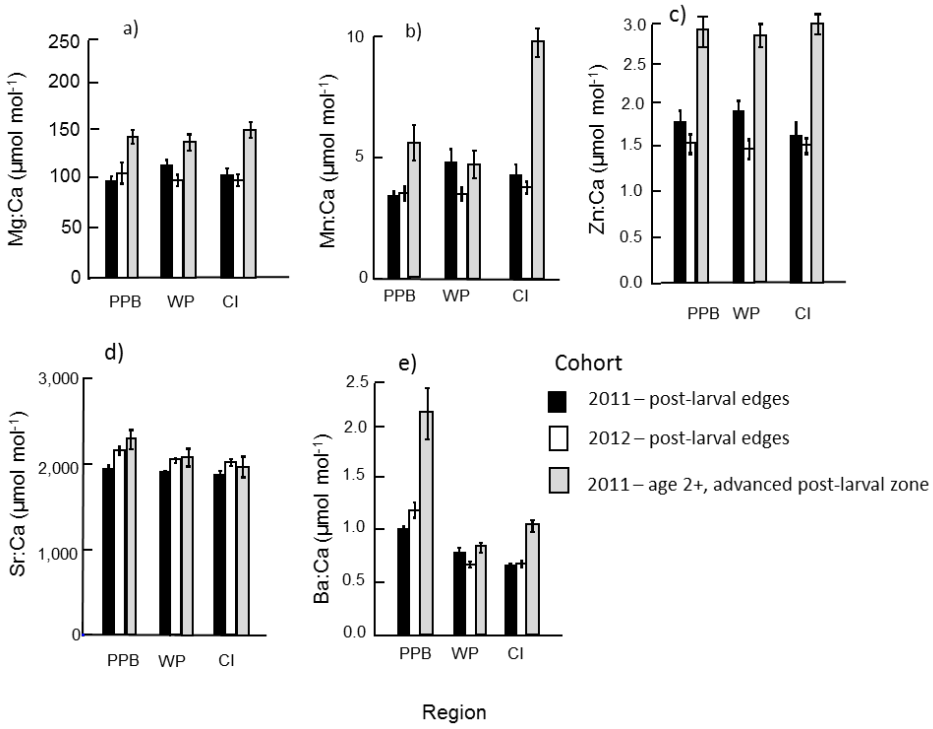


Figure 12. Comparisons of element:Ca ratios (mean ±SE) among Victorian bays, for the otolith edges of the post-larvae sampled in 2011 and 2012 (from chapter 1), and the advanced post-larval stage sampled within otoliths of age 2+ years from 2011 year-class. PPB=Port Phillip Bay, CI=Corner Inlet, WP=Western Port.

Table 6. Results of ANOVA, and post-hoc Tukey's comparisons for individual element:Ca ratios measured in the advanced post-larval otolith stage of age 2+ King George Whiting. PPB=Port Phillip Bay, CI=Corner Inlet, WP=Western Port.

Element:Ca	$F_{2,87}$	p-value, Bays	Tukeys' post-hoc comparisons: *p<0.05, **p<0.01, ***p<0.001
Mg:Ca	0.698	0.58	NS
Ln Mn:Ca	21.21	<0.001	CI>PPB***, CI>WP***
4throot Zn:Ca	0.460	0.63	NS
Sr:Ca	19.13	<0.001	PPB>CI***, PPB>WP**
Box-Cox Ba:Ca	32.84	<0.001	PPB>CI***, PPB>WP***, CI>WP*

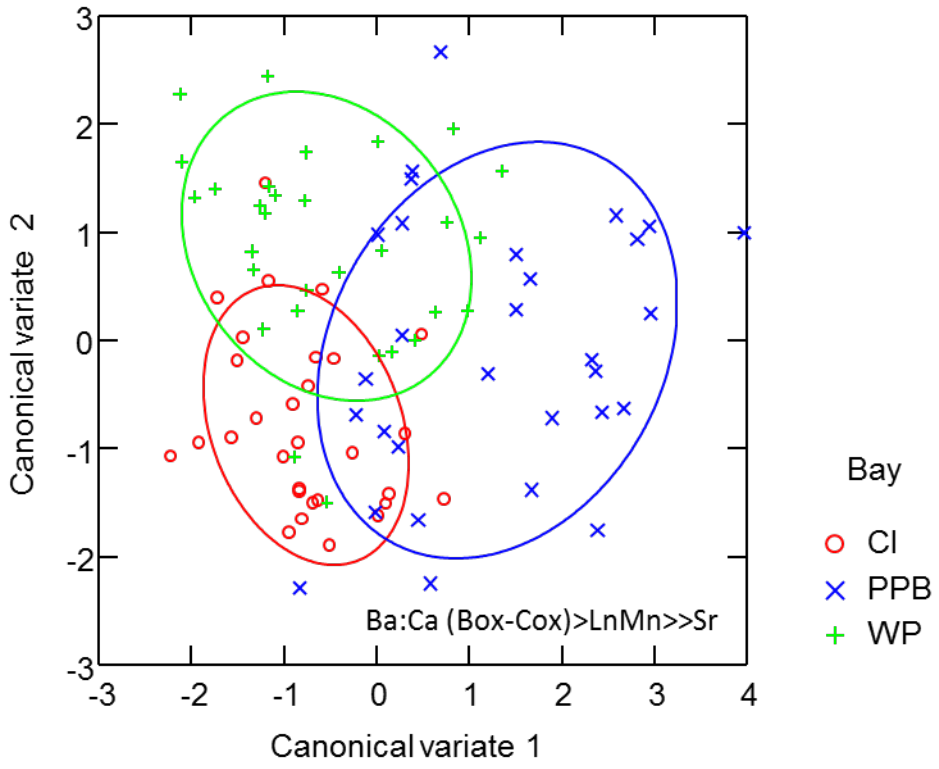


Figure 13. Canonical variate plot comparing multi-element chemistry of the advanced post-larval stage of age 2+ King George Whiting sampled from three Victorian bays in 2013/14. PPB=Port Phillip Bay, CI=Corner Inlet, WP=Western Port.

Table 7. Left – results of post-hoc pairwise comparisons between bays (after MANOVA) for the advanced post-larval stage otolith chemistry (LnMn:Ca, Box-CoxBa:ca, Sr:Ca) of age 2+ year King George Whiting sampled from three Victorian bays. Right – results of Jackknifed cross-validation classifications and Kappa index. PPB=Port Phillip Bay, CI=Corner Inlet, WP=Western Port.

Pairwise comparisons			
Bays		**Hotelling's T-Square**	**p-Value**
PPB	CI	70.764	<0.001
PPB	WP	64.965	<0.001
CI	WP	41.651	<0.001

	% samples classified to each bay		
Bay collected	CI	PPB	WP
CI n=30	80	10	10
PPB n=30	23	67	10
WP n=30	17	16	67
Grouping	**Overall classification accuracy %**	**Kappa**	
Bay	71	0.57	

Part 2: Determining contributions of Victorian and South Australian nursery source to replenishment of a known King George whiting spawning area in South Australia

Juvenile baseline signatures

Univariate graphs of variation in element:ca ratios among the major juvenile nursery bays showed among-bay variation for most element:Ca ratios (except for Mg:Ca), and the two stable isotope ratios (Figure 14). Variation was evident among bays within states (i.e. Ba:Ca), (Figure 14) and among States (i.e. Mn:Ca) (Figure 15).

Canonical variate plots showed variation among bays (Figure 16a, b) and States (Figure 16c, d) for both the 2005 and 2006 cohorts. Variation among the nursery bays (regions) was highly significant. Most of the significant pairwise comparisons were between bays from the two States, as opposed to the comparisons of bays within States (Table 8). At the State level there were clear and similar differences between the baseline signatures for both cohorts (Figure 16c, d). Mn:Ca and Sr:Ca were the two most important variables for discrimination between States for both cohorts (Figure 16c, d).

Jackknifed cross-validation classification accuracy at the bay level was only moderate for most bays in each cohort (i.e. 40-60%), but was higher for PPB in 2005, and CI and NSG in 2006 (Table 9a, b). Most of the misclassifications among bays were generally to other bays within the same State (Table 9a, b). At the State level, classification accuracy was high for both cohorts (79-89%), demonstrating strong discrimination between the baseline signatures for the Victorian and South Australian nursery areas sampled (Figure 16c, d).

State of nursery origin of adults

For both cohorts the maximum likelihood estimation of adult origins estimated that virtually all of the adult spawning samples collected in South Australia originated from nursery areas in that State (Table 10). However, comparison of the adult otolith chemistry data with the juvenile baselines indicated that many adult samples, while being more similar to the South Australian baselines, were distributed outside of the 95% confidence ellipses of both the South Australian and Victorian baseline data (Figure 17). The differences between the adult and juvenile baselines appeared largely related to the higher $\delta^{18}O$ and Sr:Ca levels in the adult samples (Figure 14d, f).

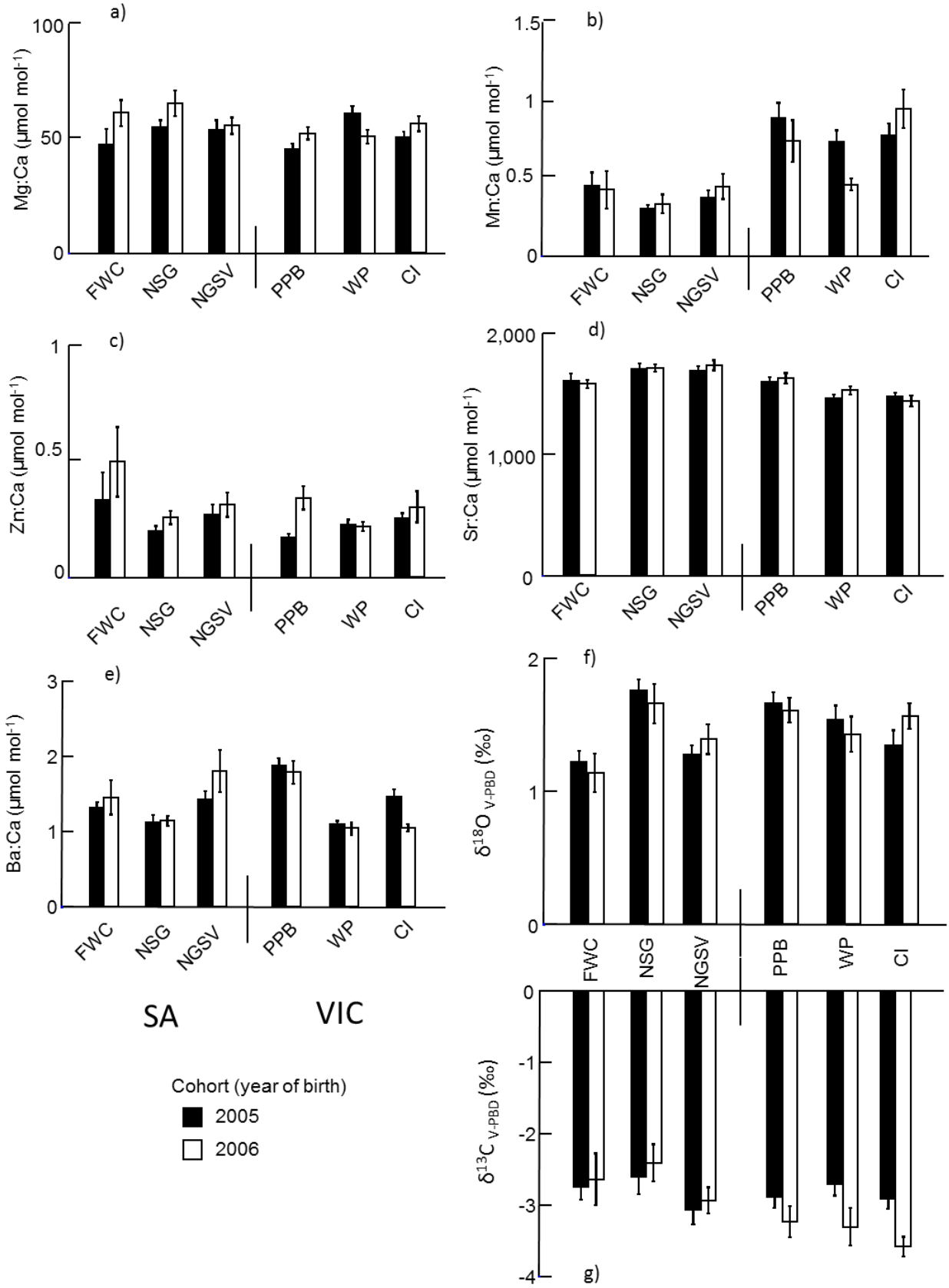


Figure 14. Comparisons of mean (± SE) element:Ca (a-e) and stable (f) isotope ratios among six sampling regions (bay nursery areas) in Victoria (VIC) and South Australia (SA) for the first to second annual increment zones of juvenile King George Whiting, and adults sampled from known spawning areas in South Australia from the 2005 and 2006 cohorts. NSG = Spencer Gulf, NGSV = North Gulf StVincent, FWC = far west coast of SA, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet, ADLT=adults.

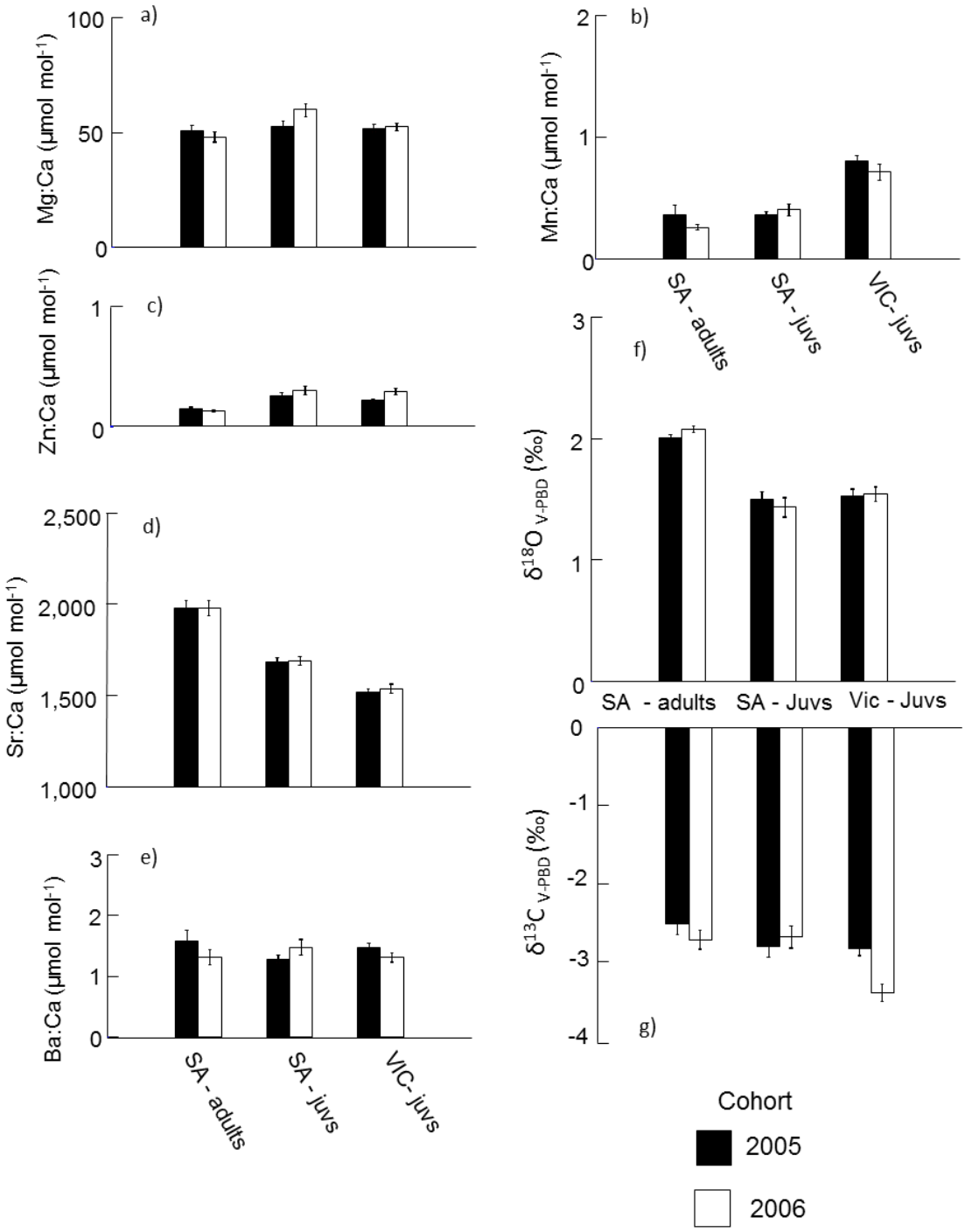


Figure 15. Comparisons of mean (± SE) element:Ca (a-e) and stable (f, g) isotope ratios among States for the first to second annual increment zones of juvenile King George Whiting, and adults sampled from known spawning areas in South Australia from the 2005 and 2006 cohorts. SA = South Australia, VIC = Victoria.

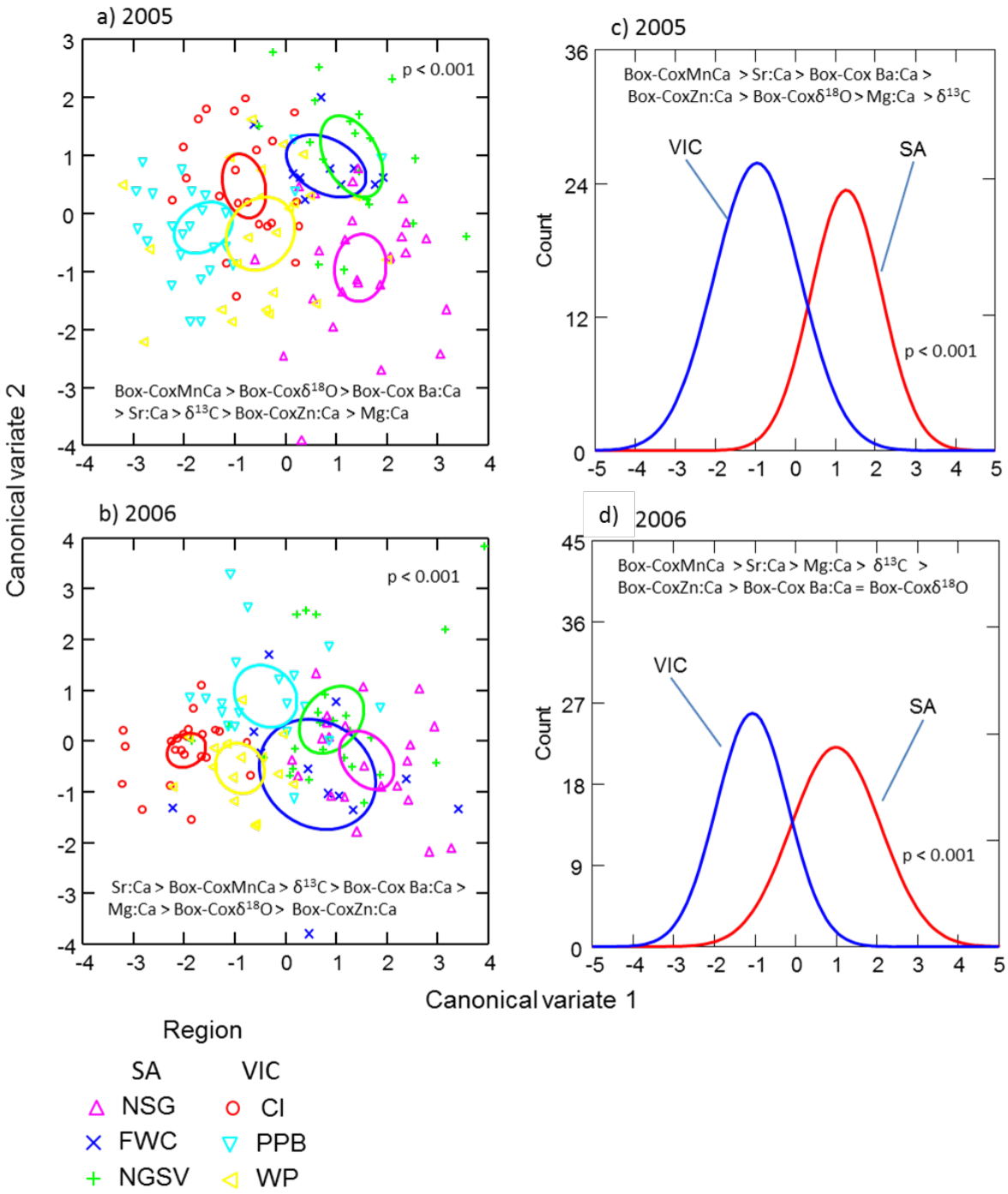


Figure 16. Canonical variate plots from quadratic discriminant function analyses of multi-variate element:Ca and stable isotope ratio data for the first to second annual increment zone of juvenile King George Whiting sampled from two cohorts, a) 2005 and b)2006 and six nursery bays across Victoria and South Australia. NSG = Spencer Gulf, NGSV = North Gulf St Vincent, FWC = far west coast of SA, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet.

Table 8. Results of between bays post-hoc pairwise comparisons of multivariate element:Ca and stable isotope ratios (after significant MANOVA, p< 0.001)) for the first to second increment zone of juvenile King George Whiting from six nursery areas across Victorian and South Australia, and two cohorts; a) 2005, b)2006. Note: only significant comparisons are indicated.

a) 2005

Significant pairwise comparisons (p≤0.01)			
Regions		**Hotelling's T-Square**	**p-Value**
CI	NGSV	54.784	<0.001
CI	NSG	89.543	<0.001
PPB	WP	41.259	<0.001
FWC	PPB	55.438	<0.001
FWC	WP	31.487	<0.01
NGSV	NSG	43.278	<0.001
NGSV	PPB	112.311	<0.001
NGSV	WP	63.348	<0.001
NSG	PPB	131.860	<0.001
NSG	WP	64.222	<0.001

b) 2006

Significant pairwise comparison (p≤0.01)			
Regions		**Hotelling's T-Square**	**p-Value**
CI	FWC	70.750	<0.001
CI	NGSV	107.505	<0.001
CI	NSG	144.381	<0.001
CI	PPB	41.140	=0.001
FWC	PPB	32.833	<0.01
NGSV	PPB	27.697	<0.01
NGSV	WP	41.881	=0.001
NSG	PPB	62.102	<0.001
NSG	WP	60.904	<0.001

Table 9. Results of jackknifed cross-validation classification of baseline (first to second increment zone) otolith chemistry signatures for samples from six bay/gulf nursery regions of Victoria and South Australia and two cohorts (2005, 2006). Data grouped by bays (a, b), data grouped by State (c, d). NSG = Spencer Gulf, NGSV = North Gulf StVincent, FWC = far west coast of SA, PPB=Port Phillip Bay, WP=Western Port Bay, CI=Corner Inlet,

a) 2005 cohort

	% classified to region					
Region of origin	CI	PPB	WP	NGSV	NSG	FWC
CI (n=22)	**50**	14	32	5	0	0
PPB (n=25)	12	72	8	8	0	0
WP (n=23)	26	9	43	0	17	4
NGSV (n=19)	0	0	16	53	26	5
NSG (n=26)	0	0	8	35	58	0
FWC (n=10)	10	0	0	30	20	40
Overall accuracy = 54%				Kappa = 0.44		

b) 2006 cohort

	% classified to region					
Region of origin	CI	PPB	WP	NGSV	NSG	FWC
CI (n=23)	70	4	13	4	0	9
PPB (n=19)	11	42	5	16	16	11
WP (n=15)	13	7	60	7	7	7
NGSV (n=26)	4	12	4	46	12	23
NSG (n=22)	0	9	0	18	73	0
FWC (n=13)	8	8	15	23	0	46
Overall accuracy = 57%				Kappa = 0.48		

c) 2005 cohort

	% classified to State	
State of origin	SA	VIC
SA (n=55)	82	18
VIC (n= 70)	11	89
Overall accuracy = 86%		Kappa = 0.71

d) 2006 cohort

	% classified to State	
State of origin	SA	VIC
SA (n=61)	82	18
VIC (n= 57)	21	79
Overall accuracy = 81%		Kappa = 0.62

Table 10. Results of maximum likelihood estimation of nursery origins of adults sampled from known spawning areas in South Australia for two cohorts. Baselines were the age 1-2 otolith chemistry from juveniles of the same cohorts sampled from three nursery areas in Victorian and three nursery areas in South Australia.

Cohort – Adult samples	Estimated % contribution of Victorian nursery areas, mean (SD)	Estimated % contribution of South Australian nursery areas, mean (SD)
2005 (n= 32)	6 (6)	94 (6)
2006 (n=40)	0 (2)	100 (2)

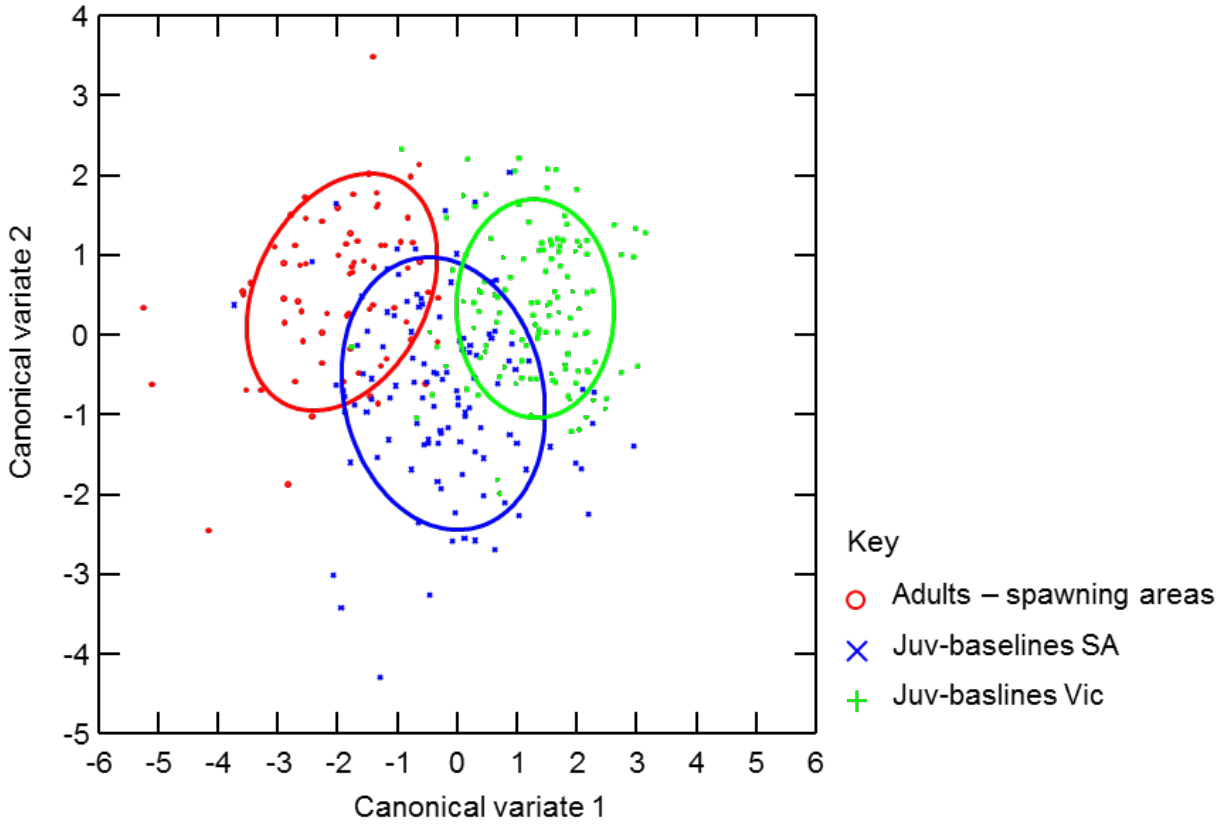


Figure 17. Discriminant function plot of adult samples from South Australian spawning areas with juveniles nursery (baseline) samples from Victorian (VIC) and South Australian (SA) bays/gulfs.

Discussion

Part 1: Mixing of juveniles among Victorian nursery bays

The analyses of the advanced post-larval stage of age 2+ King George Whiting was consistent with limited (if any) mixing of the juvenile populations among Victoria's three major nursery embayments: Port Phillip Bay (PPB), Western Port (WP) and Corner Inlet (CI). The strong separation of the otolith chemistry signatures among the three bays indicates that the populations in each bay had remained independent from the post-larval stage. The main implication of these results is that management of exploitation and catch sharing can be focussed at the bay-specific level. This is important because the King George Whiting fisheries in Victoria are largely focussed on the juvenile stages within the three main sheltered bays; and the catches in coastal waters by comparison are negligible. Each bay fishery is multispecies and has different levels of commercial and recreational targeting and dependence on King George Whiting (i.e. WP – recreational, CI – commercial and recreational, Port Phillip Bay – commercial and recreational). Management drivers for regulation of Whiting catches can therefore vary among bays, and it is important to know that management changes for one bay don't influence the Whiting fisheries in other bays.

The main drivers of the otolith chemistry discrimination among the three bays were Ba:Ca and Mn:Ca. Otolith Ba:Ca was higher in samples from PPB than CI and WP. This is highly consistent with previous studies on juveniles (0+ age) of other species (snapper, *Chrysophrys auratus* and sand flathead *Platycephalus bassensis*) across the same three bays, where Ba:Ca was consistently higher in otoliths from the PPB samples (Hamer and Jenkins, 2007). Further, these studies showed that ambient concentrations of Ba, and the Ba:Ca ratio of water samples were significantly higher in Port Phillip Bay than Western Port, Corner Inlet and coastal waters (Jones et al., 1990; Fowler and Short, 1996; Hamer et al., 2006; Hamer and Jenkins, 2007), consistent with the main driver of variation in Ba:Ca of the Whiting otoliths being the ambient concentrations. The higher Mn:Ca in the Corner Inlet King George Whiting otoliths is not consistent with the previous studies or the data on water chemistry, and indicates that other processes besides ambient water chemistry are having a greater influence on otolith Mn:Ca, or that ambient Mn:Ca has varied significantly over time.

Part 2: Determining contributions of Victorian and South Australian nursery source to replenishment of a known King George Whiting spawning area in South Australia

Clear nursery area baseline otolith signatures were developed for each State in both cohorts (2005, 2006). Consistency in the baseline signatures and degree of discrimination between States for the two cohorts indicated that the environmental or physiological processes driving the State-based discrimination were consistent over the two cohorts. Mn:Ca and Sr:Ca were particularly important in the discrimination among the States. Mn:Ca was higher in Victorian baseline samples and Sr:Ca was higher in South Australian samples.

The maximum likelihood estimation of the adult origins, suggested that virtually all the spawning adults sampled from the known South Australian spawning areas were derived from the South Australian nursery areas. However, the multi-variate chemistry data indicated that many of the adults had otolith chemistry for the age1-2 nursery stage that were outside the distribution of the South Australian baseline data for both cohorts. This may suggest that alternative juvenile source areas were important and were not included in the baseline data. It is important to have confidence that these outlying adult otolith chemistry signatures were derived from an alternative nursery area in South Australia as opposed to Victoria.

Two key observations from the adult otolith chemistry data support the idea that the outlying adults were from an uncharacterised nursery area in South Australia. Firstly, the Sr:Ca in the

baseline samples were higher in the South Australian than Victorian samples, and for the adults the Sr:Ca values were also higher than the Victorian baselines and even higher than the South Australian baselines. For Mn:Ca the adult values were clearly matched to the juvenile baselines from South Australia, with both the adults and South Australian baselines being lower than the Victorian baselines.

We are confident we have sampled the major Victorian nursery areas (Victoria's three major bays), and earlier pilot surveys of coastal areas have not found small post-larval Whiting in numbers that would lead us to believe the open coast provides a significant alternative juvenile nursery area (P. Hamer, pers. obs). However, post-larval/juvenile Whiting recruitment can occur in areas outside of the main bays/gulfs in South Australia. Further, while the main nursery areas are considered to be in the north of the Gulfs (Jones et a. 1990, Fowler and Short 1996), where we sampled most of the 3+ juveniles for the baseline data (Figure 9), the gulfs are large and there are likely juveniles recruiting in the southern regions of the gulfs also. One important area that was not sampled to construct the baselines were the bays along the northern coastline of Kangaroo Island (Figure 9). Previous tagging studies suggest that juvenile Whiting remain resident in this area and recruit into the local spawning population (Fowler et al., 2002). These bays would be expected to have cooler water than the northern Gulfs during the spring-autumn period when the juvenile baseline otolith zone was deposited (i.e. translucent zone between increments 1 and 2). Exposure to cooler water is consistent with the elevated δ18O of the adult samples (i.e. higher δ18O = otolith deposition in cooler water) (Campana, 1999), and may also partly explain the elevated Sr:Ca, either due to a kinetic effect or slower growth rate in cooler water (Sadovy and Severin, 1994; Campana, 1999). The samples of spawning adults virtually all (86%) came from the area along the north-coast of Kangaroo Island and into Investigator Strait, with the few others from southern Gulf St Vincent. It is most likely that source of these outlying juvenile signatures was the unsampled nursery areas along the north coast of Kangaroo Island.

While the known spawning areas around Kangaroo Island/Investigator Strait appear replenished from adjacent nursery bays, there is a strong likelihood of other spawning areas or "hot spots" in South Australian waters. While major spawning in Victorian waters seems unlikely (Hamer et al., 2004) , there is little knowledge of the distribution of spawning by King George Whiting in the south-east region of South Australia, and no surveys of this region have occurred. The exposed area of coastline represents the major gap in our knowledge of King George Whiting spawning. It remains possible that juveniles recruiting in Victorian bays, in particular, Port Phillip and Western Port, originate from this unsurveyed region, and emigrate back to this region as adults. Further, fish from the South Australian gulfs may also emigrate to this region, although the available tag/recapture did not indicate this (Fowler et al. 2002). However, fishing effort is relatively low in the south-east, making tag recovery difficult.

Overall, the results suggest that emigration of Whiting from Victoria's main nursery areas back to known South Australian spawning grounds around Kangaroo Island is not an important process for replenishing these spawning populations. We suggest that the most likely scenario is that Whiting from Port Phillip Bay and Western Port, and potentially even Corner Inlet, emigrate west as adults but not as far as Kangaroo Island/Investigator Strait, and replenish as yet undefined spawning areas along the coast of far west Victorian (west of Portland) and south-east South Australia.

This is consistent with early modelling studies of larval drift (Jenkins et al., 2000), which also suggest that spawning in this region would not be an important source of recruitment in to the South Australian Gulf fisheries, as larvae spawned in the south-east region of South Australia would be transported from west to east during the winter dispersal period (Jenkins et al. 2000). This implies that the Victorian population may have little influence on demographics and production of the major South Australian fisheries, but uncertainty remains as to whether or not Whiting that recruited into South Australia's Gulfs and the Kangaroo Island bays, emigrate to the south-east region of South Australia and contribute to spawning that replenishes the bay fisheries in Victoria, and if so what is the importance of the process to the demographic and production of Victoria's Whiting fisheries. One approach to resolve this uncertainty is tag recapture studies, where similar numbers of older juveniles (i.e. 3+ age) are tagged in South Australian and Victorian nursery areas, and the recapture rates of the different tagging sources are compared along the western Victorian and south-east South Australian coast. Another approach would be to use the otolith microchemistry analyses described in this chapter on adult Whiting sampled from south-eastern South Australia, to determine their nursery area origin (Victoria or central South Australia).

Chapter 3 Genetic differentiation of King George Whiting populations across Southern Australia

Introduction

Understanding the genetic structure of populations is a key component of conservation biology, particularly in terms of assessing management units for natural resources (Palsbøll et al., 2007). This is because genetic data can provide a wealth of information which can be utilised by fisheries managers, including estimates of genetic diversity and inbreeding within populations (indicators of fitness), stock identification, the source of recruits and levels of connectivity (Waples et al., 2008). Strategies for both exploitation and conservation are most effective when it is possible to identify individual stocks (Waples, 1998), however despite the vast number of population genetic studies carried out on natural populations, genetic information has been generally underused in management strategies and stock assessment for commercial and recreation fish species (Atarhouch et al., 2006).

The distribution of genetic variation across a species' range is dependent on levels of gene flow, which refers to the movement of genes between subpopulations as a result of the migration (Nei, 1977). This is most commonly inferred from the degree of genetic differentiation between two or more subpopulations, and is often represented by the statistic F_{ST} (Wright, 1949). High levels of migration and gene flow result in the genetic homogenisation of allele frequencies within each subpopulation, and thus low levels of genetic differentiation between them, whilst limited or no gene flow will result in increased genetic differentiation between subpopulations due to the divergence of allele frequencies in response to genetic drift and/or site-specific selection. As a result of their environment, marine species are often characterised by high levels of dispersal, connectivity and gene flow (Waples, 1998), which commonly results in population structure that is subtle at best, with low measures of F_{ST}.

King George Whiting, *Sillaginodes punctatus*, (Cuvier 1829) is a temperate, demersal fish species, distributed along the southern coastline of Australia from Sydney to Perth, including Tasmania. Observing the differences in the genetic composition between populations of King George Whiting provides a complimentary approach to otolith-based methods for understanding patterns of movement and connectivity between different locations. Previous work using nine microsatellite markers found little evidence of population differentiation for *S. punctatus* across Victoria and South Australia (Haigh and Donnellan, 1998), however not all sites were well represented, and Corner Inlet was not included. Corner Inlet is a noticeable gap, as based on the previously mentioned hydrodynamic modelling, this site has the highest likelihood to be replenished from alternative spawning grounds, and thus potentially be a discrete population. Phylogeographic analysis of *S. punctatus* from Western Australia, South Australia and Victoria using mitochondrial DNA also showed no evidence of long-standing population structure, or population differentiation between regions (Haigh and Donnellan, 1998). High variability was observed for the King George Whiting mitochondrial control region, which is what would be expected for a recently expanded population, and the majority of genetic variance occurred within regions (99.26%), with only a small amount between regions (0.74%) (Haigh and Donnellan, 1998).

Genetic studies have undergone a revolution in the past decade, with next-generation sequencing (NGS) methods becoming more readily available and cost effective. The recent improvement in NGS and bioinformatics has prompted a shift from analyses of microsatellite markers to direct sequence variation including single nucleotide polymorphisms (SNPs);

single base pair variations that occur widely throughout the genome. This allows for wider, more detailed genome coverage than other types of markers, and as a result, datasets containing thousands of loci can now be examined, resulting in increases in power and accuracy (Allendorf et al., 2010). These techniques have already been used to assess genetic differentiation and adaptation in a number of marine species (Nielsen et al., 2009; Hohenlohe et al., 2010; Benestan et al., 2015).

This study expands on the previous genetic work using the microsatellite markers described by Haigh and Donellan (2000) to examine genetic structure across prominent nursery areas across South Australia and Victoria, with the addition of samples from Corner Inlet. Genotype by Sequencing was also used to create a SNP dataset for individuals from numerous sites across the species' geographic range, including Western Australia and Tasmania, to provide more power and accuracy in detecting fine-scale genetic structuring.

Methodology

Sample collection

For microsatellite analysis *S. punctatus* post larvae were collected from three regions in South Australia (Spencer Gulf, Gulf St Vincent and Kangaroo Island) and from three bays in Victoria (Port Phillip Bay, Western Port and Corner Inlet) during November and December 2011 (Figure 2, Figure 18). Samples of post larvae for SNP analysis were collected from these same areas during November-December 2012. In addition, fin clips from large adult Whiting were provided, which were caught in deeper offshore habitat, north of Kangaroo Island in April 2013 (Figure 2, Figure 18).

In Western Australia, post larvae were provided from Mangles Bay and Leschenault (sampled December 2013) and fin clips were provided from juvenile fish in Albany (sampled December 2013) (Figure 18). In Tasmania, fin clips were collected from large adults in Stanley, and from juvenile *S. punctatus* in St Helens (Figure 18) during December 2014. Post larvae and juvenile samples are representative of the *S. punctatus* populations in each collection location, as individuals reside in these sheltered bays for approximately 4 years. A summary of all samples used for genetic analyses is detailed in Table 11.

Table 11. Summary of *S. punctatus* samples used for genetic analysis.

Location	Sample type	Microsatellite analysis	SNP analysis
Western Australia			
Mangles Bay	post-larvae	-	24
	post-larvae	-	40
Albany	fin clips (juv.)	-	11
South Australia			
Spencer Gulf	post-larvae	90	55
Gulf St Vincent	post-larvae	90	75
Kangaroo Island	post-larvae	90	50
Kangaroo Island (offshore)	fin clips (adult)	-	25
Victoria			
Port Phillip Bay	post-larvae	90	61
Western Port	post-larvae	90	60
Corner Inlet	post-larvae	60	60
Tasmania			
Stanley	fin clips (adult)	-	23
St Helens	fin clips (juv.)	-	17

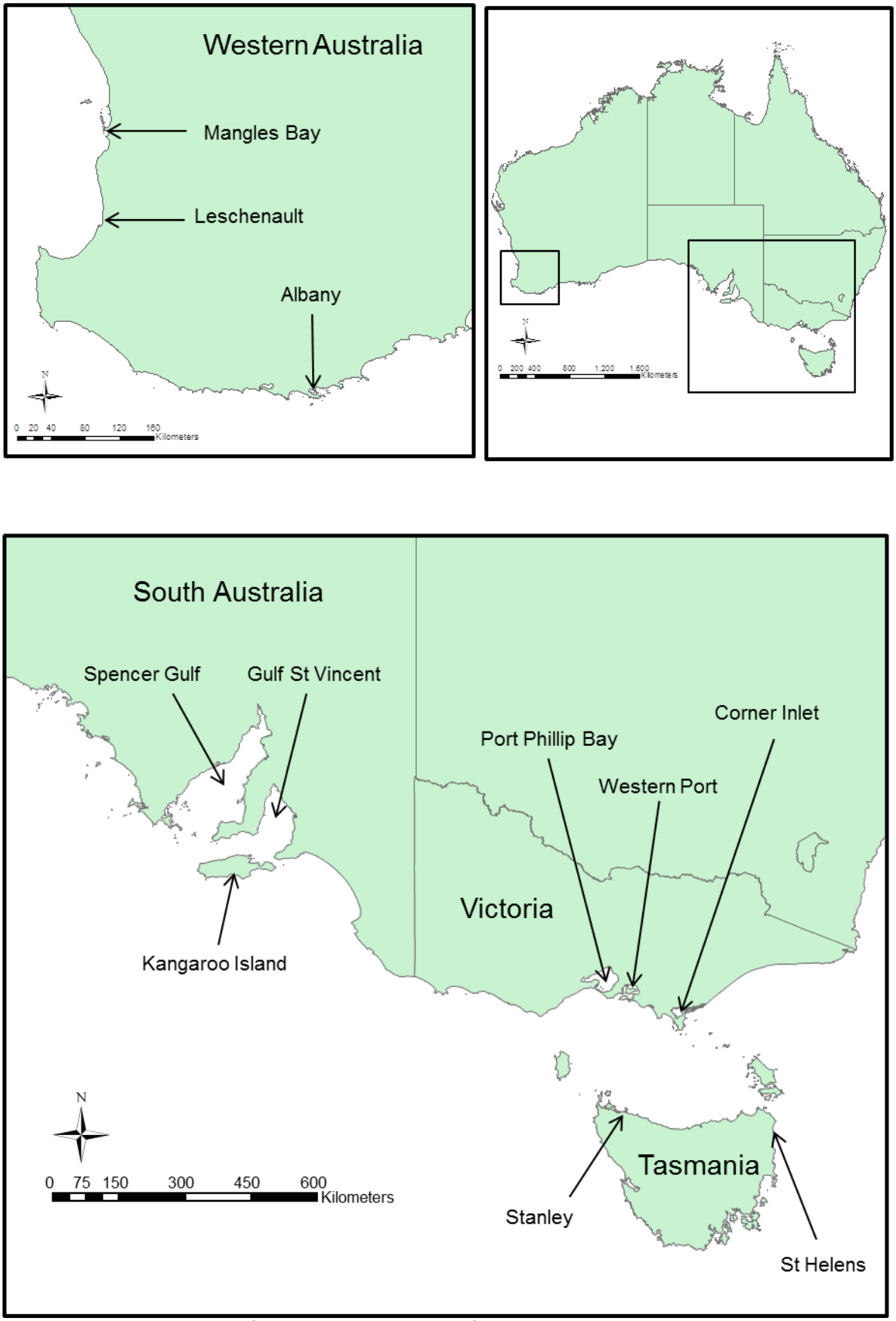


Figure 18. Areas sampled for King George Whiting for genetic analyses

Laboratory methods

Genomic DNA was extracted from all individuals using QIAGEN DNEasy Blood and Tissue kits, as per the manufacturer's instructions.

Microsatellite analysis

Levels of connectivity were assessed using seven polymorphic microsatellite markers: Sp19, Sp22, Sp32, Sp35, Sp36, Sp38 and Sp39 (Haigh and Donnellan, 2000). Only seven out of nine microsatellite markers described by Haigh and Donnellan were used, as two did not amplify sufficiently. Microsatellites were amplified using a polymerase chain reaction (PCR) touchdown program using the following thermal cycling conditions; initial hot start at 94°C for 15min; five cycles of 94°C for 45 s, 65°C for 45 s, 72°C for 45 s; five cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s; ten cycles of 94°C for 45 s, 57°C for 45 s, 57°C for 45 s, 72°C for 45 s; twenty cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 45 s; final elongation at 72°C for 15min. PCR was conducted in 11-µl volumes containing; 10 ng of genomic DNA; 5 µl PCR Master Mix (Qiagen, USA), 4 µl primer multiplex consisting of 0.26µM of each forward primer with a fluorescent dye associated tag (FAM-GCCTCCCTCGCGCCA; NED-GCCTTGCCAGCCCGC; PET-CGGAGAGCCGAGAGGTG; VIC-CAGGACCAGGCTACCGTG) and 0.13 µM of reverse primer. PCR amplicons were electrophoresed on an ABI 3130xl Genetic Analyzer, incorporating LIZ 500 (-250) size standard (Applied Biosystems). Alleles were scored using GeneMapper, v3.7 (Applied Biosystems).

SNP analysis

DNA was quantified and standardised to100 ng/µL before being sent to the Genomic Diversity Facility at the Cornell University Institute of Biotechnology. Samples were submitted in 96-well plate format and individually barcoded. Restriction enzymes were used to reduce the complexity of the genome, and Genotype-by-sequencing (GBS) libraries were constructed based on methods described by Elshire et al. (2011). GBS libraries were sequenced on the Illumina HiSeq 2000/2500 (100 base pair, single-ended reads).

Data Analysis

Microsatellite analysis

Each pairwise combination of loci was tested for linkage disequilibrium within each population using the program GENEPOP v4.2 (Rousset, 2008). Conformity to Hardy-Weinberg Equilibrium (HWE) was tested using Genalex (Peakall and Smouse, 2012), with significant deviations determined using exact tests and a sequential Bonferroni correction applied for multiple tests. Number of migrants and inbreeding coefficients were also calculated to assess migration levels and the extent of non-random mating across populations. To determine population structure F_{ST} values were calculated across all sites and between all pairwise comparisons of sites using ARLEQUIN v3.5 (Excoffier and Lischer, 2010).

SNP analysis

A SNP discovery pipeline called UNEAK (Universal Network-Enabled Analysis Kit) was used, which is for SNP-calling in species without a reference genome – an extension of the java program TASSEL (Lu et al., 2013). The SNP dataset underwent a number of filtering steps using VCFtools (Danecek et al., 2011) which are summarised in Table 12. All sites with a minor allele frequency of less than 0.5 were excluded. Allele frequency is defined as the number of times an allele appears over all individuals at that site, divided by the total number of non-missing alleles at that site (i.e. the minor allele frequency is the frequency at which the

least common allele occurs in a given population). Any genotypes with less than 5, or more than 58 (ten times the mean) reads were excluded from the dataset. This is to ensure sufficient read depth, and to eradicate repetitive DNA sequences. Poorly represented individuals with too few sites (less than 10% of the total number of SNPs) were not used in further analysis. Sites were then also excluded based on the proportion of missing genotype rate, with only 20% missing data allowed (i.e. a coverage of 80% across all individuals). Sites which were out of Hardy Weinberg equilibrium, after False Detection Rate, in more than 2 populations were identified and removed.

Table 12. Number of SNPs retained after each filtering step

Filtering step	SNP count
TASSEL GBS pipeline	165876
Minor allele frequency (MAF) > 0.05	105737
Minimum depth filter:	105737
Genotypes >5 reads (max is 10x the mean coverage; 58)	
Exclude individuals with < 10% total # sites	105737
Exclude sites with <80% coverage	11207
Hardy-Weinberg Equilibrium filter:	
Filter sites out of HWE in >2 populations	11174

The R PopGenome package (Pfeifer et al., 2014) was used to calculate general population statistics including global F_{ST} values based on minor allele frequencies (Hudson et al., 1992). Pairwise F_{ST} comparisons between all locations were calculated using ARLEQUIN v3.5 (Excoffier and Lischer, 2010). Patterns of isolation by distance were assessed using a MANTEL test for correlation between genetic and geographic distances using Isolation By Distance on the web (Jensen et al., 2005), using pairwise F_{ST} comparisons and the shortest geographic distance between each site within the ocean. FastStructure (Raj et al., 2014) was used to examine the number of genetic clusters (K's) from the SNP genotype dataset. The fastStructure algorithm was run over multiple choices of K (2-10) using a simple prior (a flat beta-prior over population-specific allele frequencies at each locus). The chooseK.py function was used to identify which K best explained the structure in the data. The fastStructure was then run over this K 100 times using a logistic prior, in which the population-specific allele frequency is generated by a logistical normal distribution, which can be used to detect more subtle genetic structuring in the dataset (Raj et al., 2014). The marginal likelihood values were calculated for each run, and the top twenty-five logistic runs were identified and the mean value of likelihood for each sample as dictated by the best K were calculated. For this step a K value of 6 was used, as chooseK.py suggested the best K is somewhere between 2 and 6.

Results

Microsatellite Results

Analyses showed that levels of genetic diversity did not vary significantly between populations or regions (Table 13). The number of alleles per locus varied from 2 to 19 with a mean of 3.93 ± 0.33 (SE) across all loci and populations. This pattern was similar to that seen for expected heterozygosity (H_e) which varied from 0.29 to 0.48 with a mean of 0.4 ± 0.019 (SE) across all populations and loci (Figure 19). The low H_e values show that there is not a high level of diversity within these microsatellite markers. Overall levels of inbreeding were low with mean F_{IS} -0.016 ± 0.0007 (SE).

Tests for conformity to Hardy-Weinberg equilibrium, after sequential Bonferroni adjustments, showed the majority of populations were in equilibrium, although significant departures from Hardy-Weinberg proportions were observed in 6 populations (Table 13). Overall the levels of deviation were not large and were not consistent across loci or populations.

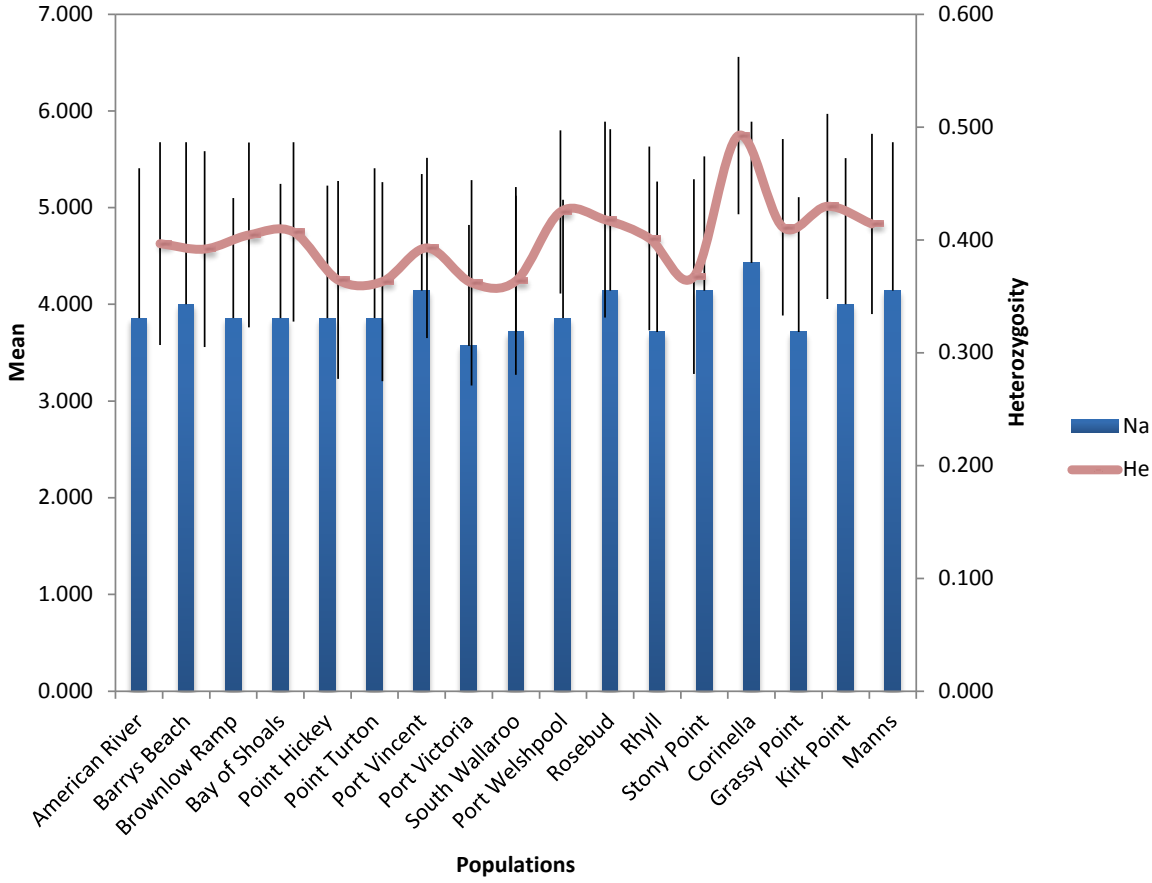


Figure 19. Patterns of genetic diversity across populations; number of alleles (Na) and expected heterozygosity (H_e).

Overall estimates of genetic differentiation were significant but low, with a global FST estimate of 0.024 ± 0.003 (SE) (Table 14). Pairwise population comparisons for F_{ST} were also small (ranging from 0.000–0.052), with the highest value of 0.052 observed between the Port Victoria and Bay of Shoals populations (Table 15). Stony Point and Corinella (both in Western Port, Victoria) were significantly different from 9 and 7 other sites respectively (Table 15). Out of a possible seventy two pairwise comparisons between South Australia and Victoria, seventeen were significantly different (Table 15). The average number of migrants being exchanged between populations per generation is fairly high (11.146 ± 1.252; Table 2), indicating that genes are well mixed across sampled locations. The analysis of molecular variance (AMOVA) showed that the majority of variance (98%) was due to differences within populations with little difference detected between populations (2%) and none of the variance attributable between regions (0%).

Table 13. Sample number (N), observed allele number (Na), observed heterozygosity (H_o), expected heterozygosity (H_e), and conformity to Hardy-Weinberg proportions after Bonferroni adjustmust (p-adj) for seventeen King George Whiting populations.

Region	Population	N	Na	H_o	H_e	HWE p-adj
Kangaroo Island	American River	30	3.857	0.371	0.397	**0.004**
	Bay of Shoals	27.857	3.857	0.484	0.407	0.01
	Brownlow Ramp	29.857	3.857	0.408	0.404	0.006
	Mean	**29.238**	**3.857**	**0.421**	**0.403**	
Gulf St Vincent	Barry's Beach	27.857	4.000	0.402	0.392	0.050
	Port Vincent	28.857	4.143	0.370	0.393	0.007
	Point Hickey	28.571	3.857	0.292	0.364	**0.003**
	Mean	**28.429**	**4.000**	**0.355**	**0.383**	
Spencer Gulf	Point Turton	29.286	3.857	0.309	0.363	0.005
	Port Victoria	27.714	3.571	0.347	0.362	**0.004**
	South Wallaroo	26.429	3.714	0.322	0.364	**0.004**
	Mean	**27.810**	**3.714**	**0.326**	**0.363**	
Port Phillip Bay	Grassy Point	29.286	3.714	0.378	0.411	**0.003**
	Kirk Point	29.571	4.000	0.406	0.43	0.006
	Rosebud	29.714	4.143	0.456	0.418	0.025
	Mean	**29.524**	**3.952**	**0.413**	**0.420**	
Western Port	Rhyll	28.857	3.714	0.402	0.401	0.005
	Stony Point	29.143	4.143	0.393	0.367	0.013
	Corinella	29.857	4.429	0.482	0.492	**0.003**
	Mean	**29.286**	**4.095**	**0.426**	**0.420**	
Corner Inlet	Port Welshpool	29.143	3.857	0.425	0.425	0.008
	Manns	29.429	4.143	0.423	0.414	0.017
	Mean	**29.286**	**4.000**	**0.424**	**0.419**	
	Mean	**28.908**	**3.933**	**0.392**	**0.4**	
	SE	**0.188**	**0.325**	**0.017**	**0.019**	

Table 14. Level of inbreeding (F_{IS}), estimates of genetic differentiation (F_{ST},) and number of migrants per generation (Nem) for seven microsatellite loci.

Locus	F_{IS}	F_{ST}	Nem
Sp32	-0.005	0.025	9.578
Sp36	-0.012	0.018	13.974
Sp38	0.011	0.021	11.849
Sp39	0.212	0.022	11.275
Sp19	-0.018	0.026	9.376
Sp22	-0.395	0.041	5.903
Sp35	0.094	0.015	16.065
Mean	-0.016	0.024	11.146
SE	0.07	0.003	1.252

Table 15. Pairwise FST values for S. punctatus post-larvae sampled across major nursery areas in South Australia and Victoria (*p < 0.05, **p < 0.01).

	SOUTH AUSTRALIA									VICTORIA							
	Spencer Gulf			Gulf St Vincent			Kangaroo Island			Port Phillip Bay			Western Port			Corner Inlet	
	South Wallaroo	Port Victoria	Point Turton	Port Vincent	Point Hickey	Barker Inlet	Bay of Shoals	Brownlow Ramp	American River	Kirk Point	Grassy Point	Rosebud	Stony Point	Corinella	Rhyll	Port Welshpool	Manns Beach
South Wallaroo	0.000																
Port Victoria	0.000	0.000															
Point Turton	0.000	0.000	0.000														
Port Vincent	0.000	0.002	0.005	0.000													
Point Hickey	0.000	0.005	0.003	0.000	0.000												
Barker Inlet	0.000	0.016	0.000	0.000	0.000	0.000											
Bay of Shoals	0.000	**0.052	**0.026	**0.020	*0.022	0.004	0.000										
Brownlow Ramp	0.000	*0.020	0.013	0.012	0.005	0.000	0.001	0.000									
American River	0.000	**0.022	0.007	*0.018	0.003	0.000	0.004	0.000	0.000								
Kirk Point	0.000	0.000	0.002	0.007	0.011	0.000	0.004	0.003	0.007	0.000							
Grassy Point	0.000	0.007	0.002	0.015	0.007	0.000	*0.014	0.001	0.001	0.002	0.000						
Rosebud	0.000	**0.038	**0.023	*0.028	*0.016	0.001	0.000	0.000	0.000	0.010	0.006	0.000					
Stony Point	0.006	**0.051	**0.031	0.012	**0.026	0.008	0.000	*0.014	**0.019	**0.022	**0.029	**0.013	0.000				
Corinella	0.000	*0.023	**0.029	**0.033	**0.026	0.012	0.003	*0.013	**0.015	0.010	0.011	0.006	**0.046	0.000			
Rhyll	0.000	0.007	0.011	0.000	0.000	0.000	0.008	0.002	0.010	0.000	0.007	**0.012	0.009	*0.018	0.000		
Port Welshpool	0.000	0.003	0.005	0.004	0.005	0.002	0.002	0.000	0.001	0.007	0.001	0.007	**0.020	0.006	0.009	0.000	
Manns Beach	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.008	**0.021	0.009	0.000	0.000	0.000

SNP results

Overall genetic differentiation was low across all *S. punctatus* populations sampled, with a global F_{ST} of 0.007. Pairwise comparisons of F_{ST} among populations were also low, ranging from 0-0.007 (Table 16), with the highest comparisons between Leschenault and St Helens (0.007) and Mangles Bay and Stanley (0.006). Overall there were twenty one pairwise comparisons which were significantly different from zero. Both sites on the west coast of Western Australia (Mangles Bay and Leschenault) were significantly differentiated from seven other locations, with highest pairwise F_{ST} values recorded for comparisons with the Tasmanian locations Stanley and St Helens (Table 16). Pairwise F_{ST} comparisons for Stanley and St Helens were significantly different for five and seven locations respectively, which included the comparison between the two Tasmanian sites (Table 16). Out of a possible twenty one pairwise comparisons between South Australia and Victoria, there were none which were significantly different.

Genetic distance was significantly correlated with geographic distance, with more distant sites being less genetically similar ($p = 0.004$, $R^2 = 0.712$; Figure 20). FastStructure analysis resulted in a maximum likelihood of two genetic clusters in the data (K=2), with six populations (K=6) revealing more minor structure in the data. The fastStructure plot shows a distinct genetic signature of Western Australia locations compared to the other locations (Figure 21).

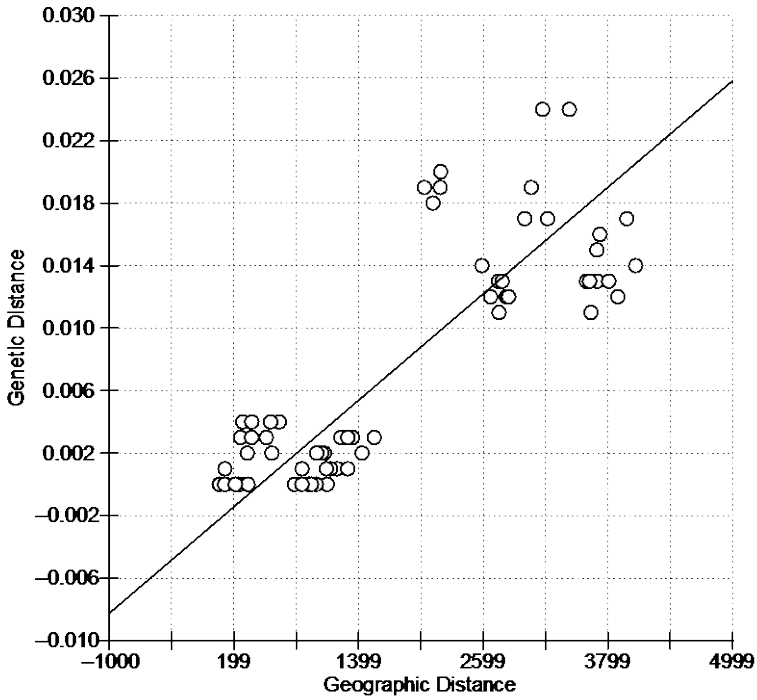


Figure 20. Levels of genetic diversity in relation to geographic distance for all *S. punctatus* populations across southern Australia (line shows RMA regression).

Table 16. Pairwise F_{ST} values for *S. punctatus* sampled in Western Australia, South Australia, Victoria and Tasmania (*p < 0.05).

	Western Australia			South Australia				Victoria			Tasmania	
	Mangles Bay	Leschenault	Albany	Spencer Gulf	Gulf St Vincent	Kangaroo Island	KI Adult	Port Phillip Bay	Western Port	Corner Inlet	Stanley	St Helens
Mangles Bay												
Leschenault	0.000											
Albany	0.000	0.000										
Spencer Gulf	***0.003**	***0.003**	0.000									
Gulf St Vincent	***0.001**	***0.001**	0.000	0.000								
Kangaroo Island	***0.003**	***0.004**	0.000	0.000	0.000							
KI Adult	0.000	0.000	0.000	0.000	0.000	0.000						
Port Phillip Bay	***0.003**	***0.003**	0.000	0.000	0.000	0.000	0.000					
Western Port	***0.003**	***0.002**	0.000	0.000	0.000	0.000	0.000	0.000				
Corner Inlet	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
Stanley	***0.006**	***0.005**	0.000	0.001	***0.001**	0.000	0.000	***0.001**	0.000	0.000		
St Helens	***0.006**	***0.007**	0.000	0.001	***0.002**	***0.003**	0.000	***0.003**	***0.001**	0.000	***0.002**	

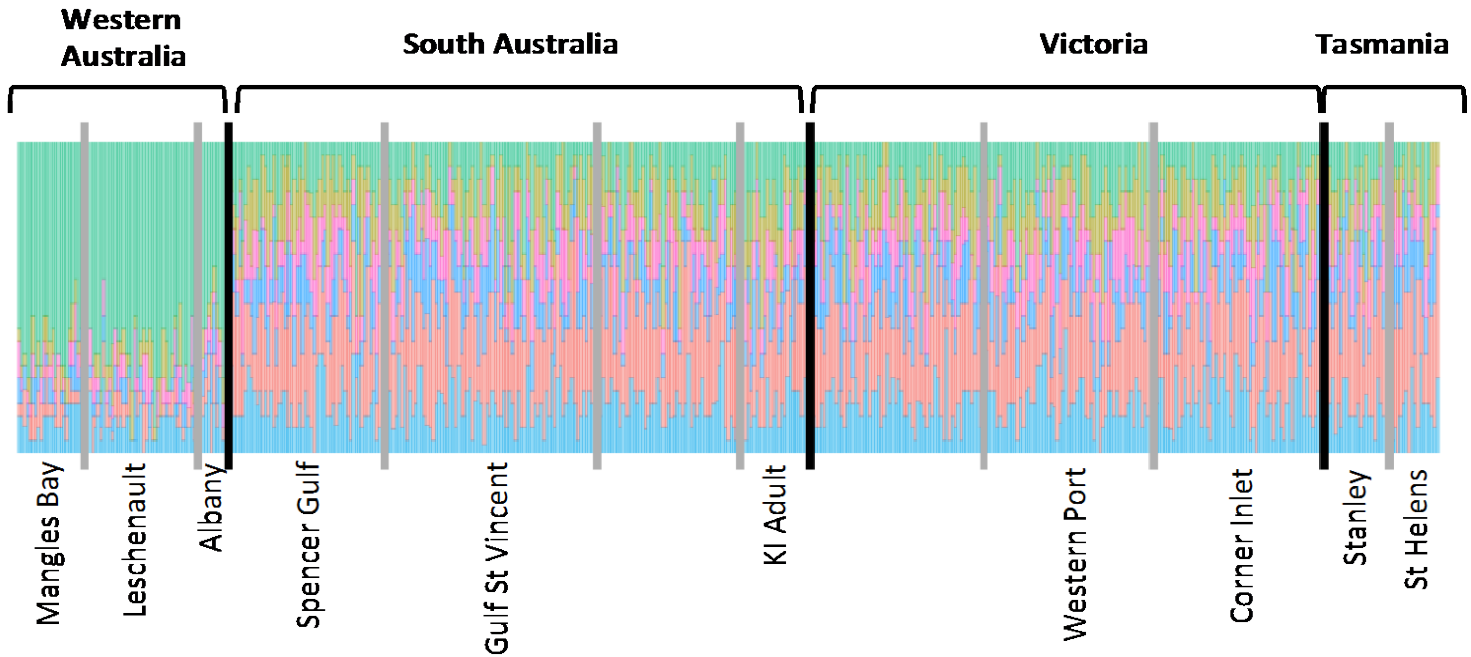


Figure 21. FastStructure plot showing population structure for *S. punctatus* across southern Australia (K=6)

Discussion

The low levels of genetic differentiation observed between most of the sampling locations of *S. punctatus* in this study are consistent with those found for other marine organisms with high dispersal potential (Palumbi, 2003; Corander et al., 2013). In many marine species, substantial dispersal or movement can occur across multiple life stages resulting in low genetic structuring (Waples, 1998), and F_{ST} values are generally found to be considerably lower for marine fish species even compared with freshwater fishes (Ward et al., 1994). Nevertheless, low but significant levels of structuring can still occur across large geographic regions, but requires the use of power of large numbers of genetic markers to detect this underlying structure (Benestan et al., 2015). The reduced costs associated with generating large amounts of sequencing data using next generation sequencing approaches now means we have the tools to identify management and conservation units for a variety of aquatic natural resources (Waples et al., 2008; Milano et al., 2011).

Levels of gene flow and genetic diversity can influence the resilience and persistence of populations over time (Miller et al., 2009). Therefore, an understanding of the levels of genetic connectivity amongst populations is an integral part of developing effective management and/or conservation strategies (Beger et al., 2014). In this study we detected no significant genetic differentiation among *S. punctatus* sampled across Victoria and South Australia, suggesting a high level of gene flow and connectivity amongst populations within and between these regions. Although the power of the microsatellite markers to detect subtle genetic structuring is impeded by low levels of variability in the marker set, the vast number of SNP loci generated provide ample power to detect shallow genetic sub-structuring, which was not present between these two regions. These findings are consistent with previous work, which found little genetic difference between populations across both states (Haigh and Donnellan, 1998).

The absence of significant genetic structuring across Victorian and South Australian regions suggests that the post-larvae arriving in Victorian bays may have originated from the same spawning areas in South Australia, or from spawning areas with high levels of connectivity to South Australian populations. In South Australia, King George Whiting is divided into three adjacent stocks for management purposes (west coast of Eyre Peninsula, Spencer Gulf, and Gulf St Vincent/Kangaroo Island) based on the movement patterns of Whiting in specific areas (Fowler et al., 2014). Hydrodynamic modelling has also suggested there are numerous discrete self-recruiting populations in South Australia (Fowler et al., 2000a). Different populations over a species' range can be linked by exchange of individuals at any point during the life cycle, and only low numbers of migrants per generation are necessary to maintain genetic homogeneity (Wang, 2004). Ocean currents may transport larvae from spatially distinct spawning areas to quite different locations, resulting in the low levels of genetic differentiation observed here within and between South Australian and Victorian stocks.

As spawning sites appear to be very specific (Fowler et al., 1999; 2000b), it is plausible that an as yet undiscovered spawning location exists somewhere off the coast of western Victoria or south-eastern South Australia, as previous modelling results would suggest (Jenkins et al., 2000). Some level of genetic connectivity would have to exist (possibly through adult movement) between such a spawning area and the known spawning areas in central South Australia. Spawning areas responsible for replenishing the bays of the west coast of South Australia are also yet to be determined (Fowler et al., 2014).

Significant genetic differentiation was detected across the geographic range of King George Whiting. This differentiation was driven by strong differences between Western Australia and all other locations, and highlighted in the fastStructure analysis. Pairwise F_{ST} comparisons also confirmed that *S. punctatus* sampled in Western Australia were most different from all other locations. The pattern of isolation by distance showed a significant positive trend for populations situated in closer proximity to be more genetically similar. Abrupt genetic change can occur at a past or present biogeographical barrier, which may be limiting gene flow and allowing Western Australian populations to become

genetically differentiated (Hellberg et al., 2002). These barriers can be in the form of strong currents, upwelling, or large expanses of open ocean which may prevent individuals from reaching suitable habitat (Gaither et al., 2010). The Great Australian Bight, a large oceanic area off the central and western sections of the southern Australian coastline, does not contain suitable habitat for juvenile and/or adult King George Whiting, and likely contributes to the observed break in genetic homogeneity between Western Australia and other locations. Mismatches between phenotype and environment may also occur when larvae reach a particular location, but do not survive because they are not adapted to local conditions (Marshall et al., 2010). Barriers to dispersal are capable of restricting gene flow over relatively small distances (Bernal-Ramírez et al., 2003).

S. punctatus from the two Tasmanian locations sampled were not only significantly different to locations in other States, but were also genetically distinct from each other, and potentially represent discrete breeding populations. This may be an example of small-scale population sub-structuring such as is thought to occur in central South Australia. The genetic results suggest that the spawning population of Whiting in north-west Tasmania (Chapter 4) does not supply larvae to the nursery area near St Helens on the east coast. Another spawning source for the eastern Tasmanian population may exist but is yet to be identified. Modelling of ocean currents in the area show it is possible that large adult Whiting off the north west coast of Tasmania are a potential source of recruits for Victorian bays (Jenkins et al., 2000), however, given the small but significant level of differentiation, it is unlikely that they are the main contributor to these populations. Further analysis of different markers, such as otolith chemical signatures of fish from these localities, may aid in elucidating movement patterns in and between these areas.

Sound fisheries management depends on robust scientific information, which should aim to include accurate definition of demographically independent populations (Waples et al., 2008). Genetic methods can be useful here, and are now incorporated in a wide range of applications pertaining to marine resource management. Genetic data represent historical patterns of gene flow and average genetic signatures over time, and may not reveal recently isolated populations or regions; therefore it is preferable that they be complimented by other techniques to assess patterns of connectivity. King George Whiting in Victoria and South Australia represent a single stock from a genetic perspective, however individuals in particular locations are likely to exhibit characteristic movement patterns. Levels of migration between populations necessary for stock structure to appear genetically homogenous are unlikely to be enough to rapidly replenish populations that are depleted (Waples, 1998), so more detailed information on specific movement is needed to inform how conservative an approach needs to be adopted by management.

Chapter 4 Age, growth and reproduction of King George Whiting from northern Tasmania

Introduction

In south-eastern Australian the only known spawning area for King George Whiting is the Investigator Strait area of South Australia (Fowler et al., 1999; 2000b) and this area is the likely source of juvenile Whiting in the South Australian Gulfs (Fowler et al., 2000a). Large, adult King George Whiting are rare in Victorian waters (Hamer et al., 2004), and studies using reverse hydrodynamic modelling of larval dispersal suggest that the spawning source for Victorian nursery areas is likely to be the coast from far west Victoria into south-eastern South Australia, but another possible, albeit less likely, source was the north-western coast of Tasmania (Jenkins et al., 2000).

Three decades ago, King George Whiting were considered to be rare in Tasmanian waters south of the Furneaux group of Islands in Bass Strait (Last et al., 1983). However, in recent years there have been increasing reports of King George Whiting in Tasmanian waters, including a small-scale mesh net fishery for large Whiting on the north-west coast, and recreational fisheries for sub-adult King George Whiting in embayments with seagrass such as St Georges Bay on the northeast coast.

In terms of understanding the population structure and connectivity of King George Whiting in south-eastern Australia, a key question is whether the recently identified population of large Whiting on the north-west coast of Tasmania represent a spawning population. The aim of this study, therefore, was to determine the age structure and reproductive characteristics of King George Whiting in this area, for comparison with these characteristics for Whiting from the known spawning areas in South Australia and Western Australia. The age structure of sub-adult Whiting from an embayment on the upper east coast of Tasmania was also examined for comparison with similar populations in embayments on the Victorian and South Australian coasts.

Methodology

Sampling Methods

Samples were collected along the north-west Tasmanian coast between Wynyard and Robbins Island, as well as from Georges Bay on the north-east Tasmanian coast (Figure 22).

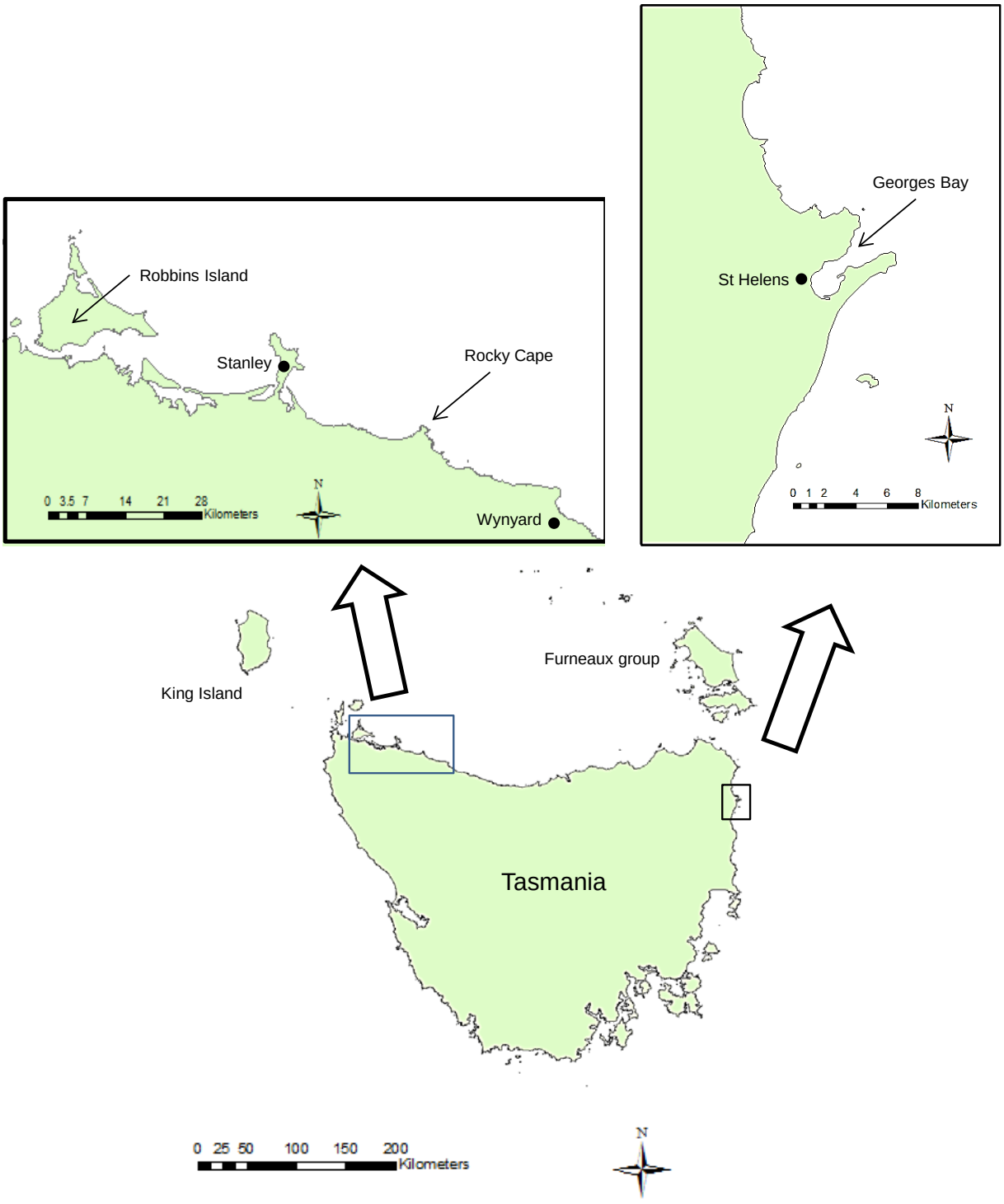


Figure 22. Map of the sampling areas for King George Whiting on the north-west coast of Tasmania and Georges Bay

On the north-west coast, King George Whiting were captured opportunistically in mesh nets (7.5 cm mesh) by a commercial fisher from September 2013 to June 2015. Samples from north-west Tasmania were mostly collected over shallow (2-5 m depth) seagrass habitat. Project researchers participated in fishing operations in December 2013 and March 2014. Fish were measured (Total length) and from May 2014 were also weighed, before fillets were removed and the frames were frozen for later analysis. Samples from Georges Bay were collected opportunistically by recreational fishers using rod and line in January and February 2012 and December 2013, generally in patchy seagrass/sand habitat. Project researchers participated in fishing operations in December 2013. Fillets were removed and the frames were frozen for later analysis.

For ageing samples, frames were thawed and re-measured (Fork length) in December 2013 and July 2014. Sagittal otoliths were dissected from frames of fish collected from January 2012 to July 2014 and stored dry in paper envelopes. For reproductive analysis, frames were thawed and re-measured (Fork length) in July 2014, March 2015 and June 2015. Gonads from frames collected from May 2014 to June 2015 were photographed 'in viscera' and then removed and weighed (0.1 g) before preservation in 10% formalin.

In December 2013, opportunistic sampling for post-larval and juvenile King George Whiting was conducted in shallow *Zostera* seagrass beds with a 10 x 2 m, 1 mm mesh seine net with 10 m hauling ropes. Sampling was conducted at 2 sites near Stanley, 1 site at Beauty Point on the Tamar River, 3 sites near Bridport and 7 sites in Georges Bay near St Helens. A minimum of 5 hauls was conducted at each site.

Ageing Methods

For age estimation, otolith (sagittae) transverse sections of the primordial plane were prepared. Otoliths were embedded into a two part Epofix resin (Struers Inc., Denmark). Sections (~250 µm) were cut from resin blocks with a low-speed diamond wheel saw (South Bay Technology Inc.,United States) and, subsequently, mounted onto microscope slides using Crystalbond 555 (ProSciTech, Australia). Sections were then polished on a lapping wheel (South Bay Technology Inc.) by using 9-µm diamond lapping film and CeO polishing film (3M, Australia) until otolith increments were of sufficient clarity for age estimation.

Annual increment formation has previously been validated for King George Whiting by Fowler and Short (1998). The first opaque zone is formed in the spring-summer of the second year of life and the algorithm developed by Fowler and Short (1998) was used to determine the age in months and assign fish to year classes.

Age data was used to determine the age structure and year-class strength in King George Whiting populations from north-west Tasmania and Georges Bay. Growth characteristics were determined using the von Bertalanffy growth curve (*1*) for the pooled age and length data from the two sampling areas:

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

where L_t is the length at time t, L_∞ is the asymptotic length - the mean length the fish of a given stock would reach if they were to grow indefinitely, K is the growth rate parameter, or the rate at which L_∞ is approached and t_o is the age of the fish at zero length if it had always grown in a manner described by the equation. The parameters were estimated using the non-linear regression routine in the Systat 13 statistical software package.

Reproductive Biology Methods

Gonadosomatic indices (GSIs) were calculated as $[GSI] = [W_g/W_f]*100$, where W_g is the gonad weight and W_f is the gonad-free fish weight (Fowler et al., 1999).

Macroscopic examinations involved classification based on colouration and size of gonads, and the visibility and appearance of oocytes (eggs) in females. For microscopic examination the gonads were preserved in 10% neutral buffered formalin and stored for at least 2 weeks. The samples were sent to the histology facility in the School of Biomedical Sciences, University of Melbourne for mounting and sectioning and then returned for subsequent histological examination. Briefly, a section of the transverse medial material was blocked in paraffin wax and 6 µm sections were cut, mounted and stained in Harris' haematoxylin and eosin. This enabled examination of the processes occurring at cellular level during the different stages of the reproductive cycle.

Ovaries and testes were classified into one of five stages based on macroscopic and microscopic characteristics (Table 17,Table 18). These characteristics were based on previous studies on King George Whiting (Hyndes et al., 1998; Fowler et al., 1999; Fowler and McGarvey, 2000; Hamer et al., 2004) and Red Cod, *Pseudophycis bachus* (Kemp et al., 2012).

Table 17. Reproductive development stages of female *S. punctatus* based on macroscopic and microscopic characteristics (from Fowler et al. (1999))

Stage	Macroscopic appearance	Microscopic characteristics
1 Immature	Ovaries small, undeveloped, clear, jelly-like or glassy, grey-pink	Only unyolked and non-atretic oocytes
2 Developing	Ovaries small, opaque, light yellow in colour; individual oocytes not discernible	Mainly unyolked and a few partially yolked oocytes, with no major atresia
3 Developed	Ovaries relatively large and quite turgid, yellow-orange; individual oocytes discernible	Oocytes at several phases: unyolked, partially yolked, but dominated by advanced. Maybe some minor atresia of advanced yolked oocytes
4 Gravid or/ running ripe	Ovaries large, orange. Clear hydrated oocytes visible among opaque oocytes. Oocytes may be ovulated	Oocytes present at all stages from unyolked to hydrated. Some atretic oocytes and post-ovulatory follicles may be present, but generally dominated by advanced yolk and hydrated oocytes
5 Regressing or resting	Ovaries small to medium, mustard yellow/orange/reddish. More flaccid than previous stages, and with granular appearance	Oocytes of all stages may be present; however, there is a high incidence of atresia suggesting the end of spawning. Post-ovulatory follicles not found

Table 18. Reproductive development stages of male *S. punctatus* based on macroscopic and microscopic characteristics (adapted from Fowler and McGarvey (2000); Hamer et al. (2004); Kemp et al. (2012)).

Stage	Macroscopic appearance	Microscopic characteristics
1 Immature	Testes very small, flat, black and thread like	Testes undergoing spermatogenesis where spermatogonia are predominant within lobules
2 Developing	Testes flat/rounder in shape, testes occupy 20 – 70% of the length of the body cavity	Various stages of spermatogenesis, with ripe spermatozoa within cysts and the lumen of lobules, but not within spermatic ducts
3 Developed	Testes lobed in formation, marked groove in the middle of each testis visible, testes occupy 40 - 70% of the length of the body cavity, creamy or white colouration with milt sometimes present	Testes show various stages of spermatogenesis with ripe spermatozoa within cysts, the lumen of lobules, and spermatic ducts
4 Gravid or/ running ripe	Testes very large and lobed/multilobed, testes occupy 40 – 70% of the body cavity, free flowing milt, testes white or pink and sometimes bloodshot	Ripe testes show an abundance of spermatozoa with little or no spermatogenic activity
5 Regressing or resting	Testes very bloodshot, testes occupy 20 – 50% of the length of the body cavity, milt sometimes present, testes brownish and rubbery as they regress to resting stage	Evidence of discharge of spermatozoa from spermatic ducts

Results

Length and Age

The King George Whiting collected from north-west Tasmania were markedly larger than those collected from Georges Bay with only a small amount of overlap between the two length-frequency distributions (Figure 23). King George Whiting from north-west Tasmania ranged from 380 to 585 mm FL with most in the range of 450 to 530 mm FL (Figure 23A). In contrast, King George Whiting from Georges Bay ranged from 265 to 445 mm FL with most in the range of 350 to 430 mm FL (Figure 23B).

The King George Whiting sampled from the north-west Tasmanian coast spanned a broad range of ages from 4 to19 years (Figure 24). The population was dominated by only 3 to 4 age-classes, indicating that recruitment is variable (Figure 24). The age structure was similar over the two sampling years indicating that the sample population was sampled (Figure 24). The progression of the dominant year classes by one year over the two years of sampling (Figure 24) supports the annual formation of otolith increments.

The King George Whiting sampled from St Georges Bay spanned a narrow age range from 2 to 4 years (Figure 25). The sample from 2012 was dominated by 4 year olds while in 2013 was dominated by 3 year olds (Figure 25).

In terms of year classes, the King George Whiting population from north-west Tasmania was dominated by fish spawned in 2001, 2003 and 2007 (and to a lesser extent fish spawned in 2005), while the fish collected from Georges Bay were dominated by fish spawned in 2007 and 2010 (Figure 26). The oldest fish sampled were spawned in 1995 (Figure 26).

The von Bertalanffy growth curve for fish pooled from both sampling areas described the age-length data well with an R^2 value of 0.998 and a mean Corrected R^2 of 0.897 (Figure 27). The estimated parameters were L_∞ = 519 mm (Wald 95% C.I.: 511-528 mm), K = 0.347 (Wald 95% C.I.: 0.299-0.395), and t_o = -0.419 (Wald 95% C.I.: -0.838-0.000). King George Whiting from Georges Bay were from a rapid growth phase up to about 400 mm in length and 4 years of age, while the larger and older fish from north-west Tasmania were in a low growth phase and many were near the asymptotic length (Figure 27).

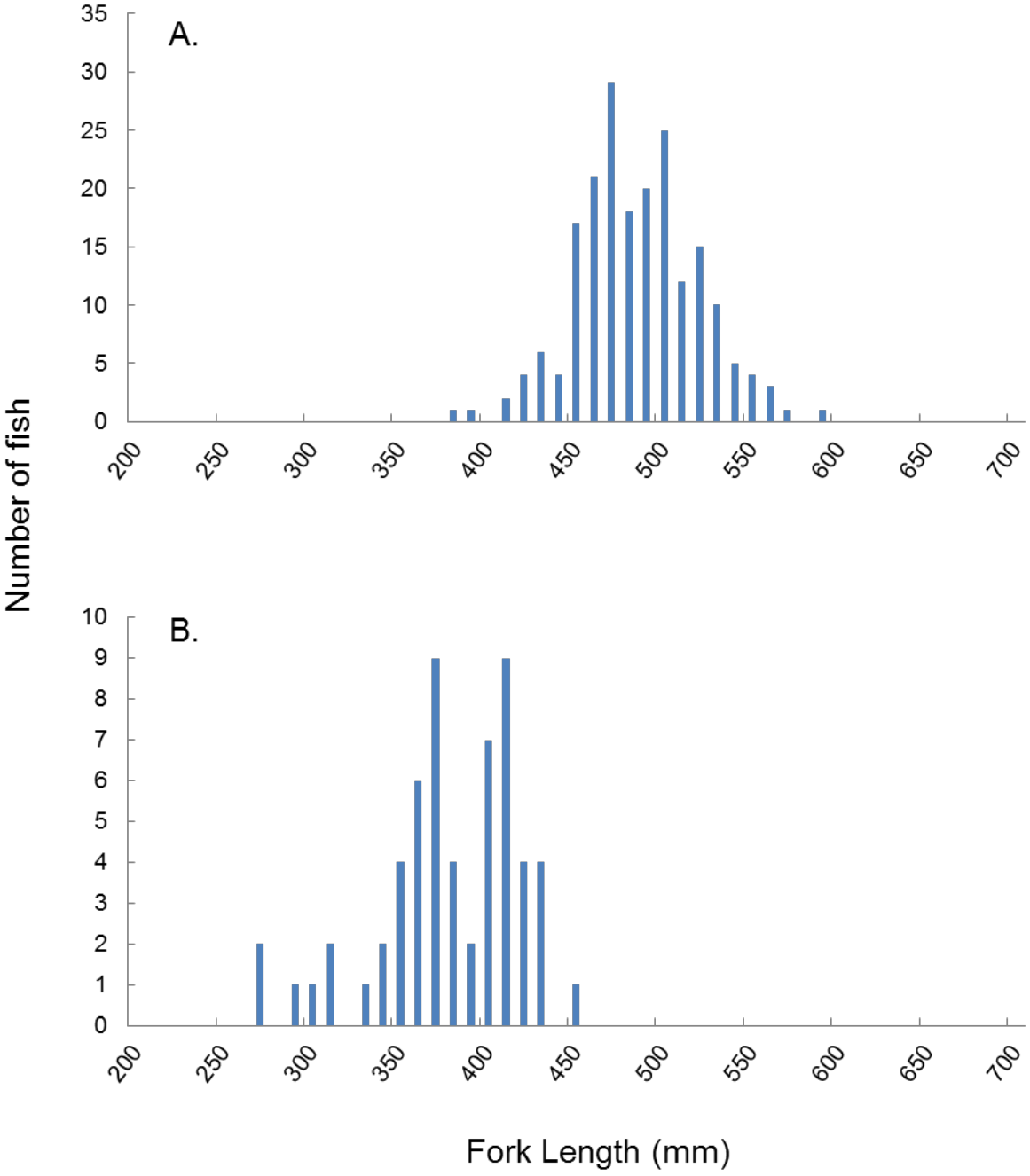


Figure 23. Length-frequency distributions of King George Whiting sampled from A) north-west Tasmania and B) Georges Bay

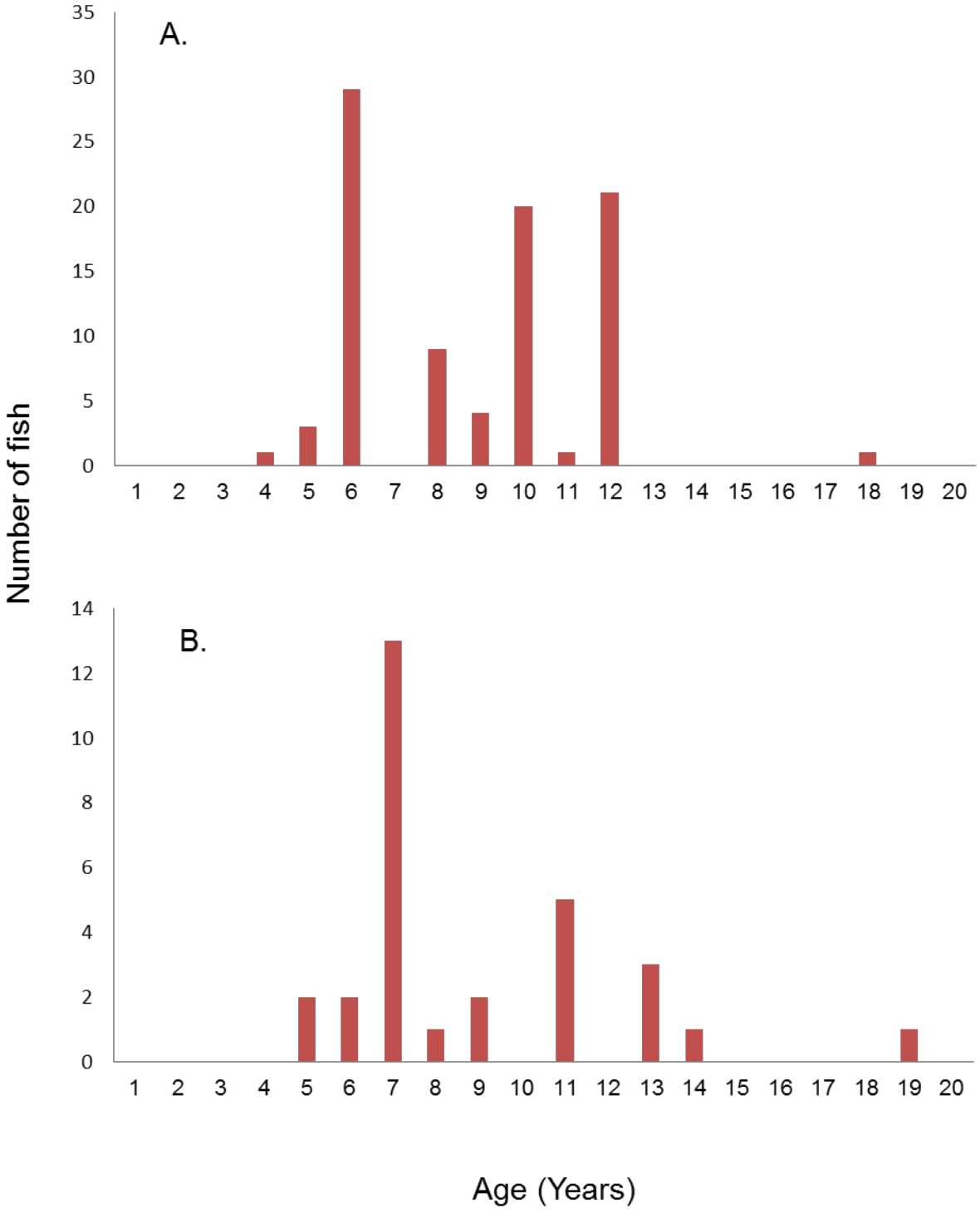


Figure 24. Age structure of the King George Whiting population sampled from North-west Tasmania, A) fish collected from September to December 2013, B) fish collected from May to July 2014.

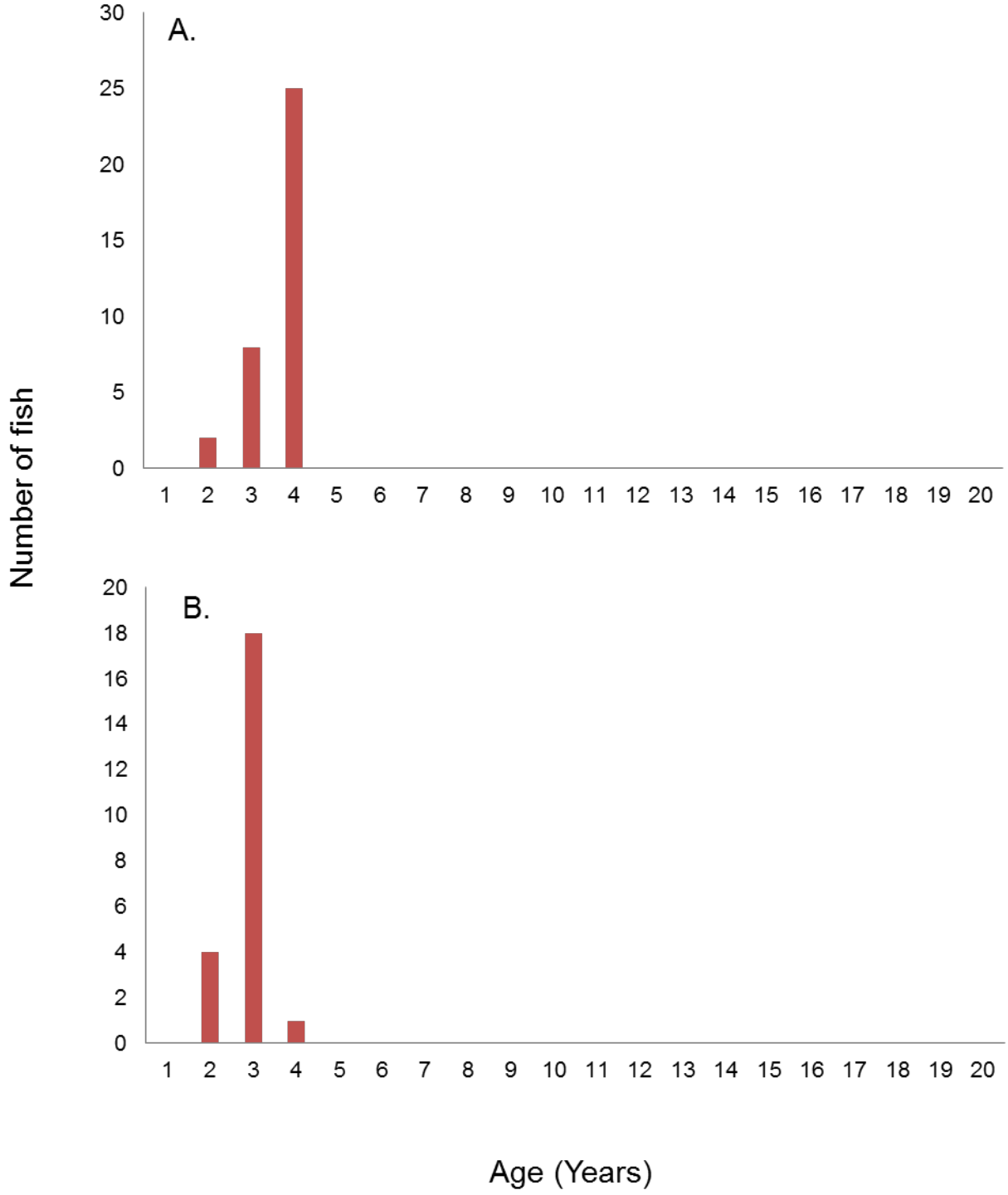


Figure 25. Age structure of the King George Whiting population sampled from Georges Bay, A) fish collected from January and February 2012, B) fish collected in December 2013.

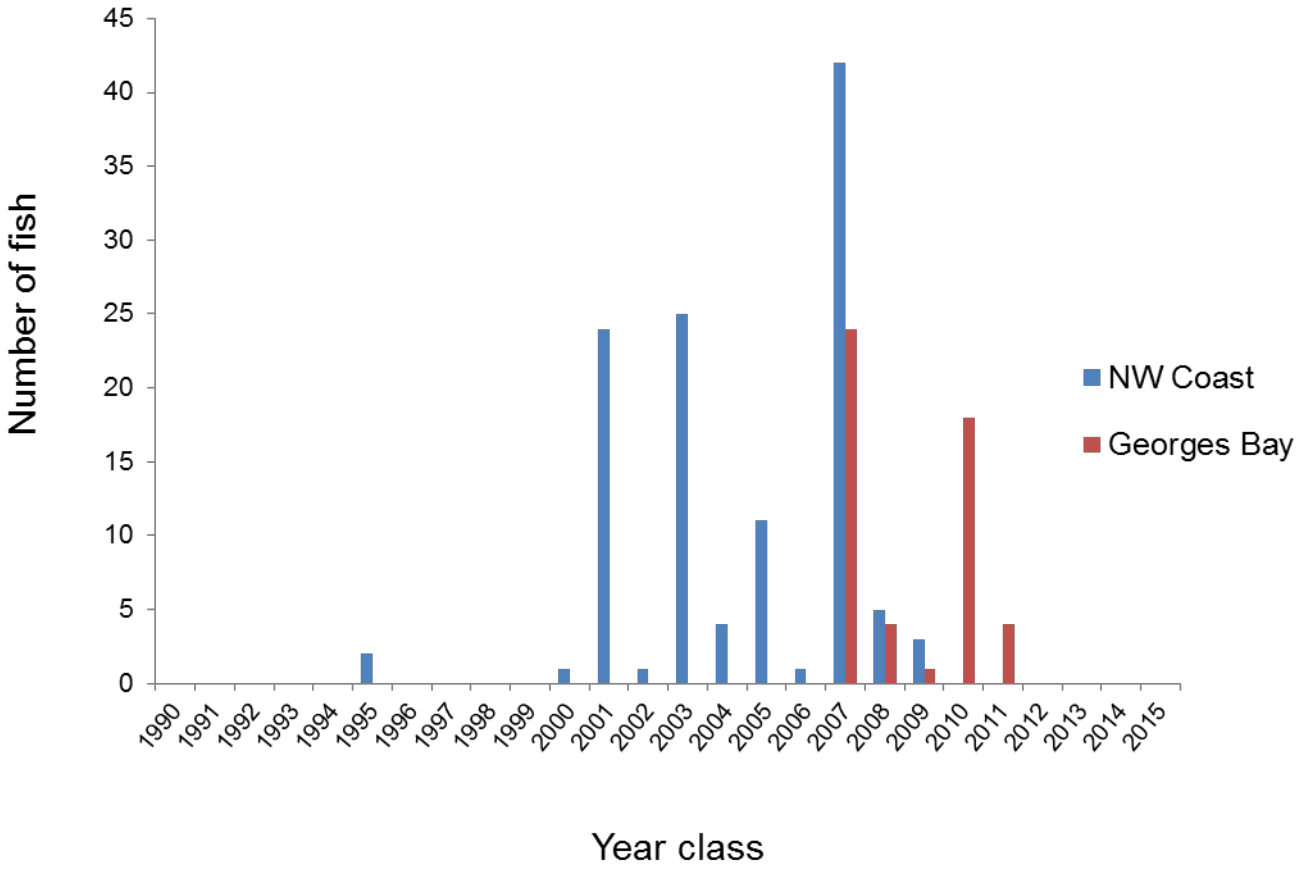


Figure 26. Age structure of the King George Whiting population sampled from Georges Bay, A) fish collected from January and February 2012, B) fish collected in December 2013.

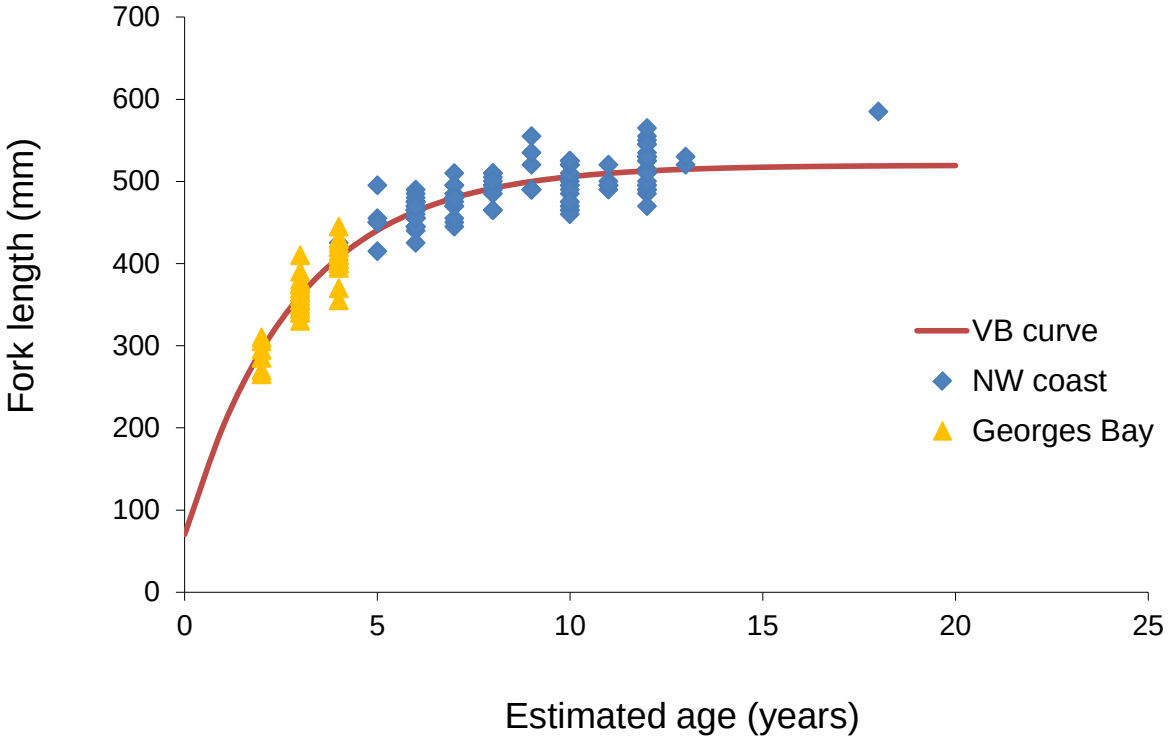


Figure 27. Age versus length of King George Whiting with data pooled from the north-west Tasmania coast and Georges Bay and the fitted Von Bertalanffy growth curve.

Reproductive Biology

For the 108 King George Whiting collected from the NW coast of Tasmania from May 2014 to June 2015 that were examined for reproductive condition, it was possible to determine the sex of all but one specimen. The majority of the sampled fish (71) were female, resulting in a male:female sex ratio of 51:100.

The GSI values for female King George Whiting showed a strong seasonal pattern, with relatively low values from June through to February, and higher values from March to May, with peak values of approximately 12% in late March (Figure 28). The seasonal pattern was less clear for male King George Whiting, recognising the small sample size involved. Lowest GSI values for males were recorded from September to December, and slightly higher values of around 2% were recorded from December to May (Figure 28). The heaviest ovaries sampled were 112.6 g while the heaviest testes were 16.8 g. Samples of King George Whiting for reproductive analysis were not available for April (Figure 28), despite significant fishing effort, indicating a drop in catchability or movement away from the sampling area (and possible aggregation for spawning).

Gonad development of female King George Whiting based on macroscopic and microscopic staging showed a strong seasonal pattern (Figure 29). The only female fish that was classified as immature was sampled in December (Figure 29). From September to January, the gonad development of the female population was dominated by fish at Stage 2 (developing) (Figure 29). These fish generally had ovaries that were small and opaque, and were pale pink/yellow in colour. Microscopically these ovaries had mainly unyolked oocytes and a few partially yolked oocytes up to about 150 µm in diameter. Females of this stage showed evidence of having spawned previously with the ovaries in a 'resting' stage. In March, most females had ovaries classified as Stage 3 (developed) (Figure 29). These ovaries were large, relatively turgid and yellow or yellow/orange in colour. Microscopically the ovaries were dominated by advanced yolk oocytes up to about 550 µm in diameter. One female collected in March was identified as Stage 4 where spawning would have been imminent (Figure 29). This fish was 540 mm FL and weighed 1553 g, and the ovaries were the heaviest of any fish, weighing 113 g. The ovaries were large and orange in colour, and microscopically were dominated by advanced yolk and hydrated oocytes. In May and June, females were mainly a mixture of Stage 3 (developed) and Stage 5 (regressing) (Figure 29). Fish classified as stage 5 had ovaries that were generally medium size, pale yellow in colour and were semi-flaccid. Microscopically these ovaries were dominated by advanced oocytes and atretic oocytes (generally > 50% atresia). Post ovulatory follicles (POFs) were not observed in ovaries, indicating that no females were sampled immediately after spawning.

Gonad development of male King George Whiting based on macroscopic and microscopic staging also showed a strong seasonal pattern (Figure 30). The only male fish that was classified as immature was sampled in December (Figure 30). From September to March, the gonad development of the male population of was dominated by fish at Stage 2 (developing) (Figure 30). These fish generally had testes that were small and opaque, and were brown in colour. Microscopically these testes displayed various stages of spermatogenesis, with some spermatozoa present in cysts and the lumen of lobules. In May and June some males had testes classified as Stage 3 (developed) (Figure 30). These testes were medium size and milky white in colour. Other males sampled from May to July were classified as Stage 5 (regressing) (Figure 30). Fish classified as Stage 5 had testes that were small to medium and opaque, and were brown in colour but with milky white near ends and edges. Microscopically the testes had abundant spermatozoa in the main sperm ducts (sometimes with evidence of discharge) and no spermatogenic activity.

Post-larval sampling

A single post-larval King George Whiting of 31 mm total length was sampled from Georges Bay near St Helens (Figure 31). This indicates that at least part of the population of King George Whiting in Georges Bay is derived from larval settlement into shallow seagrass beds.

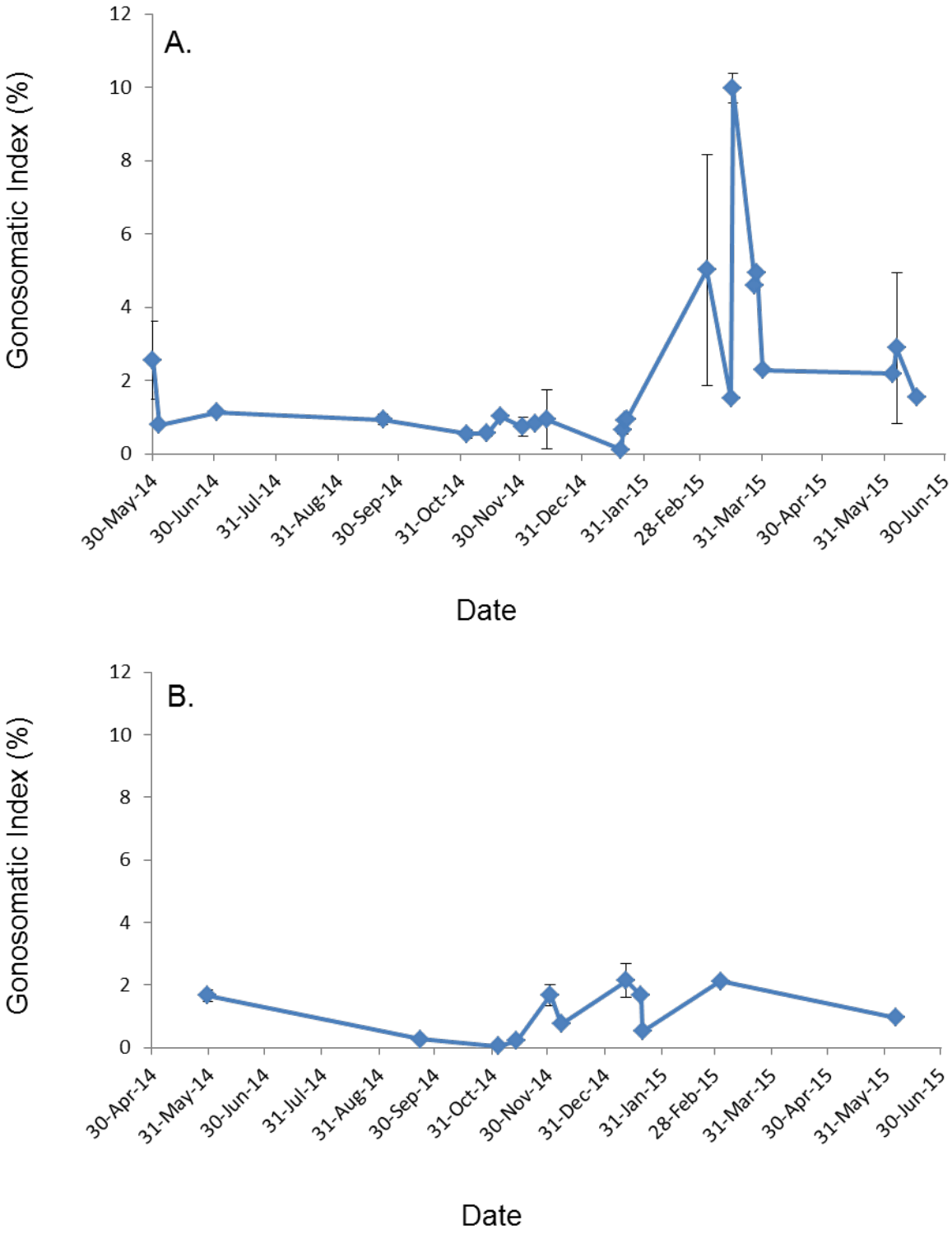


Figure 28. Gonadosomatic index (GSI) values for King George Whiting from the north-west coast of Tasmania, A) Females, B) Males.

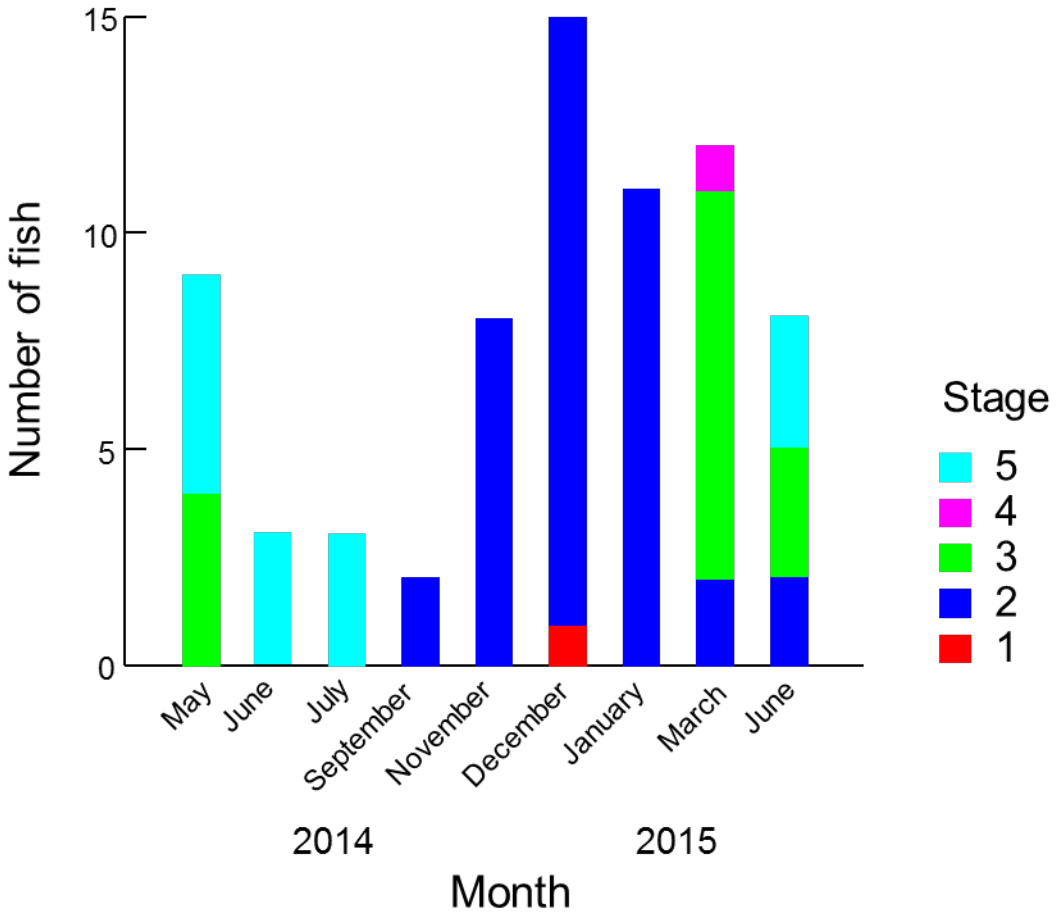


Figure 29. Reproductive stages of female King George Whiting sampled from the north-west coast of Tasmania based on macroscopic and microscopic characteristics of gonads.

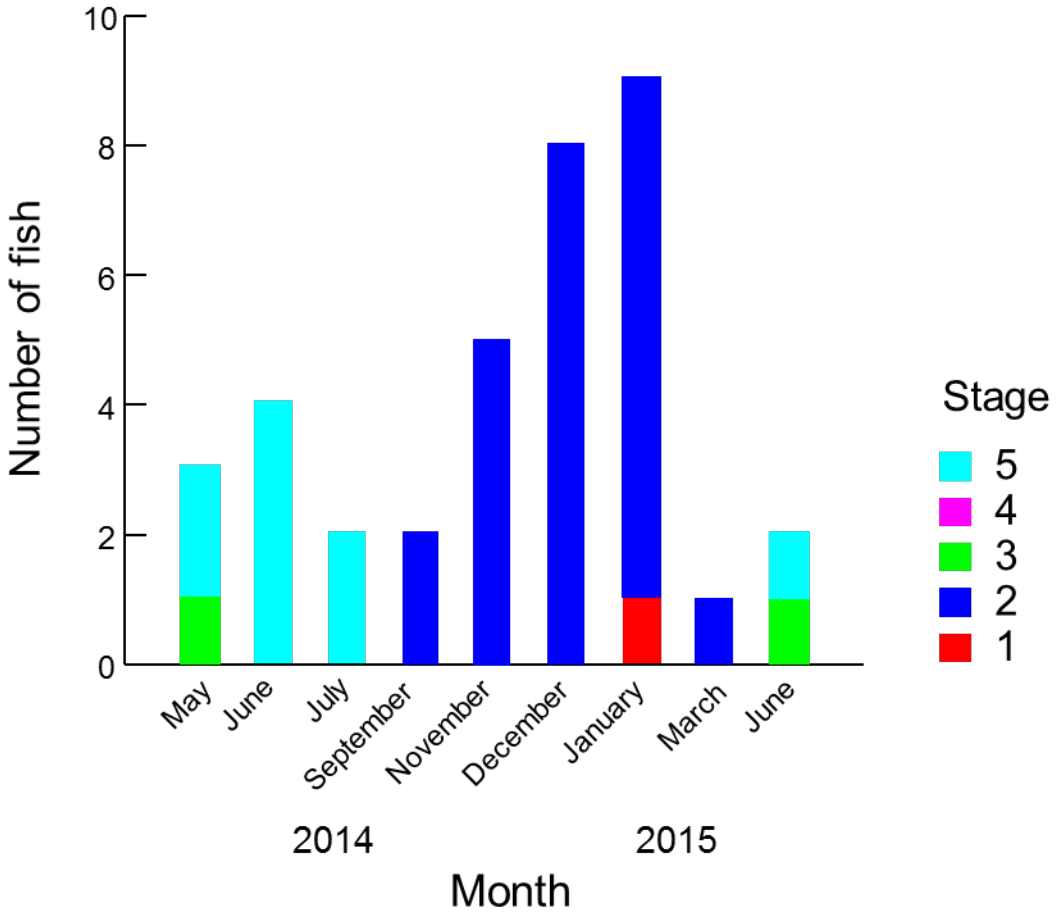


Figure 30. Reproductive stages of male King George Whiting sampled from the north-west coast of Tasmania based on macroscopic and microscopic characteristics of gonads.

Figure 31. Post-settlement King George Whiting, 31 mm Total Length, from Georges Bay near St Helens

Discussion

Up until this study the only spawning area previously identified in south-eastern Australia is in the Investigator Strait area of South Australia (Fowler et al., 1999; Fowler et al., 2000b), while another spawning area has been identified off Perth in Western Australia (Hyndes et al., 1998). Extensive sampling of King George Whiting along the Victorian coast failed to identify populations of adult, spawning fish (Hamer et al., 2004). Several lines of evidence based on population age structure and reproductive characteristics indicate that the north-west coast of Tasmania is also a spawning area for the species.

Ageing of fish collected from the north-west coast of Tasmania indicates that this population is comprised of adult fish, including one specimen, at 19 years of age, the oldest King George Whiting recorded. The age-structure of the population is similar to that in the known spawning areas in South Australia and Western Australia, dominated by fish greater than three years of age. Sexual maturity in King George Whiting is known to occur in the third to fourth year of life (Hyndes et al., 1998; Fowler et al., 1999; Fowler et al., 2000b), and in South Australia is strongly related to the location (i.e. fish in exposed offshore areas tend to be at least 3 years age and are all sexually mature) (Fowler et al., 1999; Fowler et al., 2000b). The population of fish from North-west Tasmania had a greater proportion of older (> 5 years) and larger (> 500 mm FL) fish than on the spawning areas in South Australia, where adult populations were dominated by 3 and 4 year old fish less than 500 mm FL (Fowler et al., 1999; Fowler et al., 2000b). This difference may reflect the difference in sampling methods, where fish in South Australia were sampled by hook and line, while fish in north-west Tasmania were sampled with a 7.5 cm mesh gill net. It is also possible that the difference partly relates to lower fishing pressure in north-west Tasmania, and/or greater recruitment variability. The von Bertalanffy growth curve for King George Whiting from north-west Tasmania indicated a mean asymptotic length of 520 mm, which was higher than for King George Whiting populations in South Australia (McGarvey and Fowler, 2002) and similar to the population in Western Australia (Hyndes et al., 1998) where King George Whiting are known to spawn.

The GSI index for female King George Whiting from northwest Tasmania further supported the contention that this is a spawning area for the species. The peak GSI of approximately 12% in late March was similar to the results from spawning areas in South Australia where mean GSI for females peaked at approximately 7% (1 standard deviation approximately 4 to 10 %) in April (Fowler et al., 1999). The heaviest ovaries recorded in northwest Tasmania (112.6 g) weighed more than the heaviest ovaries recorded in the South Australian spawning area (97.3 g) (Fowler et al., 1999). In general, the pattern of female GSI in north-west Tasmania, albeit with smaller sample sizes, appears to be similar to that in South Australia, with GSI increasing in March, peaking in April and declining in May (Fowler et al., 1999).

In contrast to females, the GSI index for male King George Whiting from northwest Tasmania showed no clear seasonal pattern, although there was a slight trend for GSI to be higher from December to May. The maximum male GSI of approximately 2% was lower than the approximately 6% GSI recorded for male King George Whiting from the South Australian spawning area in April (Fowler et al., 1999). This result may largely relate to the lower sample size of male King George Whiting from north-west Tasmania, and, in particular, no male King George Whiting were collected between early March and late May, when fish would be expected to be in spawning condition. The heaviest testes recorded in northwest Tasmania (16.8 g) weighed considerably less than the heaviest testes recorded in the South Australian spawning area (44.0 g) (Fowler et al., 1999).

Macroscopic and microscopic staging of gonads from female King George Whiting indicated that spawning was likely to be occurring near the sampling area in northwest Tasmania. Female fish sampled from March to June had fully developed ovaries dominated by advanced yolk oocytes and, in one case, hydrated oocytes, indicating that the fish was gravid/ running ripe and spawning was imminent. Again, this seasonal pattern of macroscopic and microscopic staging is very similar to that found on the spawning grounds in South Australia (Fowler et al., 1999). Unlike the results for South

Australia (Fowler et al., 1999), however, no post ovulatory follicles (POFs) were identified in ovaries, indicating that spawning had very recently occurred. In South Australia, fish with POFs were mainly collected in April, however, in Northwest Tasmania, King George Whiting were not collected in April despite significant sampling effort (C. Garland, Pers. Comm.).

For male King George Whiting from northwest Tasmania, fish with fully developed testes were identified in May and June, and fish with regressing testes were identified from May to July, but no fish were identified as gravid / running ripe. As mentioned, this most likely relates to the lack of samples of male fish from early March to late May. Some of the males identified as Stage 5 in May and June had abundant spermatozoa in the main sperm ducts and may still have been capable of late season spawning.

As mentioned, samples of King George Whiting from north-west Tasmania were not collected despite significant sampling effort. Reproductive characteristics, and comparative data from South Australia (Fowler et al., 1999; Fowler et al., 2000b), suggest that this would have been the peak of the spawning season. Although it is possible that catchability may have decreased through a change of behaviour at the time of spawning, it is more likely that fish moved from the fishing grounds to spawning grounds outside the normal fishing area. Mesh netting for King George Whiting in northwest Tasmania is primarily conducted in relatively shallow, protected nearshore waters over seagrass habitat. However, evidence from South Australia suggests that the preferred spawning habitat is likely to be near offshore reefs, shoals or large mounds in relatively deep water in exposed locations that experience medium to high wave energy (Fowler et al., 2000b). This suggests that in April the fish are moving offshore from the fishing grounds to spawning locations in deeper water. Sampling of these offshore habitats over the April period would be required to confirm this hypothesis.

In contrast to the King George Whiting population on the northwest Tasmanian coast, the population sampled in Georges Bay was restricted to young, 2 – 4 year old fish. This is the typical pattern where juvenile Whiting up to about 4 years of age occur in protected embayment or gulf habitats (Hyndes et al., 1998; Fowler et al., 2000b). After a long larval dispersal phase of 3 to 5 months, post-larvae settle in these inshore nursery habitats (Jenkins and May, 1994; Fowler and Short, 1996; Jenkins et al., 2000) before moving offshore with sexual maturity at 3 to 4 years of age (Hyndes et al., 1998; Fowler et al., 2000b). Georges Bay, with significant areas of protected seagrass habitat, is likely to represent a similar inshore nursery area to that found in the mainland States. This is supported by the collection of a single post-larval King George Whiting in Georges Bay, indicating that fish in the Bay are likely to be derived from post-larval settlement rather than migration.

The identification of a second King George Whiting spawning area in south-eastern Australia has significant implications for our understanding of population connectivity. Reverse hydrodynamic modelling of dispersal pathways to Victorian nursery bays suggested that the primary spawning area would be the coastline from far western Victoria into south-eastern South Australia, however, a second possible source was identified as the north-west coast of Tasmania (Jenkins et al., 2000). Thus, based on hydrodynamics and larval duration, it is possible for King George Whiting larvae spawned in northwest Tasmania to settle in bays of central Victoria, creating connectivity in the population across Bass Strait (Jenkins et al., 2000). However, modelling predicted that the spawning area would be off the northern part of the west coast of Tasmania, whereas residual currents in the model suggested that larvae from the sampling area in this study would be advected east along the north coast of Tasmania (Jenkins et al., 2000). It is possible that adults may move west to the northern part of the west coast for spawning in April. However, genetic results (Chapter 3) indicate that King George Whiting from Victoria are genetically separated from those from the north-west coast of Tasmania, suggesting that the identified spawning population does not contribute larvae to Victorian nurseries. Residual currents running east along the north coast of Tasmania (Jenkins et al., 2000) may suggest the north-west Tasmania could be the source of post-larvae entering Georges Bay, however, this is also contradicted by genetic evidence that King George Whiting from the northwest coast and Georges Bay come from genetically distinct populations (Chapter 3). Large King George Whiting have been reported from the Furneaux group of islands in eastern Bass Strait, and this is a possible spawning location for King

George Whiting (yet to be confirmed through scientific study) that could potentially be the source of larvae in Georges Bay.

In conclusion, this study has identified a second spawning population of King George Whiting in south-eastern Australia based on age-structure and reproductive characteristics. King George Whiting from the northwest coast of Tasmania show a similar seasonal spawning cycle to those in South Australia, with the main spawning season from March to May with a peak in April. Although fish sampled on the fishing grounds off north-west Tasmania showed evidence of spawning, the primary spawning locations, likely to be offshore, are yet to be identified. Hydrodynamic and dispersal modelling suggests that larvae settling in bays of central Victoria could be derived from spawning off northwest Tasmania, however this pathway is contradicted by genetic evidence that Victorian and Tasmanian King George Whiting are genetically distinct.

Chapter 5 Otolith chemistry life history transects for King George Whiting from south-eastern Australia

Introduction

In many fish species, juveniles and adults are known to undertake significant migrations, including ontogenetic migrations from juvenile nursery habitats to adult habitats including spawning areas (Harden Jones, 1968; Secor, 2015). In the process of migration, fish can cross major environmental gradients, for example catadromous and anadromous species where a major change in salinity as well as other environmental factors is experienced in the migration process (Secor, 2015). Marine fishes are likely to experience more subtle but still significant changes in environmental factors, for example in undertaking ontogenetic migration from estuarine and coastal nursery habitats to adult habitat in the open ocean (Gillanders et al., 2003; Verweij et al., 2008). Variation in migration patterns can occur amongst individuals and in groups of fish ('contingents') in a population, where there is partial migration such that some fish do not migrate (Chapman et al., 2012; Gahagan et al., 2015; Gillanders et al., 2015).

Rapid advances in technology have seen the development of a suite of new research tools in recent decades that can address questions related to fish migration (Secor, 2015). One tool that has been used extensively is otolith chemistry, where chemical elements incorporated into fish otoliths are used as 'natural tags' (Elsdon et al., 2008; Kerr and Campana, 2013). One common application of otolith chemistry in relation to migration is to identify specific chemical signatures for nursery areas and then to retrospectively classify older juveniles or adults that have migrated to other locations back to their source nursery area (Hamer et al., 2005; Elsdon et al., 2008; Kerr and Campana, 2013) (See chapter 2). The method assumes that all source populations have been sampled and that either chemical signatures are temporally stable or that single cohorts are followed through the analysis (Elsdon et al., 2008). Another application that is used to examine movement and migration is profile analysis to describe movements through different environments (Elsdon et al., 2008). This method uses a life history transect of chemical concentrations across the otolith to define different groups of fish that have had different migration histories (Elsdon et al., 2008). The method is a type of stock discrimination analysis and the underlying causes of the chemical variation do not need to be known.

Profile analysis has most commonly been used for examining movement between freshwater and estuarine or seawater. Commonly found positive correlations between Sr:Ca and salinity, and negative correlations between Ba:Ca and salinity mean that these elements often form the basis of studies, although relationships vary and should be validated on an individual study basis (Elsdon et al., 2008; Macdonald and Crook, 2010; Walther and Limburg, 2012; Conroy et al., 2015). Because of the more homogeneous environment, less variation in profiles may be expected for marine fish (Sturrock et al., 2012), however significant variation can still occur, for example some coastal embayment environments may have higher Ba:Ca than the open ocean, potentially allowing movement patterns between bay and ocean to be inferred from otolith chemistry profiles (Hamer et al., 2006).

In this chapter we use otolith chemical life-history transects of adult *S. punctatus* from the Kangaroo Island area of South Australia, western Victoria, and north-western Tasmania, to determine if these groups have similar or different migration histories and therefore whether they represent separate population units or whether there is connectivity amongst these groups of fish. In this way we address the question of population connectivity across State borders and the potential need or otherwise for cross-jurisdictional management.

Methodology

Sample collection

This study compared otolith chemistry life-history profiles across the chronological structure of transverse sections of adult Whiting otoliths from three coastal water regions in south-eastern Australia – Kangaroo Island (South Australia), Portland (Western Victoria), Stanley (north east Tasmania) (Figure 32). These Whiting ranged in age from approximately 4+-9+ years of age. It was not possible to collect samples from all regions for the same cohorts; however, samples from at least two cohorts from each region were available to investigate inter-cohort variation in the regional otolith chemistry profiles **(**Table 19**).** Otoliths were generally removed from chilled fish within 24 hours of capture, cleaned and stored in paper envelopes until processing.

Table 19 Summary of samples used for life-history otolith chemistry profiles

Region	Size TL cm (mean, range)	Age years (mean, range)	Cohort (sample number)
Kangaroo Island	41 (33-48)	5+ (47+)	2005 (13), 2006 (15)
Portland	45 (41-49)	4+ (3-5+)	1997 (16), 1998 (5)
Stanley	53 (50-56)	8+ (7-9+)	2003 (15), 2005 (6)

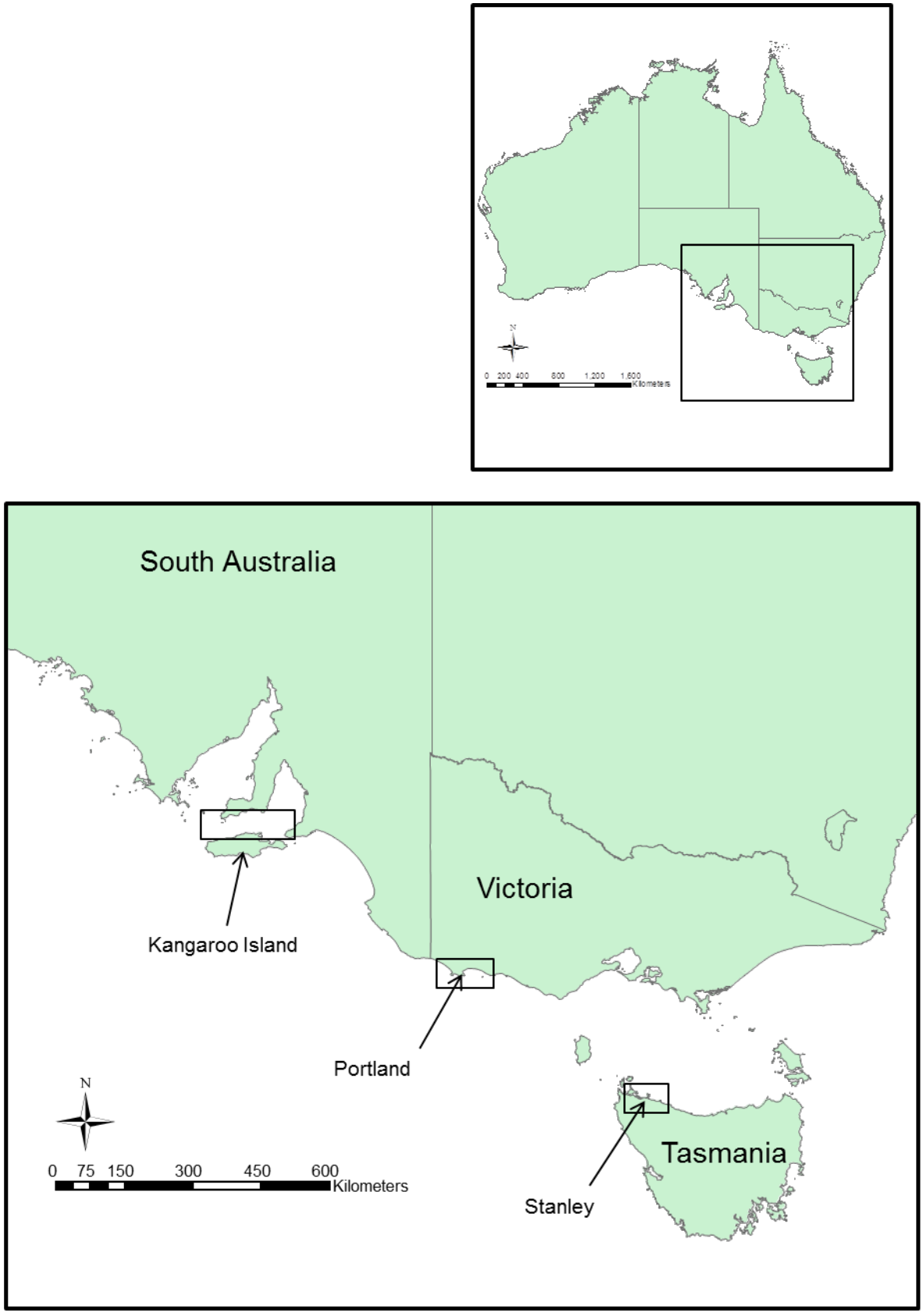


Figure 32. Sampling areas for adult King George Whiting used for life-history transect analysis

Otolith preparation

One whole sagitta was selected for each fish. It was embedded separately in epoxy resin (Struers Epofix), sectioned to approximately 400 µm in thickness in the transverse plane to incorporate the core, and polished with grades of aluminium oxide lapping film lubricated with Milli-Q water. Each polished section was sonicated in Milli-Q water for 5 minutes, liberally rinsed prior to drying in a laminar flow cabinet and stored in a plastic container. The birth year (i.e. year class) was determined from the count of annual increments.

Elemental analysis

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS, Laser - New Wave UP213, ICP-MS – Thermo Scientific Element 2, Fisheries Victoria, Queenscliff, Victoria, Australia) was used to obtain age-related element:Ca profiles. The laser was programmed to traverse the otolith section from the core to the margin along the ventral side of the sulcus (Figure 33). This axis was chosen for analysis because it is one of the clearest for differentiating the opaque zones for fish ageing. Laser settings were: beam diameter 40 µm, fluence 10 J cm-2, and repetition rate 5 Hz, with 8 µm s^{-1} stage movement along the transect path. Ablation occurred in helium that was mixed with argon for injection to the plasma. Each transect path was pre-ablated as a final surface cleaning step (fluence 6 J cm^{-2}, repetition rate 5 Hz, and 60 µm s^{-1} stage movement along the transect path). The ICP-MS measured the isotopes of ^{25}Mg, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, and ^{43}Ca. The latter was used as the internal standard to adjust for variation in ablation yield. The Ca concentration of otolith matrix was 38.8% by weight (Yoshinaga, et al. 2000). Blanks were obtained by analysing sample gases for approximately 50 x ICP-MS scans of the selected isotopes prior to sample ablation, and the averages of the blank counts were subtracted from the sample counts prior to calibration. Calibration was achieved with the National Institute of Standards (NIST) 612 certified reference pellet (Lahaye et al., 1997). Data are presented as molar ratios to Ca.

The profile for each element:Ca ratio was matched to fish age (i.e. yearly growth zones) using the opaque zones in the otolith macrostructure as temporal references. After analysis by laser ablation ICP-MS, a digital image of the otolith was recorded from which the increment widths were measured from the core to the otolith margin adjacent to the trench left by the ablation path on the surface of the otolith section (Figure 33). Using these distances, the known rate of movement of the laser beam across the otolith and the time taken for individual ICP-MS scans of the isotopes the consecutive element:Ca measurements were divided into consecutive age (years) zones. The element:Ca measurements for each age zone were then integrated to provide average element:Ca ratio data for each year of life that were used for further statistical analysis (Fowler et al., 2005).

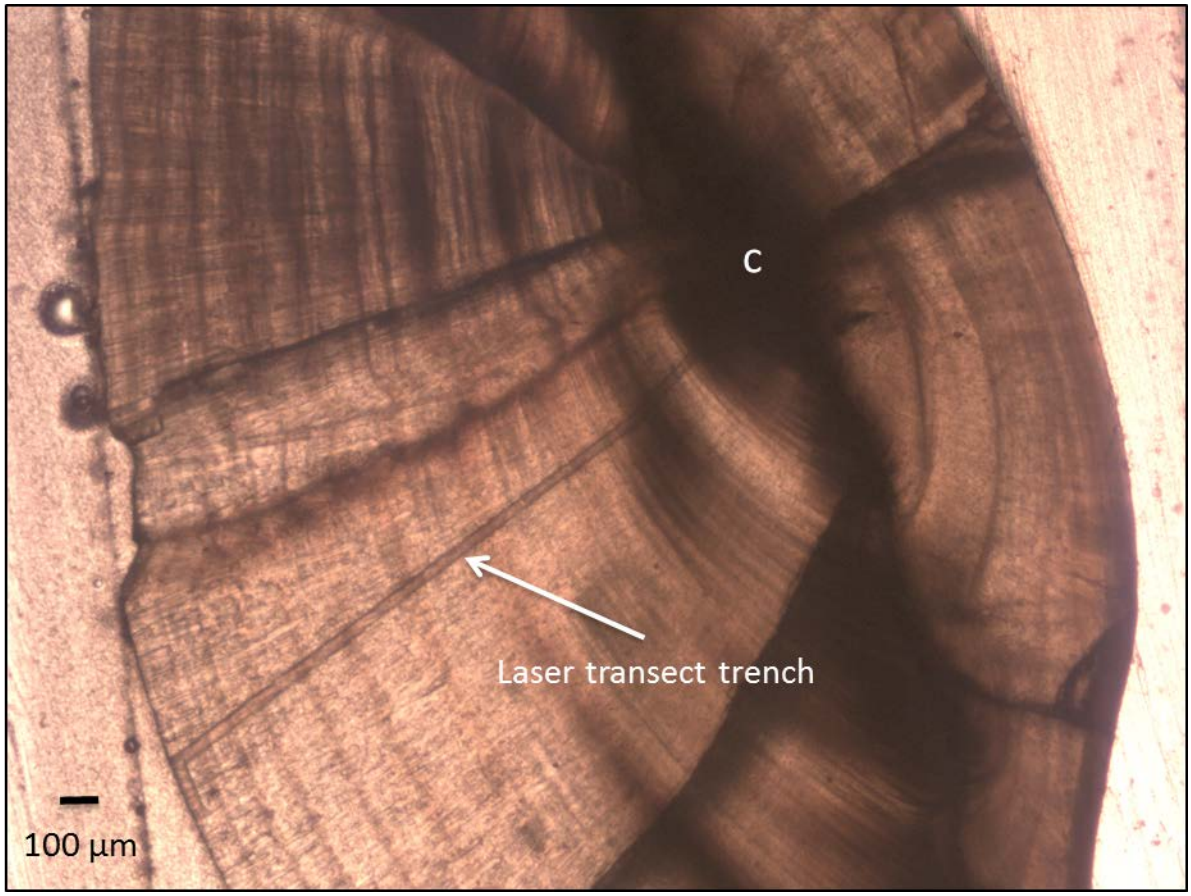


Figure 33 Image of transverse section of an age 8+ year adult King George Whiting showing the 40 µm width laser ablation path used to construct the life-history otolith chemistry profiles. C=core

Data analysis

The focus of this study was to compare differences among regions for the individual ages rather than comparing between the different ages across the profiles. Therefore, the regional comparisons for each age were conducted as separate analyses, as opposed to a repeated measures approach. The similarities and differences among regions for each age were used to address specific questions about spatial separation with age and infer stock structure. To achieve these comparisons, it was necessary to simplify the transect data. For each otolith and element, an annual, age-related mean was calculated from the series of elemental concentrations that were assigned to each year of life. For each otolith this provided profiles of age-related mean estimates of concentrations of the element:Ca ratios for Ba, Sr, Zn, Mn and Mg, from all increments from the otolith core to its outside edge. These increments are labelled 0-1, 1-2, 2-3 and so on, relating to the otolith material deposited between the consecutive opaque zones from the otolith core to the outside edge.

For each age the among-region comparisons were achieved using analyses of variance, multivariate analyses of variance and discriminant function analyses of the age-specific annual averages. For individual element:Ca ratios and each increment (year), a single factor analysis of variance (ANOVA) was used to compare amongst regions, and a Tukey's pairwise post-hoc test was used for a posteriori tests to identify which regional means were significantly different. Subsequently, the data from the five element:Ca ratios were considered together in a multivariate analysis of variance (MANOVA), followed by Hotellings' T-square pairwise tests to identify which regions differed. Also, the multi-elemental data for each increment were presented by plotting the regional 95% confidence ellipses around the mean canonical scores for canonical variates 1 and 2 from a quadratic discriminant function

analysis (QDFA). Assumptions of normality and homogeneity of variances were assessed qualitatively using box plots, frequency histograms and residual plots. To meet these assumptions, the data for Mn:Ca, and Ba:Ca were transformed using ln(x+1), Zn:Ca was 4^{th} root transformed.. While individual variables satisfied the assumptions of the univariate analyses, assumptions of multivariate analyses are problematic to formally test (Quinn and Keough, 2002). Qualitative comparisons of within-group, scatterplot matrices did not indicate major heterogeneity of within-group variance-covariances, however, quadratic discriminant functions were applied as a conservative approach as they do not assume equal, within-group covariances (Quinn and Keough, 2002).

Finally, because we could not match cohorts across all sampling regions we conducted the above outlined univariate analysis for all element:ca ratios but comparing different cohorts within each sampling region. Clear and significant difference among cohorts for a sampling region would suggest that conclusions of regional difference could be confounded by inter-cohort variation.

Results

Univariate element/Ca ratios for individual cohorts within sampling areas were largely similar (Table 20, Figure 34, Figure 35, Figure 36). The only significant differences between cohorts were for Mg/Ca in increment zone 1-2 of fish sampled from South Australia, and for Sr/Ca in increment zone 6-7 for fish sampled in Tasmania (Table 20). Overall, similar chemical transect signatures between cohorts within areas justifies the comparison of different cohorts amongst areas.

Highly significant univariate differences occurred between sampling areas for all elements analysed, although differences were less distinct for Zn/Ca compared to other elements (Table 21). Mg/Ca ratios for fish sampled in West Victoria were significantly higher than for north-west Tasmania and South Australia which were similar (Table 21, Figure 37). Mg/Ca ratios declined with age for fish from all areas (Figure 37). A similar pattern occurred for Mn/Ca ratios, with the exception that ratios for the 0-1 increment zone were not significantly different (Table 21, Figure 37). Mn/Ca ratios declined with age, showing a relatively linear decline for west Victoria but an exponential decline for samples from north-west Tasmania and South Australia (Figure 37). Zn/Ca ratios declined exponentially with growth, with significantly higher values for increment zones 1-2 and 2-3 in fish sampled from west Victoria than north-west Tasmania or South Australia (Table 21, Figure 37). Sr/Ca ratios for fish sampled in South Australia were significantly higher than those from west Victoria that were in turn significantly higher than in fish from north-west Tasmania (Table 21). The pattern for Sr/Ca showed an increasing trend for South Australia so that levels were much higher than the other two States in older fish (Figure 37). Ba/Ca ratios for fish sampled in west Victoria and South Australia were significantly higher than those from Tasmania for the first 4 increment zones but for increment zone 4-5, ratios were higher for fish from South Australia than the other two States (Table 21). The Ba/Ca ratio for fish sampled from west Victoria declined slightly over the first four increments and then more rapidly for increment zones 4-5 and 5-6 to fall to the same level as fish sampled from Tasmania (Figure 37).

Like univariate analyses, multivariate MANOVA showed no significant differences between multi-element signatures in otolith increment zones for different cohorts within sampling areas (Table 22). Overall, similar multi-element transect signatures between cohorts within areas justifies the comparison of different cohorts amongst areas.

Pairwise comparisons of multi-element signatures amongst the different sampling areas from Multivariate MANOVA showed highly significant differences for all area comparisons in all otolith increment zones (Table 23). Discriminant function plots of the multi-element signatures in each otolith increment zone showed clear separation of the 95% confidence ellipses for the mean (Figure 38). It was notable, however, that the ellipses for west Victoria and South Australia were less separated in the 0-1 increment than for older fish (Figure 38).

Table 20. Single factor ANOVA comparisons of element/Ca ratios in otolith increment zones for two cohorts in each of the three sampling areas. Significance level adjusted (Bonferroni) for the number of otolith zones analysed for each combination of State and element. Significant P-values in bold.

Element/Ca	Otolith zone	South Australia		Western Victoria		North-west Tasmania	
		Main effects	p – value: Cohorts	Main effects	p – value: Cohorts	Main effects	p – value: Cohorts
Ln Mg:Ca	0-1	2005, 2006	0.047	1997, 1998	0.923	2003, 2005	0.141
	1-2	2005, 2006	0.251	1997, 1998	0.298	2003, 2005	0.148
	2-3	2005, 2006	0.154	1997, 1998	0.185	2003, 2005	0.507
	3-4	2005, 2006	0.412	1997, 1998	0.541	2003, 2005	0.849
	4-5	2005, 2006	0.757			2003, 2005	0.691
	5-6	2005, 2006	0.017			2003, 2005	0.365
	6-7	2005, 2006	0.061			2003, 2005	0.424
Ln Mn:Ca	0-1	2005, 2006	0.072	1997, 1998	0.967	2003, 2005	0.792
	1-2	2005, 2006	**0.003**	1997, 1998	0.690	2003, 2005	0.811
	2-3	2005, 2006	0.128	1997, 1998	0.582	2003, 2005	0.586
	3-4	2005, 2006	0.293	1997, 1998	0.128	2003, 2005	0.978
	4-5	2005, 2006	0.830			2003, 2005	0.698
	5-6	2005, 2006	0.155			2003, 2005	0.272
	6-7	2005, 2006	0616			2003, 2005	0.606
4^{th} root Zn:Ca	0-1	2005, 2006	0.701	1997, 1998	0.345	2003, 2005	0.247
	1-2	2005, 2006	0.441	1997, 1998	0.504	2003, 2005	0.393
	2-3	2005, 2006	0.879	1997, 1998	0.687	2003, 2005	0.349
	3-4	2005, 2006	0.335	1997, 1998	0.605	2003, 2005	0.435
	4-5	2005, 2006	0.413			2003, 2005	0.835
	5-6	2005, 2006	0.123			2003, 2005	0.416
	6-7	2005, 2006	0.103			2003, 2005	0.741
Sr:Ca	0-1	2005, 2006	0.991	1997, 1998	0.211	2003, 2005	0.998
	1-2	2005, 2006	0.648	1997, 1998	0.266	2003, 2005	0.301
	2-3	2005, 2006	0.323	1997, 1998	0.195	2003, 2005	0.633
	3-4	2005, 2006	0.013	1997, 1998	0.562	2003, 2005	0.902
	4-5	2005, 2006	0.458			2003, 2005	0.707
	5-6	2005, 2006	0.137			2003, 2005	0.184
	6-7	2005, 2006	0.037			2003, 2005	**0.002**
Ln Ba:Ca	0-1	2005, 2006	0.056	1997, 1998	0.735	2003, 2005	0.330
	1-2	2005, 2006	0.178	1997, 1998	0.072	2003, 2005	0.890
	2-3	2005, 2006	0.508	1997, 1998	0.879	2003, 2005	0.437
	3-4	2005, 2006	0.442	1997, 1998	0.335	2003, 2005	0.327
	4-5	2005, 2006	0.823			2003, 2005	0.698
	5-6	2005, 2006	0.227			2003, 2005	0.294
	6-7	2005, 2006	0.573			2003, 2005	0.804

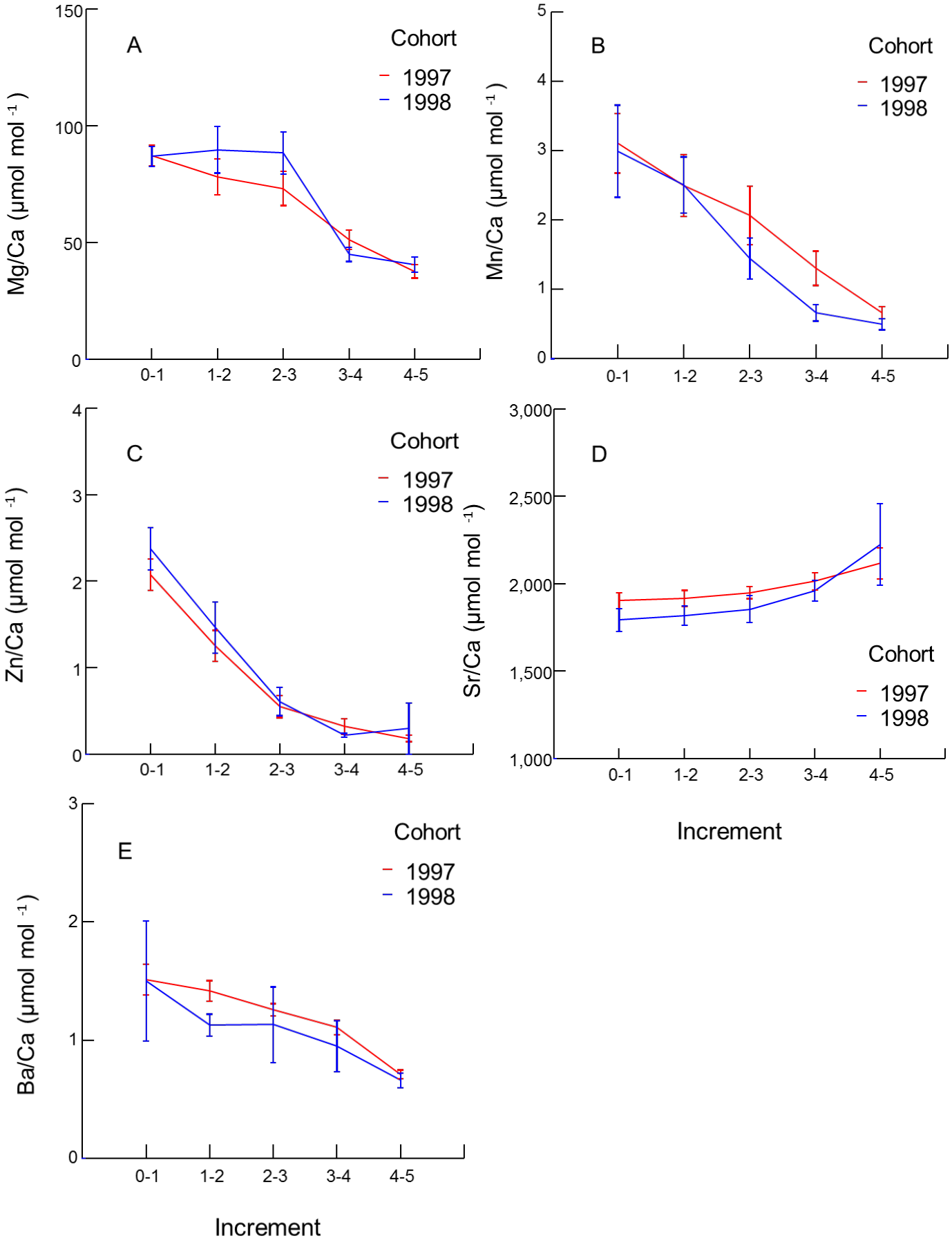


Figure 34. Mean (+/- S.E.) element/Ca ratios in otolith increment zones for two cohorts in West Victoria

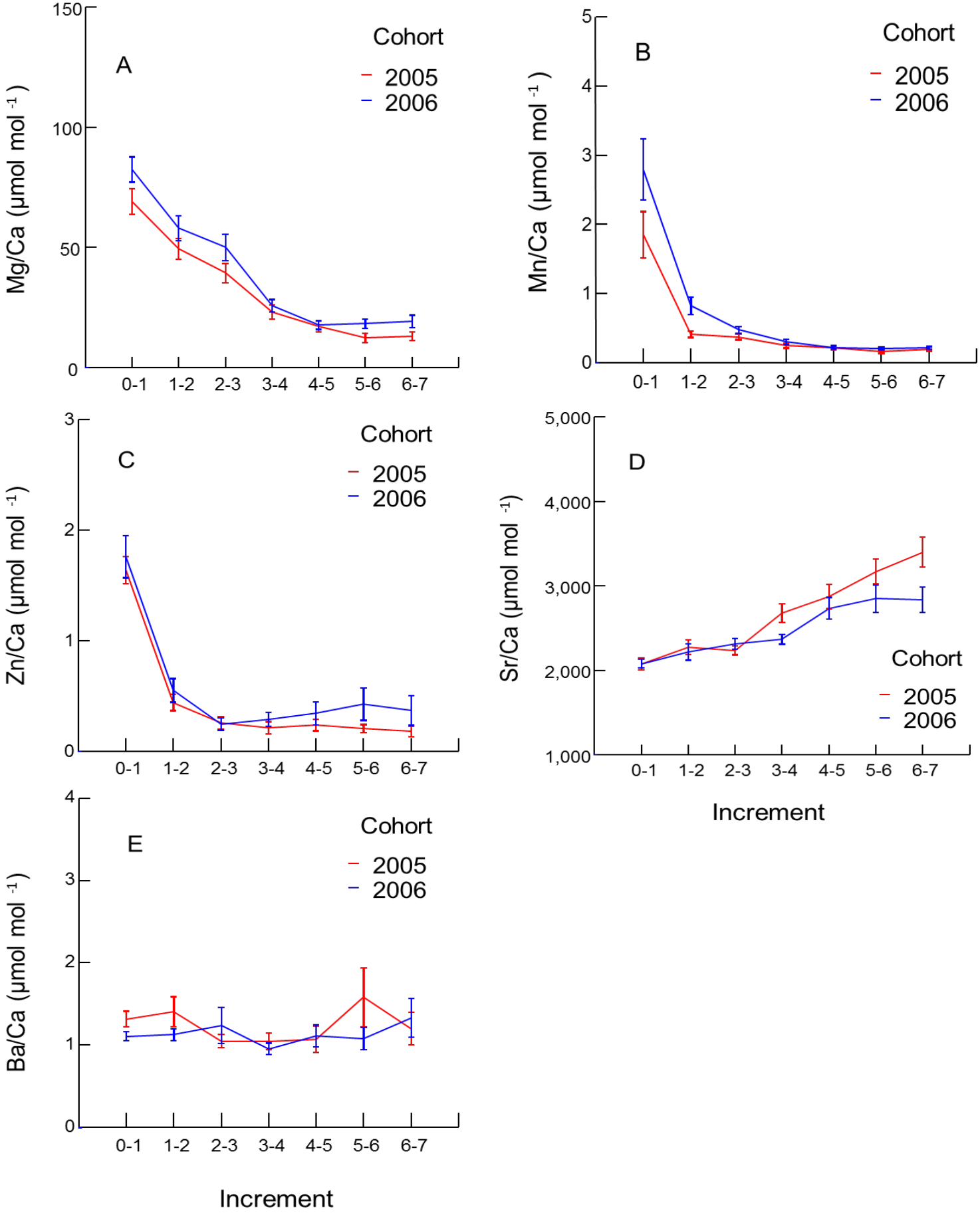


Figure 35. Mean (+/- S.E.) element/Ca ratios in otolith increment zones for two cohorts in South Australia

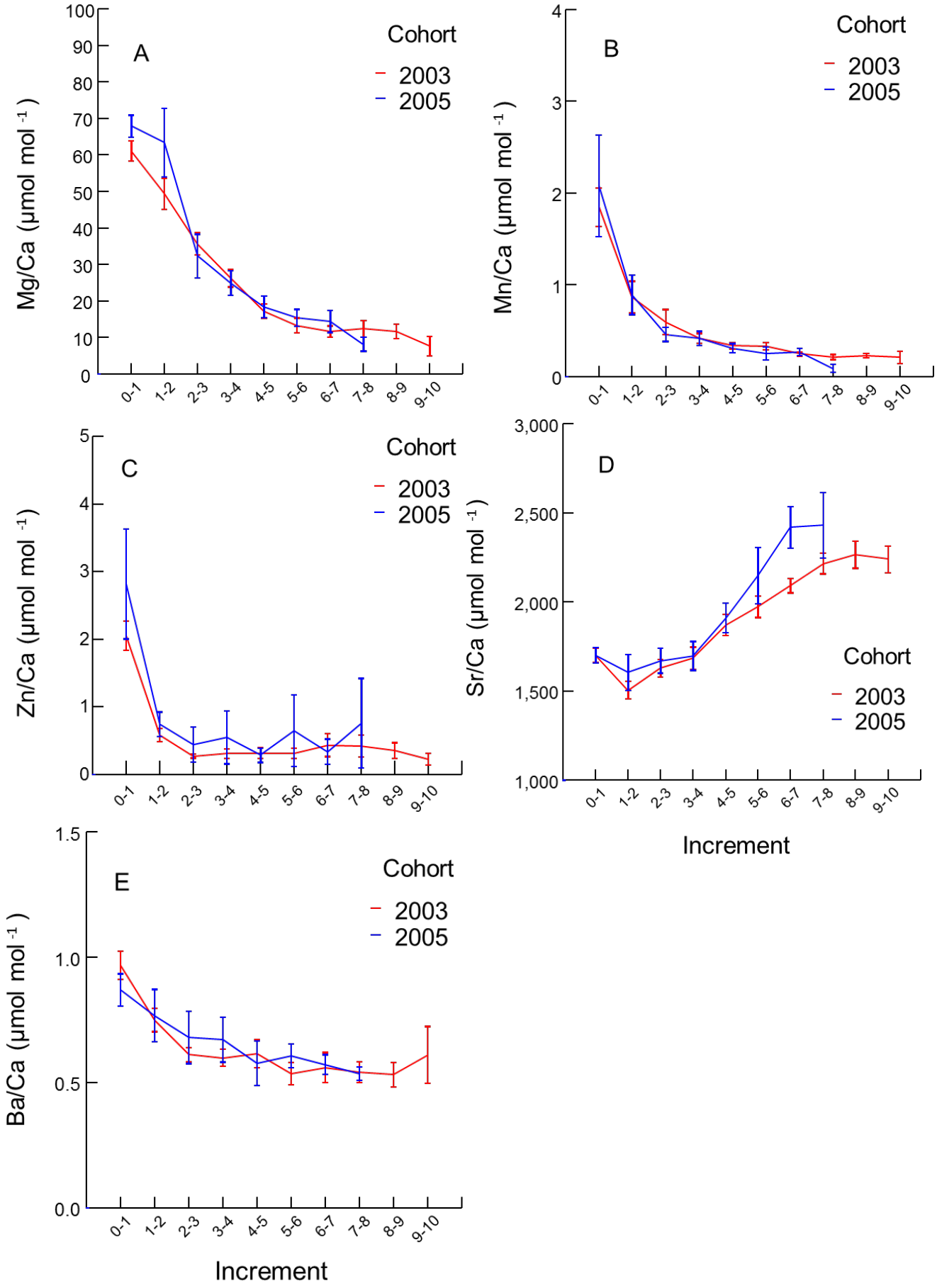


Figure 36. Mean (+/- S.E.) element/Ca ratios in otolith increment zones for two cohorts in north-west Tasmania

Table 21. Single factor ANOVA comparisons of element/Ca ratios in otolith increment zones for three sampling areas in south-eastern Australia. Significance level adjusted (Bonferroni) for the number of otolith zones analysed for each element. Significant P-values in bold.

Element/Ca	Otolith zone	Main effects	p – value: Areas	Significant Tukey's pairwise comparisons between Areas
Ln Mg:Ca	0-1	WestVic, NWTas, SA	**<0.001**	WestVic > NWTas
	1-2	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	2-3	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	3-4	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	4-5	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	5-6	NWTas, SA	0.617	
	6-7	NWTas, SA	0.195	
Ln Mn:Ca	0-1	WestVic, NWTas, SA	0.024	
	1-2	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	2-3	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	3-4	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	4-5	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	5-6	NWTas, SA	**<0.001**	
	6-7	NWTas, SA	0.064	
4^{th} root Zn:Ca	0-1	WestVic, NWTas, SA	0.055	
	1-2	WestVic, NWTas, SA	**<0.001**	WestVic > SA, NWTas
	2-3	WestVic, NWTas, SA	**0.002**	WestVic > SA
	3-4	WestVic, NWTas, SA	0.534	
	4-5	WestVic, NWTas, SA	0.429	
	5-6	NWTas, SA	0.649	
	6-7	NWTas, SA	0.365	
Sr:Ca	0-1	WestVic, NWTas, SA	**<0.001**	SA > WestVic > NWTas
	1-2	WestVic, NWTas, SA	**<0.001**	SA > WestVic > NWTas
	2-3	WestVic, NWTas, SA	**<0.001**	SA > WestVic > NWTas
	3-4	WestVic, NWTas, SA	**<0.001**	SA > WestVic > NWTas
	4-5	WestVic, NWTas, SA	**<0.001**	SA > WestVic, NWTas
	5-6	NWTas, SA	**<0.001**	
	6-7	NWTas, SA	**<0.001**	
Ln Ba:Ca	0-1	WestVic, NWTas, SA	**<0.001**	WestVic > NWTas
	1-2	WestVic, NWTas, SA	**<0.001**	WestVic > NWTas, SA > NWTas
	2-3	WestVic, NWTas, SA	**<0.001**	WestVic > NWTas, SA > NWTas
	3-4	WestVic, NWTas, SA	**<0.001**	WestVic > NWTas, SA > NWTas
	4-5	WestVic, NWTas, SA	**<0.001**	SA > WestVic, SA > NWTas
	5-6	NWTas, SA	**<0.001**	
	6-7	NWTas, SA	**<0.001**	

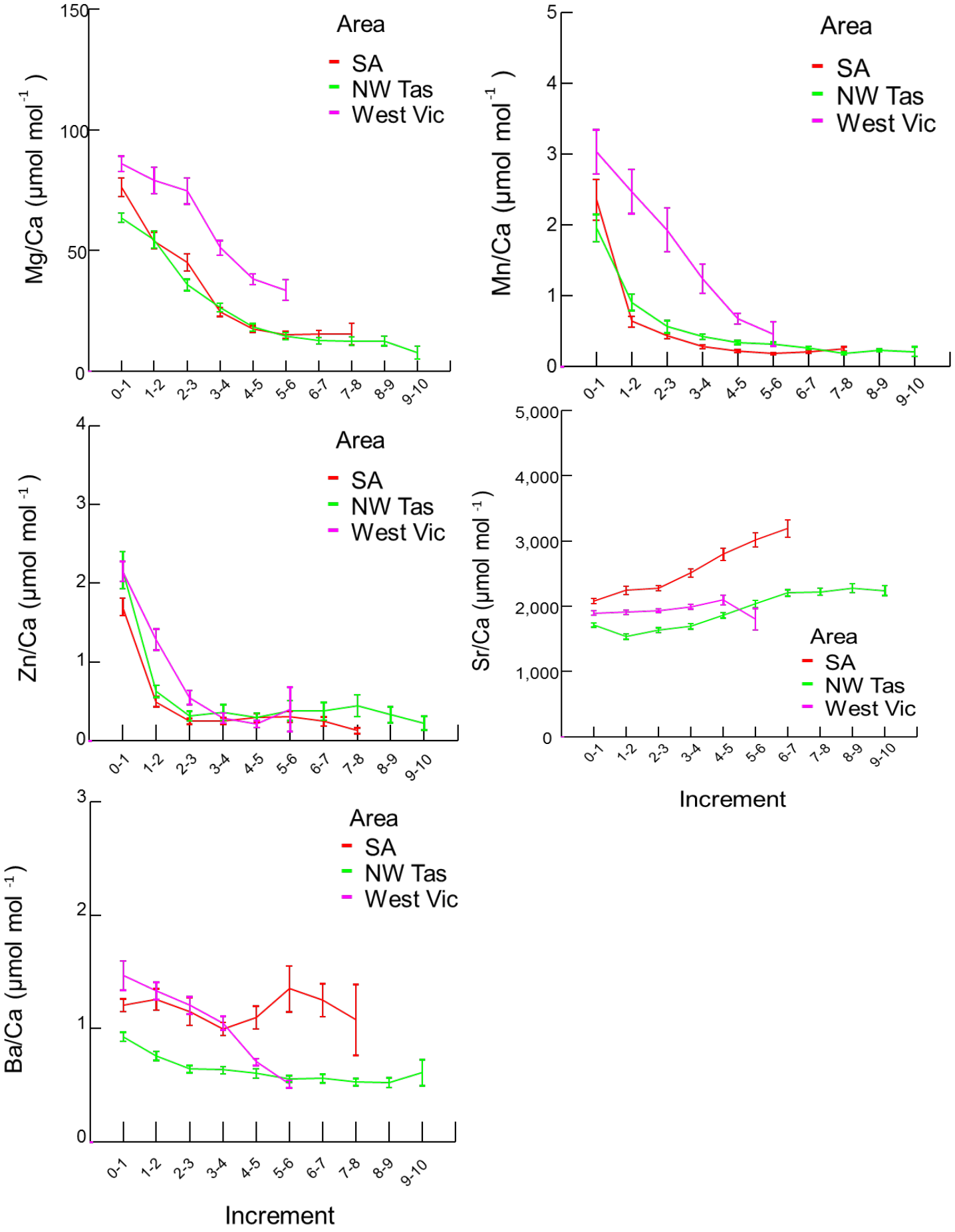


Figure 37. Mean (+/- S.E.) element/Ca ratios in otolith increment zones for three sampling areas in south-eastern Australia

Table 22. Single factor MANOVA multi-element comparisons of element/Ca ratios in otolith increment zones for two cohorts in each of the three sampling areas. P-values based on Hotelling's T-Square. Significance level adjusted (Bonferroni) for the number of otolith zones analysed for each State. Significant P-values in bold.

Element/Ca	Otolith zone	South Australia		Western Victoria		North-west Tasmania	
		Main effects	p – value: Cohorts	Main effects	p – value: Cohorts	Main effects	p – value: Cohorts
Ln Mg:Ca	0-1	2005, 2006	0.025	1997, 1998	0.682	2003, 2005	0.437
Ln Mn:Ca	1-2	2005, 2006	0.082	1997, 1998	0.603	2003, 2005	0.397
4^{th} root Zn:Ca	2-3	2005, 2006	0.604	1997, 1998	0.140	2003, 2005	0.614
Sr:Ca	3-4	2005, 2006	0.225	1997, 1998	0.697	2003, 2005	0.891
Ln Ba:Ca	4-5	2005, 2006	0.709			2003, 2005	0.983
	5-6	2005, 2006	0.023			2003, 2005	0.423
	6-7	2005, 2006	0.023			2003, 2005	0.102

Table 23. Single factor MANOVA multi-element comparisons of element/Ca ratios in otolith increment zones showing pairwise comparisons of the three sampling areas using Hotelling's T-Square. Significance level adjusted (Bonferroni) for the number of otolith zones analysed for each comparison of States. Significant P-values in bold.

Element/Ca	Otolith zone	WestVic v NWTas		WestVic v SA		NWTas v SA	
		Hotelling's T-Square	p – value	Hotelling's T-Square	p – value	Hotelling's T-Square	p – value
Ln Mg:Ca	0-1	89.077	**<0.001**	38.528	**<0.001**	114.863	**<0.001**
Ln Mn:Ca	1-2	124.940	**<0.001**	83.840	**<0.001**	139.167	**<0.001**
4^{th} root Zn:Ca	2-3	117.140	**<0.001**	115.622	**<0.001**	193.904	**<0.001**
Sr:Ca	3-4	110.182	**<0.001**	122.075	**<0.001**	142.581	**<0.001**
Ln Ba:Ca	4-5	85.156	**<0.001**	161.392	**<0.001**	94.615	**<0.001**
	5-6					102.474	**<0.001**
	6-7					85.180	**<0.001**

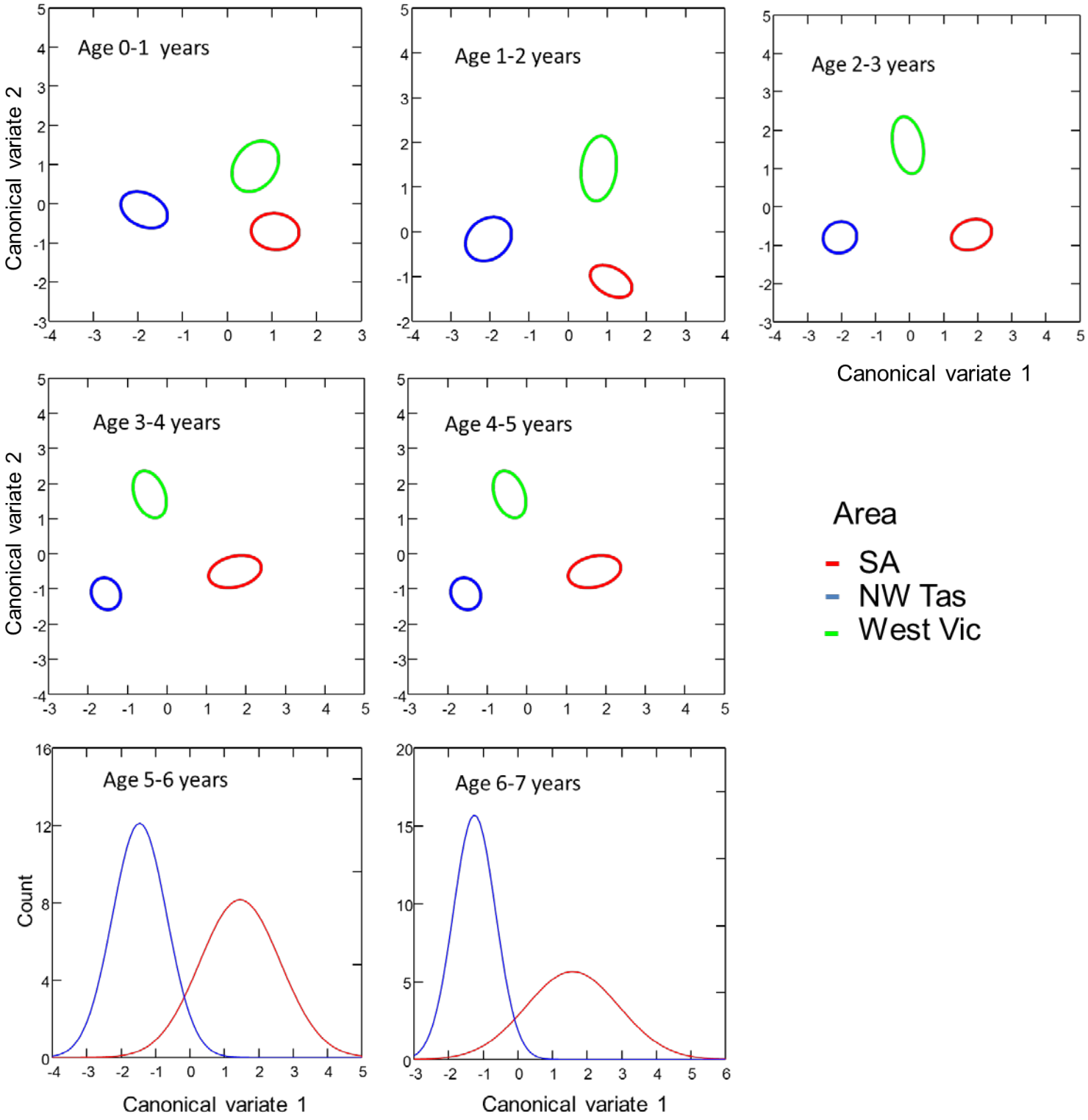


Figure 38. Canonical variate plots from the discriminant function analyses for the multi-elemental datasets from otolith increment zones for three sampling areas in south-eastern Australia. Data shown in each plot are the 95% confidence ellipses around the regional means.

Discussion

Analysis of otolith chemical profiles provides further evidence that King George Whiting from Victoria, South Australia and Tasmania represent separate populations. For fish sampled within each State, univariate and multivariate chemical profiles were similar for fish from different cohorts, indicating that the environmental conditions experienced by fish were relatively consistent from year to year. This similarity in chemical profiles amongst cohorts within States meant that profiles from different cohorts could be compared amongst States to assess State-based differences. These State-based differences were substantial and were consistent with results from chemical core and nursery area signatures differentiating Victorian and South Australian populations (Chapters 1 & 2) and genetic analyses differentiating Tasmanian from mainland populations (Chapter 3).

Reasons for these differences in chemical profiles are likely to be complex, and for the purposes of stock/population discrimination do not necessarily need to be understood (Elsdon et al., 2008). Differences may relate to changing ambient chemical environment experienced in the process of movement and migration, most pronounced for movement between freshwater and seawater (Elsdon et al., 2008; Macdonald and Crook, 2010; Walther and Limburg, 2012; Conroy et al., 2015). Differences can also relate to environmental conditions potentially affecting incorporation of elements, for example differences in temperature or salinity (Elsdon et al., 2008). Finally, differences may have a physiological basis and reflect different growth and metabolic rates (Martin and Thorrold, 2005; DiMaria et al., 2010; Woodcock et al., 2012).

We found highly significant variation in univariate chemical transects amongst States even though less variation in profiles may be expected for marine fish because of the more homogeneous environment (Sturrock et al., 2012). Variation in elements such as Ba:Ca may be explained by movements between coastal embayments and the open coast. For example, high Ba concentrations in Port Phillip Bay, a largely enclosed bay with a water residence time of over a year, leads to a distinct change in Ba:Ca profile in Snapper, *Pagrus auratus*, otoliths when fish move to and from the bay (Hamer et al., 2006). Port Phillip Bay is one of the major nursery areas for Victorian King George Whiting, and juvenile fish remain in the bay for 3 to 4 years before moving offshore onto the coast and gradually moving west (Hamer et al., 2004). This life history is consistent with the Ba:Ca profile found for Whiting collected from west Victoria, where Ba:Ca levels were elevated in otolith increments up to 3-4 and then declined in otolith increments 4-5 and 5-6 (Figure 37). This change most likely reflects movement of Whiting from a Ba rich environment in Port Phillip Bay to a Ba poor environment on the west Victorian coast. Some of this variation may be reduced by the fact that some fish sampled in west Victoria may have come from the other major nursery areas in Victoria (Western Port and Corner Inlet) that have less elevated levels of Ba than Port Phillip Bay (Hamer and Jenkins, 2007). The relatively stable Ba:Ca profile for fish sampled in South Australia may reflect the fact that the ontogenetic movement of Whiting in central SA is much more limited, primarily a movement from the northern to southern sections of the major Gulfs (Fowler et al., 2000b; 2002). Or alternatively limited movement at all if the adults had originated in the Kangaroo Island near where they were sampled, as postulated in chapter 2 and indicated in Fowler et al. (2002).

Multivariate elemental profiles also showed highly significant variation amongst States. Although differences for all otolith increment zones were highly significant, it was notable that, based on Hotelling's T-Square values and canonical variate plots, the profiles for fish sampled from west Victoria and South Australia were more similar for the first few increments compared to later increments. This is likely to be a reflection of similar ambient water chemistry and/or environmental conditions such as temperature and salinity in shallow seagrass nursery areas in South Australia and Victoria. The youngest juvenile Whiting in these areas tend to occur in shallow, protected seagrass habitats and gradually move into deeper water as they age (Fowler et al., 2000b; 2002; Jenkins, 2010). Fish from these States were always distinctly different from fish in Tasmania, possibly reflecting a lack of large bays or gulfs on the Tasmanian coastline that would provide equivalent nursery area conditions to those in Victoria and South Australia.

Although the results are consistent with otolith chemical core and nursery area signature differences between fish sampled from Victoria and South Australia (Chapters 1& 2), and genetic differences between fish sampled from Tasmania and the mainland States, they are not consistent with the lack of genetic differentiation between fish sampled in Victoria and South Australia (Chapter 3). Modelling studies based on hydrodynamics and larval duration based on daily otolith increments have indicated that spawning of fish recruiting to central Victorian bays is most likely to occur from western Victoria to south-eastern South Australia but not as far west as the known spawning area in central South Australia (Jenkins et al., 2000). A possible explanation for the lack of genetic differentiation between Whiting from Victoria and South Australia is that there is a low level of mixing of adult fish between the known spawning area in central South Australia and the spawning area for Victorian fish, notionally straddling the Victorian-South Australian border. Ecologically distinct populations could be maintained even if a low amount of exchange occurred, however such exchange would result in genetic homogeneity.

In terms of the question of whether State jurisdictional management is most appropriate for the King George Whiting fishery, our study largely supports State-based management as each State appears to be fishing independent stocks. The conclusion is very well supported for the Tasmanian fishery where there is a genetic difference from the mainland populations (Chapter 3). There is a caveat to this conclusion for South Australian and Victorian stocks, however, in that genetic results indicate that at least a small amount of mixing of the populations occurs. Otolith chemical nursery signatures indicate that adult Whiting from Victorian nurseries are not migrating to the known South Australian spawning area (Chapter 2), however, it is possible that some adult Whiting from the South Australian population may move to the area near the Victorian-South Australian border and contribute to spawning in the area where larvae are transported to Victorian nursery areas. The chemical profile analysis in this chapter indicates that if this movement occurs it must be a relatively rare event, supporting the current management by individual States. This is still an area of uncertainty though, depending on the degree of mixing, and further studies are recommended based on conventional tagging, and potentially acoustic tagging, to resolve this question.

Conclusion

Our study presents strong evidence that the King George Whiting in juvenile nursery areas of Victoria and South Australia are derived from different spawning sources (Objective 1). Evidence from otolith core chemistry and growth estimated from otolith core microstructure showed significant differences for post-larvae from the two States. Early larval growth was higher for post-larvae from South Australia than Victoria, suggesting higher water temperatures and/or productivity of food organisms in the spawning area for South Australian post-larvae. Otolith chemical differences were consistent with the microstructure results in that they were strongly driven by Mg:Ca, an element that is largely under physiological control, and incorporation is mainly influenced by factors such as temperature and growth rate. The results were consistent with previous modelling studies that predicted that the spawning area for Victorian Whiting would range from western Victoria to the south-east of South Australia.

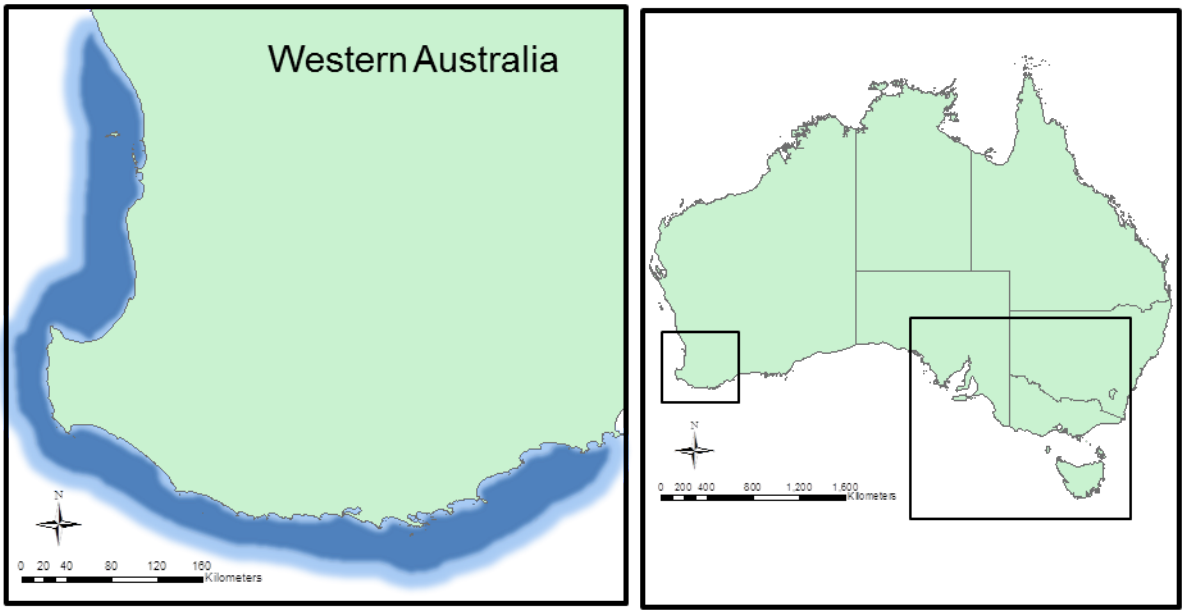
Juvenile Whiting tend to remain in nursery areas for the first 3 to 4 year of life before moving out on to the open coast with maturity, however, previously it was not known whether juveniles moved between nursery areas (Objective 2). This has significant implications for the management of fishing in individual nursery areas where most of the catch is taken. Our results based on otolith chemistry indicate that juvenile Whiting in Victorian nursery bays remain in those bays until nearing maturity, and management should treat each bay as a separate, relatively independent fishery.

The question of whether Whiting from Victorian nursery areas replenish the known spawning area in South Australia is integral to whether there is a need for cross-jurisdictional management between Victoria and South Australia (Objective 3). Our results showed that Whiting from Victorian nursery areas were not contributing to the spawning population in the known South Australian spawning area. Back-classification of adults back to putative nursery areas indicated that we had not captured all nursery areas in our survey of baseline signatures. We argue, however, that these non-characterised nursery areas were most likely to be in South Australia rather than Victoria (for example the Kangaroo Island nursery area) and therefore do not affect the conclusion in relation to the contribution of Victorian Whiting to spawning.

We were able to confirm that the large King George Whiting captured in the North-west Tasmanian commercial fishery represent a previously unknown spawning population (Objective 5). The size, age and reproductive characteristics of the population were similar to fish in the previously known spawning population in central South Australia, including the seasonal timing of spawning. Previously, modelling studies have suggested that there is a possible pathway of larval dispersal from spawning in north-west Tasmania to nursery areas in central Victoria. However, our genetic studies indicate that Whiting from Tasmania are genetically separated from populations on the mainland, so there does not appear to be any population connectivity between Tasmanian population and the mainland. Moreover, genetic studies indicated that there was population structuring within the Tasmanian jurisdiction, with genetic separation of populations on the north-west coast and the upper east coast of the State.

The results of our study represent a major advance in our understanding of the life history and stock structure of King George Whiting in southern Australia (Objective 4). Evidence from otolith chemistry and microstructure indicates that fisheries in Victoria, South Australia and Tasmania are based on separate populations and therefore management by individual jurisdictions is justified. The genetic results supported the separation of the Tasmanian population, but did not find a difference between Victorian and South Australian populations. This indicates that at least a low level of gene flow must be occurring between the Victorian and South Australian populations, even if from a management point of view the differentiation of the stocks is significant.

A conceptual diagram of King George Whiting stock structure based on our results is shown in Figure 39.



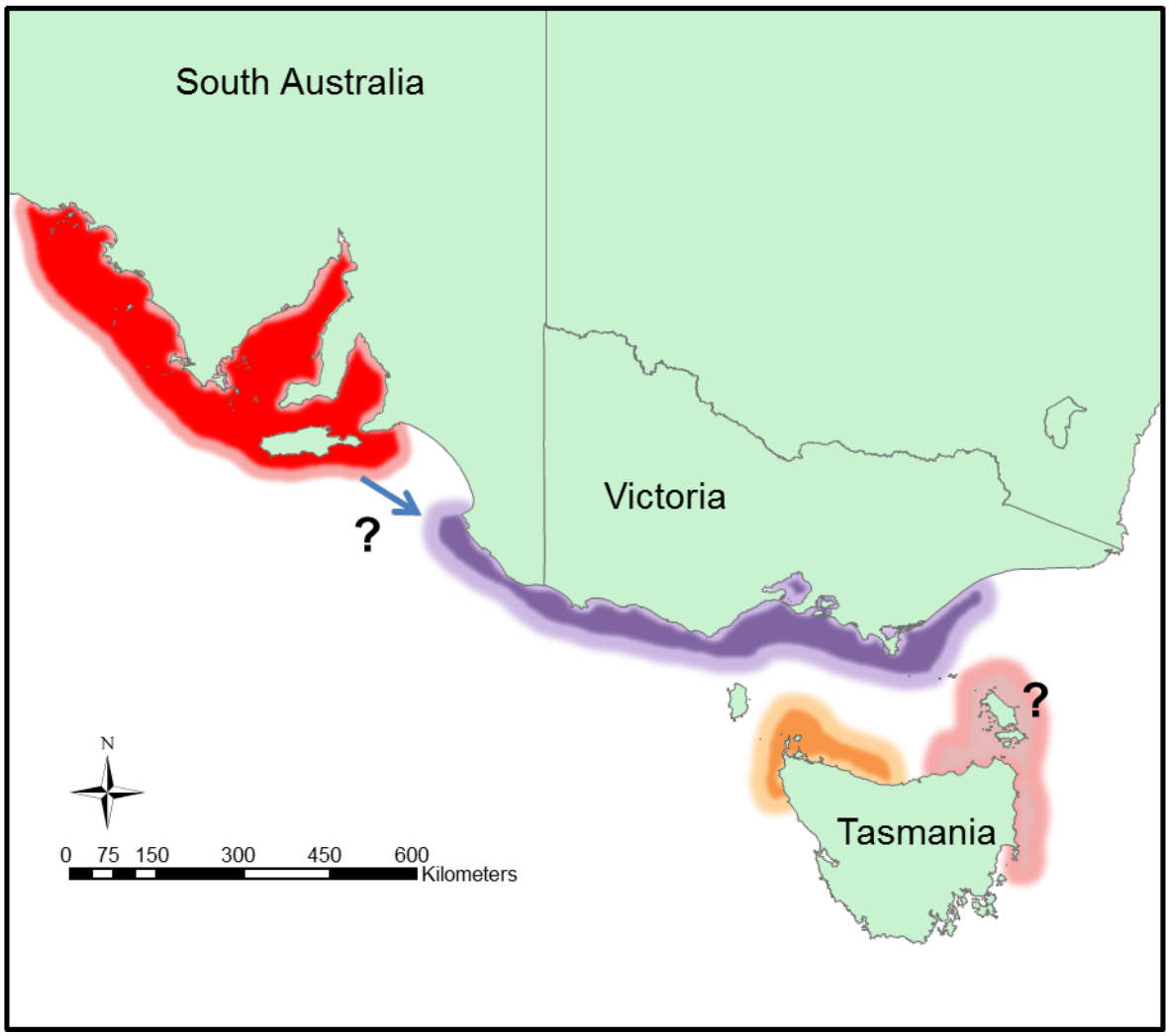


Figure 39. Conceptual diagram of King George Whiting population structure in Southern Australia based on the results of this study. Note that for fishery management purposes the population in South Australia is further divided into three sub-stocks based on movement patterns and dispersal modelling (Fowler et al. 2014). The east Tasmanian stock has been assumed to include the Furneaux island group however the population status of Whiting in this area is yet to be determined.

Implications

The possibility that King George Whiting formed a continuous population across South Australia to Victoria would call in to question the current State-based management of the fishery. Previous results showing a lack of spawning in Victoria and a movement of adult fish to the west, together with predicted spawning straddling the border of Victoria and South Australia indicated that some spawning of fish recruiting to Victorian nurseries was likely to come from South Australia. The key questions for management were whether the known spawning area for the central South Australian fishery, Investigator Strait and the southern gulfs, was also a source for the Victorian Whiting fishery, and also whether juveniles from Victorian nursery areas were migrating back to the known spawning area in South Australia. In the former case, fishery activities affecting Whiting abundances in South Australia could also potentially affect Victoria through reducing the number of larvae produced. In the latter case, fishing activities in Victoria could potentially reduce the number of adult whiting from Victoria reaching the central South Australian spawning ground.

The weight of evidence from our results, however, does not support the contention that larvae are dispersed from the known South Australian spawning ground to Victoria, or that fish from Victoria migrate back to the known central South Australian spawning grounds. This evidence supports the original modelling suggesting that spawning of Whiting in central Victorian nursery grounds is likely to come from the coastline from western Victoria to south-eastern South Australia. The caveat on this result for management, however, is that genetic results indicated no genetic differentiation between Whiting from Victoria and South Australia, suggesting that at least a small amount of gene flow must be occurring between the Victorian and South Australian populations. A hypothesis to explain this would be that some adult Whiting from the central South Australian area are at least occasionally undertaking movements along the coast and spawning in the predicted spawning area for Victorian fish (Figure 39). The coastline represented by the Coorong between Investigator Strait and Cape Jaffa may represent a barrier to movement through a lack of reef habitat, but there may be occasional movements of fish across this barrier. This movement is likely to be relatively rare so that from a management point of view, the Victorian and South Australian populations are still effectively separate stocks for management, confirming the relevance of State-based management.

State –based management would also be questioned if larvae from the newly identified spawning area off north-west Tasmania were dispersed to Victorian nursery areas as suggested by modelling (albeit at a low probability). Genetic analysis conducted as part of this project, however, show that Whiting from Tasmania are genetically distinct from mainland populations, and therefore population connectivity with the mainland must be extremely low or non-existent. The finding that populations on the north-west and the upper east coast of Tasmania are genetically distinct indicates that management of the Whiting fishery in Tasmania will have to take into account the presence of multiple stocks (similar to the South Australian fishery).

At a finer scale, the finding that Whiting in Victorian bays do not move between bays in the juvenile phase indicates that the fishery effort might best be managed on an individual bay basis. At this stage it is unknown whether Whiting in each bay form part of separate sub-populations as is thought to be the case with the South Australian gulfs. Some variation in chemistry of otolith cores from post-larvae sampled from Victoria suggests that there is a possibility that the different nursery bays are sourced from different spawning areas.

Recommendations

The weight of evidence from this project supports the contention that the Whiting fisheries in each State in southern Australia are based on different stocks. This validates the management of the individual fisheries by each jurisdiction. This conclusion assumes, based on otolith chemical and genetics results, that exchange of adults between the central South Australian spawning area and the likely spawning area for Victoria near the State border with South Australia is possible but limited. Such limited exchange means that from the point of view of management the stocks are separate. Moreover, in the case of Tasmania, and possibly Victoria pending more information, there is the potential for multiple stocks or sub-stocks that may need to be managed separately as is currently the case in South Australia. Management of the Whiting fishery in Tasmania will in future need to take into account that the north-west coast of the State is a spawning area for the species, and some level of protection for fish in this area, such as closed areas, closed seasons, or maximum size limits may be needed in the future as the fishery develops.

Further development

Some areas of uncertainty still remain that require some further research to resolve. The main question that still remains to be answered is: what is the rate of exchange of individuals, presumably adults, which mix between the spawning areas for the South Australian and Victorian fisheries that maintain genetic homogeneity across the two stocks? Our results indicate that movement of adults derived from Victorian nurseries to the known spawning area is very low. Less certain is potential movement of adults derived from South Australian nurseries to the presumed spawning area for Victorian fish. The latter relates to an additional question that still needs answered: where is the exact spawning location for the Victorian Whiting population?

Although still at a low level, there is an increasing effort to catch adult King George Whiting on the coast of western Victoria and south-eastern South Australia. This means that conventional tagging of King George Whiting could be a useful tool to address the questions above. Tagging of adult Whiting in the known South Australian spawning area would be very useful to see if individuals are re-captured in the presumed spawning area for Victorian Whiting. In addition, tagging of older juvenile Whiting in Victorian bays may help confirm the predicted spawning area if they are later captured on the coast of western Victoria and south-eastern South Australia. Tagging of fish in Victorian nursery areas may also help answer the question of whether fish in each bay are part of a separate sub-population and associated spawning area.

In relation to tagging adult fish in the known South Australian spawning area, an alternative approach would be to use acoustic tags. Curtains of listening stations could then be placed along the south-east coast of South Australia to detect the movement of any large Whiting into that area.

In addition to traditional tagging, otolith microchemistry studies could be conducted on adult Whiting sampled from south-eastern South Australia to determine their nursery area origin (Victoria or central South Australia) using the same methods described in Chapter 2.

A further recommendation for development is for studies on King George Whiting to be conducted at the Furneaux group of Islands including Flinders Island (Figure 39). There are anecdotal reports of large Whiting and this may represent a spawning area for the Tasmanian east coast population, or alternatively could link in to the Victorian population. Otolith chemical, genetics and reproductive analyses would all be useful in determining the status of Flinders Island in the population structure and connectivity in the species.

Extension and Adoption

Managers

Project scientists have been in dialogue with fisheries managers in the south-eastern States over the course of the project. The results from the project have formed part of the input to managers formulating fishery regulations for the developing King George Whiting fishery in Tasmania.

Other researchers

An oral presentation was made at the Australian Society for Fish Biology Annual Conference in 2013: Greg Jenkins, Paul Hamer, Tony Fowler, Jodie Kemp. 'Finding the spawning source of Victoria's King George Whiting'

Two oral presentations were made at the 2015 Australian Marine Sciences Annual Conference in 2015:

1. Greg Jenkins, Paul Hamer, Jodie Kemp, Julia Kent, Tony Fowler. 'Closing the life history loop on a fish species with embayment, coastal and oceanic life phases'

2. Julia Kent, Greg Jenkins, Craig Sherman, Paul Hamer, Tony Fowler. 'Genetic analyses show high levels of connectivity for King George Whiting (*Sillaginodes punctatus*) across two States'

An oral presentation was made at the International Council for the Exploration of the Sea (ICES) Annual Science Conference, Copenhagen, Denmark September 2015: Gregory Jenkins, Paul Hamer, Jodie Kemp, Julia Kent, Tony Fowler. 'Closing the life history loop on a fish species with embayment, coastal and oceanic life phases'

Industry

2012

A presentation was given on King George Whiting science and research to over 320 recreational anglers at a Whiting information night organised by the recreational fishing industry. The talk included an outline of the research proposed to be undertaken in this project

A presentation on King George Whiting to a recreational fishing climate change adaptation workshop at DPI Queenscliff also included information on this project.

2013

A presentation was given on King George Whiting science to the members of the Snapper Point Angling Club, one of the largest angling clubs in the Port Phillip Bay region. The talk included an outline of research undertaken in this project

A presentation on King George Whiting science including a description of the objectives and preliminary results of this project. The presentation was given at the VRfish workshop to develop marine fishery policy held at Torquay, Victoria.

2014

A presentation on King George Whiting science to was given to 250 recreational anglers at a Whiting information night organised by the recreational fishing industry. This presentation included results from this this project.

2016

A presentation on King George Whiting science to was given to 180 recreational anglers at a Whiting information night organised by the recreational fishing industry. This presentation included results from this this project.

Broader community

A web page was developed for the project and is available on the DEDJTR external website: http://agriculture.vic.gov.au/fisheries/science-in-fisheries/fisheries-research-findings/finding-the-source-of-victoriaas-king-george-whiting

Project coverage

In December 2013, ABC radio in northern Tasmania ran an interview on the King George Whiting research and this can be accessed at the following web link: http://www.abc.net.au/local/photos/2013/12/04/3904680.htm.

Press releases on King George Whiting fishing from Fisheries Victoria that included information on the project were released in March 2014 and February 2015.

While conducting field work in North-west Tasmania in March 2015 there was media coverage of the project by the Hobart Mercury newspaper including page 3 article and web article (this article can at the following web link http://www.themercury.com.au/news/tasmania/our-great-fish-catch/story-fnj4f7k1-1227286948603?sv=4d0cab6fbbbf92e351d2b1d7cdfb0b07.)

Project materials developed

Scientific papers will be developed for publication from this report

Appendices

References

Allendorf, F.W., Hohenlohe, P.A., Luikart, G., 2010. Genomics and the future of conservation genetics. Nat. Rev. Genet. 11, 697-709.

Anderson, J.T., 1988. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. J. Northw. Atl. Fish. Sci. 8, 55-66.

Atarhouch, T., Rüber, L., Gonzalez, E.G., Albert, E.M., Rami, M., Dakkak, A., Zardoya, R., 2006. Signature of an early genetic bottleneck in a population of Moroccan sardines (*Sardina pilchardus*). Mol. Phylogenet. Evol. 39, 373-383.

Barnes, T.C., Gillanders, B.M., 2013. Combined effects of extrinsic and intrinsic factors on otolith chemistry: implications for environmental reconstructions. Can. J. Fish. Aquat. Sci. 70, 1159-1166.

Beger, M., Selkoe, K.A., Treml, E., Barber, P.H., von der Heyden, S., Crandall, E.D., Toonen, R.J., Riginos, C., 2014. Evolving coral reef conservation with genetic information. Bull. Mar. Sci. 90, 159-185.

Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., Bernatchez, L., 2015. RAD-genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species; the American lobster (*Homarus americanus*). Mol. Ecol.

Bernal-Ramírez, J., Adcock, G., Hauser, L., Carvalho, G., Smith, P., 2003. Temporal stability of genetic population structure in the New Zealand snapper, *Pagrus auratus*, and relationship to coastal currents. Mar. Biol. 142, 567-574.

Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications [Review]. Mar. Ecol. Prog. Ser. 188, 263-297.

Chapman, B.B., Hulthén, K., Brodersen, J., Nilsson, P.A., Skov, C., Hansson, L.A., Brönmark, C., 2012. Partial migration in fishes: Causes and consequences. J. Fish Biol. 81, 456-478.

Conroy, C.W., Piccoli, P.M., Secor, D.H., 2015. Carryover effects of early growth and river flow on partial migration in striped bass *Morone saxatilis*. Mar. Ecol. Prog. Ser. 541, 179-194.

Coplen, T.B., Kendall, C., Hopple, J., 1983. Comparison of stable isotope reference material. Nature 302, 236-238.

Corander, J., Majander, K.K., Cheng, L., Merilä, J., 2013. High degree of cryptic population differentiation in the Baltic Sea herring *Clupea harengus*. Mol. Ecol. 22, 2931-2940.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., 2011. The variant call format and VCFtools. Bioinformatics 27, 2156-2158.

DiMaria, R.A., Miller, J.A., Hurst, T.P., 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. Env. Biol. Fish. 89, 453-462.

Elsdon, T.S., Gillanders, B.M., 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. Can. J. Fish. Aquat. Sci. 59, 1796–1808.

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R., Walther, B.D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Ann. Rev. 46, 297.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564-567.

Fielding, A.H., Bell, J.F., 1997. A review of methods for the measurement of prediction errors in conservation presence/absence models. Envir. Conserv. 24, 38-49.

Ford, J.R., Williams, R.J., Fowler, A.M., Cox, D.R., Suthers, I.M., 2010. Identifying critical estuarine seagrass habitat for settlement of coastally spawned fish. Mar. Ecol. Prog. Ser. 408, 181-193.

Fowler, A.J., Black, K.P., Jenkins, G.P., 2000a. Determination of spawning areas and larval advection pathways for King George whiting in southeastern Australia using otolith microstructure and hydrodynamic modelling. II. South Australia. Mar. Ecol. Prog. Ser. 199, 243-254.

Fowler, A.J., Campana, S.E., Jones, C.M., Thorrold, S.R., 1995a. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. J. Can. Sci. Halieut. Aquat. 52, 1431-1441.

Fowler, A.J., Campana, S.E., Jones, C.M., Thorrold, S.R., 1995b. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. Can. J. Fish. Aquat. Sci. J. Can. Sci. Halieut. Aquat. 52, 1421-1430.

Fowler, A.J., Jones, G.K., McGarvey, R., 2002. Characteristics and consequences of movement patterns of King George whiting (Perciformes : *Sillaginodes punctata*) in South Australia. Mar. Freshw. Res. 53, 1055-1069.

Fowler, A.J., McGarvey, R., 2000. Development of an integrated fisheries management model for King George whiting (*Sillaginodes punctata*) in South Australia. FRDC Project 95/008 Final Report.

Fowler, A.J., McGarvey, R., Carroll, J., Feenstra, J.E., 2014. King George Whiting (*Sillaginodes punctatus*) Fishery. Fishery assessment report to Primary Industries and Regions South Australia (Fisheries and Aquaculture), South Australian Research and Development Institute (Aquatic Sciences) publication F2007/000834-4, SARDI research report series 801, SARDI, Adelaide.

Fowler, A.J., McLeay, L., Short, D.A., 1999. Reproductive mode and spawning information based on gonad analysis for the King George whiting (Percoidei: Sillaginidae) from South Australia. Mar. Freshw. Res. 50, 1-14.

Fowler, A.J., McLeay, L., Short, D.A., 2000b. Spatial variation in size and age structures and reproductive characteristics of the King George whiting (Percoidei : Sillaginidae) in South Australian waters. Mar. Freshw. Res. 51, 11-22.

Fowler, A.J., Short, D.A., 1996. Temporal variation in the early life-history characteristics of the King George whiting (*Sillaginodes punctata*) from analysis of otolith microstructure. Mar. Freshw. Res. 47, 809-818.

Fowler, A.J., Short, D.A., 1998. Validation of age determination from otoliths of the King George whiting *Sillaginodes punctata* (Perciformes). Mar. Biol. 130, 577-587.

Gahagan, B.I., Fox, D.A., Secor, D.H., 2015. Partial migration of striped bass: Revisiting the contingent hypothesis. Mar. Ecol. Prog. Ser. 525, 185-197.

Gaither, M.R., Toonen, R.J., Robertson, D.R., Planes, S., Bowen, B.W., 2010. Genetic evaluation of marine biogeographical barriers: perspectives from two widespread Indo-Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). J. Biogeogr. 37, 133-147.

Gillanders, B.M., 2002. Connectivity between juvenile and adult fish populations: do adults remain near their recruitment estuaries? Mar. Ecol. Prog. Ser. 240, 215-223.

Gillanders, B.M., Able, K.W., Brown, J.A., Eggleston, D.B., Sheridan, P.F., 2003. Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. Mar. Ecol. Prog. Ser. 247, 281-295.

Gillanders, B.M., Izzo, C., Doubleday, Z.A., Ye, Q., 2015. Partial migration: Growth varies between resident and migratory fish. Biol. Lett. 11, 20140850.

Gillanders, B.M., Kingsford, M.J., 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar. Ecol. Prog. Ser. 141, 13-20.

Green, B.S., McCormick, M.I., 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Mar. Ecol. Prog. Ser. 289, 263-272.

Haigh, L., Donnellan, S., 1998. Stock structure based on analysis of mitochondrial DNA and microsatellites, in: Fowler, A.J., McGarvey, R. (Eds.), Development of an integrated fisheries management model for King George whiting, FRDC Report 1995/008, pp. 105-124.

Haigh, L., Donnellan, S., 2000. Characterization of microsatellite loci in King George Whiting *Sillaginodes punctata* Cuvier and Valenciennes (Percoidei: Sillaginidae). Mol. Ecol. 9, 2213-2215.

Hamer, P.A., Jenkins, G.P., 2007. Comparison of spatial variation in otolith chemistry of two fish species and relationships with water chemistry and otolith growth. J. Fish Biol. 71, 1035-1055.

Hamer, P.A., Jenkins, G.P., Coutin, P., 2006. Barium variation in *Pagrus auratus* (Sparidae) otoliths: A potential indicator of migration between an embayment and ocean waters in south-eastern Australia. Est. Coast. Shelf Sci. 68, 686-702.

Hamer, P.A., Jenkins, G.P., Gillanders, B.M., 2003. Otolith chemistry of juvenile snapper *Pagrus auratus* in Victorian waters: natural chemical tags and their temporal variation. Mar. Ecol. Prog. Ser. 263, 261-273.

Hamer, P.A., Jenkins, G.P., Gillanders, B.M., 2005. Chemical tags in otoliths indicate the importance of local and distant settlement areas to populations of a temperate sparid, *Pagrus auratus*. Can. J. Fish. Aquat. Sci. 62, 623-630.

Hamer, P.A., Jenkins, G.P., Sivakumaran, K.P., 2004. Identifying the spawning locations of King George whiting in Victorian waters: a recreational fishing based study. Fisheries Victoria Research Report Series No. 21.

Harden Jones, F.R., 1968. Fish Migration. Edward Arnold, London.

Haywood, M.D.E., Kenyon, R.A., 2009. Habitat shifts by decapods-an example of connectivity across tropical coastal ecosystems, in: Nagelkerken, I. (Ed.), Ecological Connectivity among Tropical Coastal Ecosystems, pp. 229-269.

Hellberg, M.E., Burton, R.S., Neigel, J.E., Palumbi, S.R., 2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. 70, 273-290.

Hohenlohe, P.A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E.A., Cresko, W.A., 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genet 6, e1000862.

Hudson, R.R., Slatkin, M., Maddison, W., 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132, 583-589.

Hyndes, G.A., Platell, M.E., Potter, I.C., Lenanton, R.C.J., 1998. Age composition, growth, reproductive biology, and recruitment of King George whiting, *Sillaginodes punctata*, in coastal waters of southwestern Australia. Fish. Bull. 96, 258-270.

Jenkins, G.P., 2010. Fisheries Adaptation to Climate Change – Marine Biophysical Assessment of Key Species. Fisheries Victoria Research Report Series No. 49.

Jenkins, G.P., Black, K.P., Hamer, P.A., 2000. Determination of spawning areas and larval advection pathways for King George whiting in southeastern Australia using otolith microstructure and hydrodynamic modelling. I. Victoria. Mar. Ecol. Prog. Ser. 199, 231-242.

Jenkins, G.P., King, D., 2006. Variation in larval growth can predict the recruitment of a temperate, seagrass-associated fish. Oecologia 147, 641-649.

Jenkins, G.P., May, H.M.A., 1994. Variation in settlement and larval duration of King George whiting, *Sillaginodes punctata* (Sillaginidae), in Swan Bay, Victoria, Australia. Bull. Mar. Sci. 54, 281-296.

Jensen, J.L., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. BMC genetics 6, 13.

Jones, G.K., Hall, D.A., Hill, K.L., Stanifford, A.J., 1990. The South Australian marine scale fishery: Stock assessment, economics, management. South Australian Department of Fisheries, Green Paper, South Australia, p. 186.

Jones, G.P., Almany, G.R., Russ, G.R., Sale, P.F., Steneck, R.S., Oppen, M.J.H., Willis, B.L., 2009. Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. Coral Reefs 28, 307-325.

Kemp, J., Jenkins, G.P., Swearer, S.E., 2012. The reproductive strategy of red cod, *Pseudophycis bachus*, a key prey species for high trophic-level predators. Fish. Res. 125–126, 161-172.

Kerr, L.A., Campana, S.E., 2013. Chemical Composition of Fish Hard Parts as a Natural Marker of Fish Stocks, Stock Identification Methods: Applications in Fishery Science: Second Edition, pp. 205-234.

Lahaye, Y., Lambert, D., Walters, S., 1997. Ultraviolet laser sampling and high resolution inductively coupled plasma-mass spectrometry of NIST and BCR-2G glass reference materials. Geostandards Newsletters 21, 205-214.

Last, P.R., Scott, E.O.G., Talbot, F.H., 1983. Fishes of Tasmania. Tasmanian Fisheries Development Authority, Hobart.

Lu, F., Lipka, A.E., Glaubitz, J., Elshire, R., Cherney, J.H., Casler, M.D., Buckler, E.S., Costich, D.E., 2013. Switchgrass genomic diversity, ploidy, and evolution: novel insights from a network-based SNP discovery protocol. PLoS Genet 9, e1003215.

Ludden, J.N., Feng, R., Gauthier, G., Stix, J., Shi, L., Francis, D., Machado, N., Wu, G., 1995. Applications of LAM-ICP-MS analysis of minerals. Can Mineralogy 33, 419-434.

Macdonald, J.I., Crook, D.A., 2010. Variability in Sr:Ca and Ba:Ca ratios in water and fish otoliths across an estuarine salinity gradient. Mar. Ecol. Prog. Ser. 413, 147-161.

Marshall, D., Monro, K., Bode, M., Keough, M., Swearer, S., 2010. Phenotype–environment mismatches reduce connectivity in the sea. Ecol. Lett. 13, 128-140.

Martin, G.B., Thorrold, S.R., 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. Mar. Ecol. Prog. Ser. 293, 223-232.

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. Fish. Bull. 100, 545–558.

Meekan, M.G., Carleton, J.H., McKinnon, A.D., Flynn, K., Furnas, M., 2003. What determines the growth of tropical reef fish larvae in the plankton: Food or temperature? Mar. Ecol. Prog. Ser. 256, 193-204.

Melancon, S., Fryer, B.J., Markham, J.L., 2009. Chemical analysis of endolymph and the growing otolith: Fractionation of metals in freshwater fish species. Environ. Toxicol. Chem. 28, 1279–1287.

Milano, I., Babbucci, M., Panitz, F., Ogden, R., Nielsen, R.O., Taylor, M.I., Helyar, S.J., Carvalho, G.R., Espiñeira, M., Atanassova, M., 2011. Novel tools for conservation genomics: comparing two high-throughput approaches for SNP discovery in the transcriptome of the European hake. PLoS One 6, e28008.

Millar, R.B., 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 44, 583-590.

Millar, R.B., 1990a. Comparison of methods for estimating stock fishery composition. Can. J. Fish. Aquat. Sci. 47.

Millar, R.B., 1990b. A versatile computer program for mixed stock fishery composition. Canadian Technical Report Fisheries and Aquatic Science No. 1753.

Miller, K., Maynard, B., Mundy, C., 2009. Genetic diversity and gene flow in collapsed and healthy abalone fisheries. Mol. Ecol. 18, 200-211.

Miller, M.B., Clough, A.M., Batson, J.N., Vachet, R.W., 2006. Transition metal binding to cod otolith proteins. J. Exp. Mar. Biol. Ecol. 329, 135-143.

Nagelkerken, I., 2009a. Ecological connectivity among tropical coastal ecosystems. Springer Dordrecht Heidelberg London New York.

Nagelkerken, I., 2009b. Evaluation of nursery function of mangroves and seagrass beds for tropical decapods and reef fishes: Patterns and underlying mechanisms, in: Nagelkerken, I. (Ed.), Ecological Connectivity among Tropical Coastal Ecosystems. Springer, Dordrecht Heidelberg London New York, pp. 357-399.

Nei, M., 1977. F-statistics and analysis of gene diversity in subdivided populations. Annals of human genetics 41, 225-233.

Nielsen, E.E., Hemmer-Hansen, J., Larsen, P.F., Bekkevold, D., 2009. Population genomics of marine fishes: identifying adaptive variation in space and time. Mol. Ecol. 18, 3128-3150.

Nikolaus Probst, W., Kraus, G., Rideout, R.M., Trippel, E.A., 2006. Parental effects on early life history traits of haddock *Melanogrammus aeglefinus*. ICES J. Mar. Sci. 63, 224-234.

Palsbøll, P.J., Berube, M., Allendorf, F.W., 2007. Identification of management units using population genetic data. Trends Ecol. Evol 22, 11-16.

Palumbi, S.R., 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl. 13, 146-158.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28, 2537-2539.

Pfeifer, B., Wittelsbürger, U., Onsins, S.E.R., Lercher, M.J., 2014. PopGenome: an efficient Swiss army knife for population genomic analyses in R. Mol. Biol. Evol., msu136.

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press.
Raj, A., Stephens, M., Pritchard, J.K., 2014. fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics 197, 573-589.
Ranaldi, M.M., Gagnon, M.M., 2008. Zinc incorporation in the otoliths of juvenile pink snapper (*Pagrus auratus* Forster): The influence of dietary versus waterborne sources. J. Exp. Mar. Biol. Ecol. 360, 56-62.
Rousset, F., 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8, 103-106.
Sadovy, Y., Severin, K., 1992. Trace elements in biogenic aragonite: correlation of body growth rate and strontium levels in the otoliths of the white grunt, *Haernulon plumieri* (Pisces: Haemulidae). Bull. mar. Sci. 50, 237-257
Sadovy, Y., Severin, K.P., 1994. Elemental patterns in red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto Rico reflect growth rate, not temperature. Can. J. Fish. Aquat. Sci. 51, 133-141.
Secor, D.H., 2015. Migration Ecology of Marine Fishes. John Hopkins University Press, Baltimore.
Sim, J., Wright, C.C., 2005. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. Phys Ther 85, 257–268.
Simpson, S.D., Harrison, H.B., Claereboudt, M.R., Planes, S., 2014. Long-distance dispersal via ocean currents connects omani clownfish populations throughout entire species range. PLoS ONE 9.
Sponaugle, S., 2010. Otolith microstructure reveals ecological and oceanographic processes important to fisheries management. Env. Biol. Fish. 89, 221-238.
Stanley, R.R.E., Bradbury, I.R., Bacco, C.D., Snelgrove, P.V.R., Thorrold, S.R., Killen, S.S., 2015. Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). ICES J. Mar. Sci. 72, 2350-2363.
Sturrock, A.M., Hunter, E., Milton, J.A., EIMF, Johnson, R.C., Waring, C.P., Trueman, C.N., 2015. Quantifying physiological influences on otolith microchemistry. Methods Ecol. Evol. 6, 806–816.
Sturrock, A.M., Trueman, C.N., Darnaude, A.M., Hunter, E., 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. 81, 766-795.
Verweij, M.C., Nagelkerken, I., Hans, I., Ruseler, S.M., Mason, P.R.D., 2008. Seagrass nurseries contribute to coral reef fish populations. Limnol. Oceanogr. 53, 1540-1547.
Walther, B.D., Limburg, K.E., 2012. The use of otolith chemistry to characterize diadromous migrations. J. Fish Biol. 81, 796-825.
Wang, J., 2004. Application of the one-migrant-per-generation rule to conservation and management. Cons. Biol. 18, 332-343.
Waples, R.S., 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity 89, 438-450.
Waples, R.S., Punt, A.E., Cope, J.M., 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish Fish. 9, 423-449.
Ward, R., Woodwark, M., Skibinski, D., 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. 44, 213-232.
Wilkinson, L., Blank, G., Gruber, C., 1996. Desktop data analysis with SYSTAT. Prentice Hall, Englewood Cliffs, NJ.
Woodcock, S.H., Munro, A.R., Crook, D.A., Gillanders, B.M., 2012. Incorporation of magnesium into fish otoliths: Determining contribution from water and diet. Geochim. Cosmochim. Acta 94, 12-21.
Wright, S., 1949. The genetical structure of populations. Annals of eugenics 15, 323-354.
Yoshinaga, J., Atsuko, N., Masatoshi, M., Edmonds, J.S., 2000. Fish otolith reference material for quality assurance of chemical analysis. Mar. Chem. **69**, 91-97.