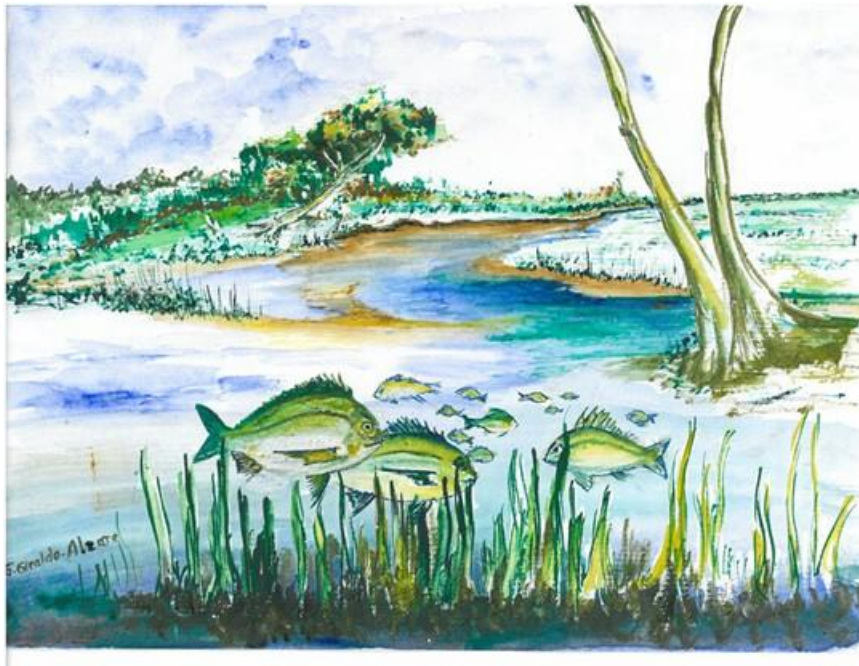


Population Structure and Movement of Black Bream

(Acanthopagrus butcheri)

in South Australian Estuaries Based on Otolith Chemistry



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Presented for the degree of Doctor of Philosophy

School of Biological Sciences

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July 2015

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Cover image: Black bream ecosystem (watercolour by Gustavo Giraldo & Judith Giraldo)

“This is what knowledge really is. It is finding out something for oneself with pain, with joy, with exultancy, with labour, and with all the little ticking, breathing moments of our lives, until it is ours which is rooted in the structure of our lives”

Thomas Wolfe (1900-1938)

Table of Contents

Declaration	2
Table of Contents	4
Abstract	8
Acknowledgements	10
Chapter Acknowledgements.....	11
Chapter 1. General Introduction	13
Estuaries	13
Use of estuaries by fish	15
Determining population structure and connectivity	18
Otolith chemistry for determining population structure and movements of fish.....	19
Objectives.....	20
Study area – Fleurieu Peninsula and Kangaroo Island.....	22
Characteristics of Kangaroo Island and Southern Fleurieu estuaries	22
Fishes of Kangaroo Island.....	28
Study species – <i>Acanthopagrus butcheri</i>	28
Chapter 2 – Statement of authorship	31
Chapter 2. Temporal and spatial variability in otolith elemental composition: a precursor for determining population connectivity	33
Abstract	34
Introduction	35
Materials and Methods	36
Sample collection	36
Otolith preparation.....	38
Otolith chemical analysis	38

Statistical analyses.....	39
Results	40
Temporal differences.....	42
Spatial differences	42
Discussion	50
Temporal and spatial variation in otolith chemistry.....	50
Grouping of estuaries and applications for describing stocks	52
Conclusion.....	54
Chapter 3 – Statement of authorship	56
Chapter 3. Temporal patterns of connectivity between juvenile and subadult areas inferred from otolith elemental fingerprints	58
Abstract	59
Introduction	60
Materials and methods	61
Sample collection	61
Otolith preparation	62
Age estimation and assignment of annual recruitment cohort	63
Multielemental otolith analyses	63
Statistical analyses.....	65
Results	67
Juvenile otolith signatures.....	67
Temporal contributions of recruits.....	68
Temporal patterns of connectivity.....	69
Discussion	71
Conclusion.....	75

Chapter 4 – Statement of authorship	76
Chapter 4. Partial migration in black bream from different estuaries on Kangaroo Island: do migratory fish represent a hybrid complex?	78
Abstract	79
Introduction	80
Materials and Methods	82
Study species	82
Sampling.....	83
Otolith preparation.....	84
Analysis of environmental history of fish	85
Determining environmental histories of individual fish.....	85
Microsatellite analyses	86
Statistical analysis	86
Results	89
Environmental histories.....	90
Hybrid fish and environmental histories	93
Discussion	94
Ba:Ca in otoliths of black bream.....	95
Variation in contingents among estuaries.....	96
Are euryhaline fish hybrids?	97
Consequences of partial migration	98
Conclusion.....	99
Chapter 5. General Discussion	100
Spatial and temporal variability in elemental signatures of black bream.....	100
Connectivity between populations of black bream on the Fleurieu Peninsula and Kangaroo Island	102

Partial migration of black bream on Kangaroo Island	103
Future Research Directions	105
Conclusion.....	106
References	108

Abstract

The elemental concentration of otoliths provides an opportunity to discriminate among fish living in different habitats, and to estimate population connectivity, life history variation and potential movements. Understanding temporal patterns of recruitment and connectivity of estuarine fish populations, as well as life history variation is fundamental to elucidating population dynamics and informing effective management and conservation efforts. Estuarine associated species utilise sheltered habitats for growth and feeding, but also face fluctuating environmental conditions. Estuaries may be temporally variable sinks or sources of juveniles and have fish moving to other areas. Otolith chemistry provides a tool to reconstruct origins and quantify connectivity between juvenile and adult populations, as well as investigate movements of fish throughout their life history.

The overall aim of this study was to assess connectivity and partial migration within black bream (*Acanthopagrus butcheri*) populations in South Australia. The key objectives were to: (1) Determine spatial and temporal variation in otolith elemental signatures to assess their usefulness for determining connectivity of black bream populations; (2) Retrospectively trace subadult fish from the Fleurieu Peninsula (FP) and Kangaroo Island (KI), South Australia, to their juvenile regions to gain an understanding of movements; and (3) Determine the proportion of migrant and resident life history types within estuarine populations and if these different contingents reflect a hybrid fish genotype.

I examined spatial and temporal variability in otolith chemistry of juvenile 0⁺ black bream to discriminate fish living in different estuaries. Fish were sampled in up to 12 estuaries on KI (7 estuaries) and the FP (5 estuaries) annually over a five year period (2007–2011). Otoliths were examined to determine if individual estuaries or groups of estuaries differed in chemical concentrations (signatures or tags), and to examine inter-annual variation in chemical tags. Tags differed among individual estuaries and in some cases adjacent estuaries shared similar chemical tags. Differences in chemical tags were detected among all estuaries in two of the year comparisons. Similarities in otolith tags were detected for five of the seven estuaries in 2010 and 2011. Grouping estuaries with similar chemical tags enhanced the classification accuracy of fish to estuaries and therefore the ability to discriminate stock structure based on otolith chemistry. Temporal differences in chemical tags were detected among years for several estuaries; however, there were no clear trends in the differences between years. The results highlight that black bream from different estuaries or groups of estuaries have unique chemical tags that can be used to trace cohorts of fish. Such differences can be used to estimate connectivity, population movements, and the function of estuaries as nursery areas for this species in subsequent years.

Otolith chemistry was then used to reconstruct origins and to quantify connectivity between juvenile and adult components of black bream populations. Trace element composition of juvenile black

bream otoliths from broad areas representing estuaries from each of KI and FP were quantified for each of four years (2007-2010) and used as baseline data to retrospectively trace the juvenile region of 1 to 3⁺ year black bream. Such an approach was possible because there were differences in otolith element concentration of young-of-year black bream among broad areas. Through LA-ICP-MS analysis of the juvenile region of subadult (1 to 3⁺ year old) black bream, I was able to estimate the proportion of juvenile fish that recruited from these two regions through time, as well as temporal patterns of connectivity between KI and FP. My results suggest variability among cohorts of the same age, among age classes within a cohort and between regions in terms of self-recruitment. These results highlight the variable nature of connectivity among populations and recruitment to adult populations suggesting that management and conservation efforts may need to consider such variability.

Partial migration, where some members of a population migrate showing life history profiles of salinity habitats was evaluated in six estuarine populations of black bream from KI, using otolith Ba:Ca ratios. Profiles of Ba:Ca across otoliths were used to broadly determine fish habitat use (freshwater, estuarine, marine) and assign euryhaline or stenohaline status. In addition, we evaluated whether migratory fish may represent hybrid fish between black bream and yellowfin bream (*Acanthopagrus australis*) using an eight locus microsatellite library. Partial migration was observed in all six estuarine populations, although for most estuaries there were greater numbers of migratory fish than resident fish. Hybrid fish were found in five of the six estuaries and comprised both migratory and resident fish. Both resident and migratory fish spent most time in marine waters, but migratory fish also moved to estuarine and freshwaters. Similar patterns were also seen for hybrid fish. The data highlight the different migratory behaviours of subadult individuals and show that hybrid bream had reached estuaries previously thought to be inhabited by pure black bream. Complex migratory patterns may allow populations to persist under adverse environmental conditions and in systems subject to change.

Otolith chemistry data provided greater insight into patterns of connectivity between broad geographic regions and life history variation of black bream in South Australia. The variable nature of connectivity among populations and recruitment to adult populations suggests that conservation efforts will be more complex than protecting a single region. However, the temporally variable contribution of recruits and broad scale connectivity between juvenile regions suggests that no one region acts as a single 'source' of recruits, potentially safeguarding the species against the loss of functional nursery habitats. In the same manner, the ability of black bream populations to possess a suite of movement behaviours (likely in response to different conditions) may be beneficial in a dynamic environment such as an estuary since it allows the persistence of populations when faced with increasing habitat degradation. Overall, these findings infer that the adoption of a flexible life history strategy likely enhances species resilience to fluctuating environmental conditions and potentially adverse impacts.

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Antechamber Bay, Kangaroo Island

Chapter 1. General Introduction

Estuaries

Worldwide, aquatic ecosystems are under pressure due to natural and geophysical processes (e.g. evaporation and climate change) and anthropogenic interventions (e.g. population growth, deforestation, irrigation) (Millennium Ecosystem Assessment 2005, Wilby and Harris 2006, Stonefelt et al. 2007, Soh et al. 2008, Gillanders et al. 2011). As a consequence water, including freshwater flows, is prioritized as a critical resource for environmental sustainability (Gillanders and Kingsford 2002).

Changes in freshwater flow to estuarine environments appear to be increasing in many parts of Australia (Walker et al. 2006, Poff and Zimmerman 2009) causing variation in the biology and ecology of habitat-forming species and their associated flora and fauna (Gillanders 2007). Estuaries are a particularly threatened ecosystem where human impacts are driving them to be among the most degraded ecosystems worldwide (Edgar et al. 2000).

Although many definitions of the term ‘estuary’ exist, a widely used one is “*a semi-enclosed body of water with free connection with the open sea and within which sea water is measurably diluted with freshwater derived from land drainage*” (Pritchard 1967, Perillo 1995). However, this definition may not be applicable to estuaries in the Southern Hemisphere, as many have openings to the adjacent marine environment (i.e. mouths) that seasonally close (Fig. 1.1). These are estuaries that are often in areas with high evaporation rates relative to freshwater input (i.e. Australia); as such these estuaries may become inverse estuaries with hypersaline waters in the upper reaches (Potter et al. 2010).

Hypersaline estuaries may occur due to a number of factors (e.g. evaporation in dry summers, size and depth of their mouths, intrusion by marine waters). Potter et al. (2010) considered that estuaries in the Southern Hemisphere required a definition that better encompassed their characteristics. Thus, they proposed a more appropriate definition may be: “*An estuary is a partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural sea water and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible*”.

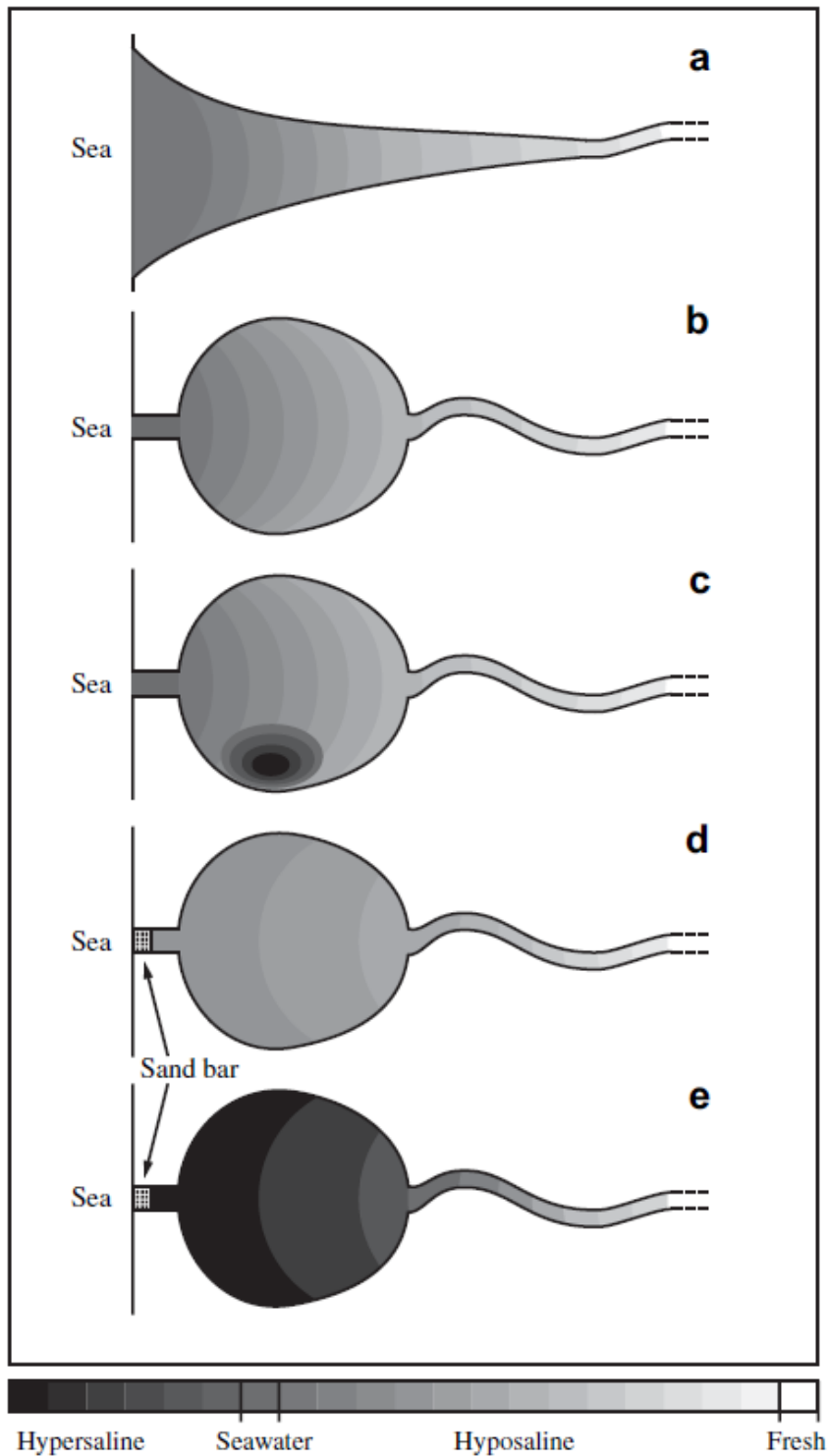


Figure 1.1. Stylised examples of (a) a classical estuary with a longitudinal salinity gradient and (b–e) typical temperate Australian estuaries, comprised of short and narrow marine openings, a wide central basin and the lower reaches of their tributary river(s). (b) Estuary permanently open to the ocean with a longitudinal salinity gradient, (c) estuary permanently open to the ocean with an overall longitudinal salinity gradient but with a region of hypersalinity, (d) estuary seasonally closed to the ocean by a sand bar across its mouth (dotted box) but remaining hyposaline and (e) estuary normally closed by a sand bar at its mouth (dotted box) and which is markedly hypersaline (from Potter et al. 2010).

Australian estuaries started forming after the last ice age and are considered to be relatively young geologically, having formed over the past 6000 years (Kench 1999). Depending on their position, configuration and stream dynamics, their geomorphology around the coast varies between the more open marine and the more closed system estuaries (Bucher and Saenger 1991). The physicochemical (abiotic) factors within the estuaries also vary (Kench 1999, Roy et al. 2001). Consequently, any change in those parameters can affect estuarine habitat-forming species and associated flora and fauna.

Seasonal inflows of freshwater are required for the ecological performance and productivity of estuaries (Whitfield et al. 2012). Additionally, seasonal abiotic factors (e.g. temperature and rainfall) can effect changes in estuarine productivity (Kennish et al. 2008, González-Ortegón and Drake 2012). For example, changes that occur in phytoplankton and zooplankton production will change the habitat complexity and biotic diversity required to maintain an ideal environment for larval survival and fish migration (Thomas 1996). Natural flows of freshwater to estuaries can be altered and reduced through the construction of weirs, barrages and other structures, leading to changes in sedimentation, nutrient input and physicochemical characteristics of the water (Gillanders 2007). A reduction of freshwater flow can directly cause the closure of estuaries and subsequent salinity alteration. Anthropogenic factors strongly influence freshwater input to estuaries often leading to significant change in abiotic parameters, which in turn can affect the health of the estuary and its associated flora and fauna (Gillanders et al. 2008).

Use of estuaries by fish

Estuarine environments play a major role in the life cycle of many fish species. Estuaries are used as nursery grounds, spawning habitat, nesting sites, feeding or refuge areas (Gillanders et al. 2008), often supporting great abundances of fish (Lenanton and Potter 1987, Beck et al. 2001, Able 2005). Estuaries provide food and shelter to fish that move to and from them during various stages of their life history (Dingle 1996, Kennish et al. 2008). Since fish use estuaries in a variety of ways and at different stages of their life history, a number of researchers have categorised estuarine fish use into multiple ecological guilds (Fig. 1.2) (Elliott et al. 2007b, Potter et al. 2015).

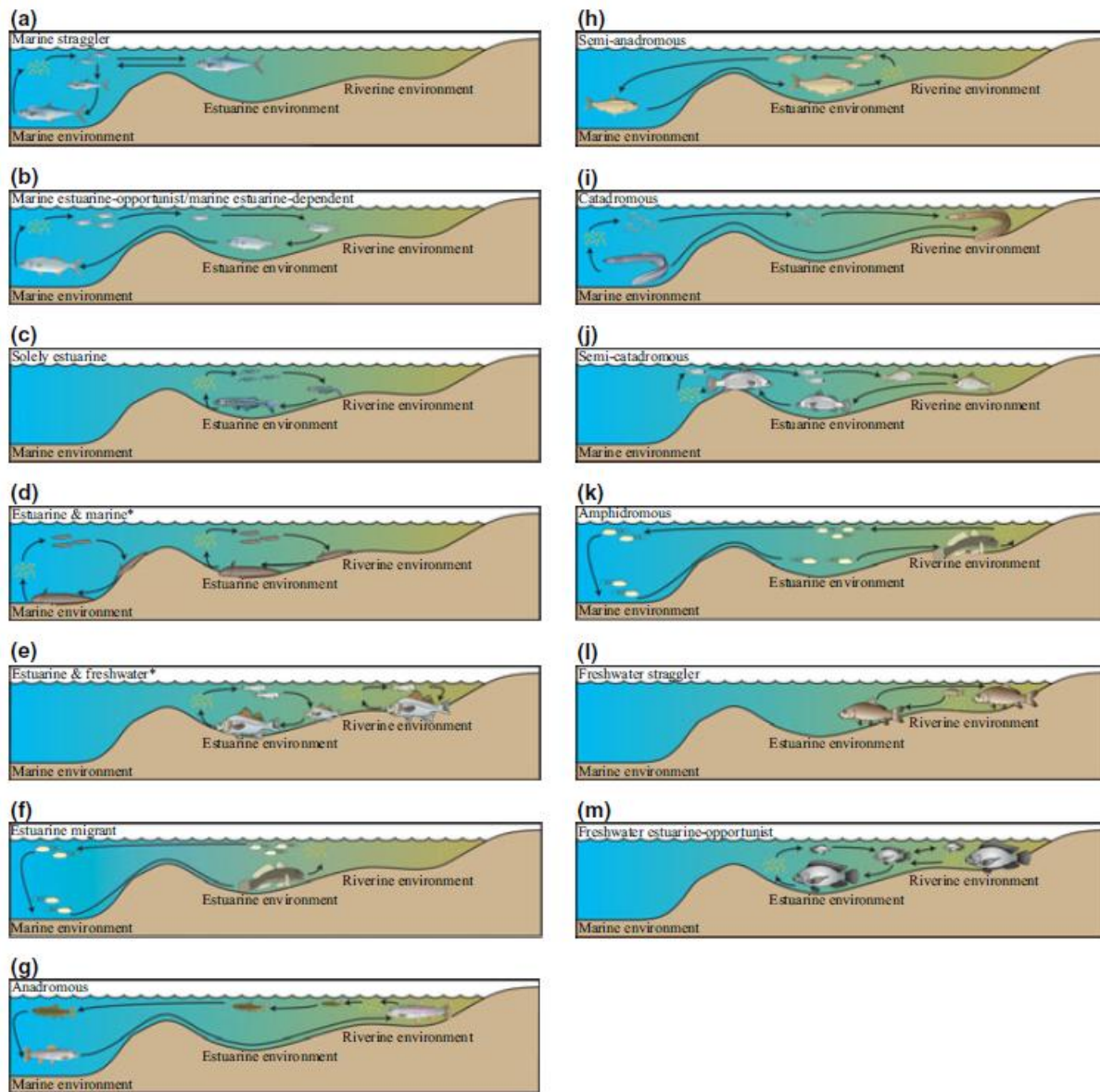


Figure 1.2. Ecological guilds of fishes found in estuaries based on estuarine usage (refers only to the estuarine populations of the guild) (from Potter et al. 2015).

The estuarine usage functional group approach recognises four categories of fishes using estuaries, namely marine, estuarine, diadromous and freshwater (Potter et al. 2015). Each broad category contains several guilds depending on the location of spawning, feeding and, or refuge (Fig. 1.2). In some cases fish use may involve movement between estuaries and other ecosystems (e.g. coastal reefs). Within the marine category three guilds were recognised ranging from those rarely found in estuaries (*marine straggler*), to those that are found in estuaries in large numbers at some stage of their life history (*marine estuarine-opportunist*), to those that are estuarine dependent at some stage of their life history (*marine estuarine-dependent*) (Fig. 1.2) (Potter et al. 2015). *Marine stragglers* are usually found near the estuary mouth where salinities remain high (Elliott and Dewailly 1995, Elliott

and Hemingway 2002). *Marine estuarine-opportunists* are frequently found in estuaries using the system for nursery areas, but may also use coastal marine waters as nursery areas (Lenanton and Potter 1987, Potter et al. 1990). Few marine species are regarded as *estuarine dependent* (Potter et al. 2015). The estuarine category comprises four guilds, *solely estuarine*, *estuarine and marine*, *estuarine and freshwater*, and *estuarine migrant* (Fig. 1.2) (Potter et al. 2015). *Solely estuarine* species obviously complete their entire life cycle within estuaries, whereas the *estuarine and marine* and *estuarine and freshwater* guilds have species whose populations complete their life cycles within an estuary but they may also have populations in marine or freshwaters, respectively (Potter et al. 2015). The final estuarine category, *estuarine migrants*, are species which spawn in estuaries where larvae may be flushed out of the estuary, but return at some other stage of their life history.

The fourth category includes diadromous species that migrate between the sea and freshwater. Five guilds have been recognised in this category including *anadromous* and *catadromous* guilds where most their life history is in the sea (*anadromous*) or freshwater (*catadromous*) and they migrate to the other environment for spawning (Fig. 1.2) (Potter et al. 2015). Both these guilds also have a *semi* guild where the spawning run is reduced. For example, the *semi-catadromous* spawning run would extend only to downstream estuarine areas and not into the marine environment (Potter et al. 2015). The final category, freshwater, contains two guilds. *Freshwater stragglers* are similar to marine stragglers but come from freshwater to the low salinity part of the estuary (Fig. 1.2) (Potter et al. 2015). The *freshwater estuarine-opportunist* is analogous to the marine estuarine-opportunist guild (Potter et al. 2015). Thus, estuaries are used by many species of fish representing different guilds (Elliott et al. 2007a).

Several guilds of fish use estuaries as nursery habitats and a number of studies have estimated the importance of different estuaries or areas within an estuary as nursery habitats (e.g. Gillanders 2002a, Reis-Santos et al. 2013b). Frequently, estuaries are cited as nursery habitats based on large numbers of juveniles found within estuarine habitats (Beck et al. 2001). However, Beck et al. (2001) suggests that a nursery habitat should comprise more than just increased abundances of juvenile fish. For example, there needed to be a greater contribution per unit area to the production of individuals that recruit to the adult population. The greater contribution to adult populations may arise as a combination of increased density and growth that leads to increased survival of juveniles and their recruitment to an adult habitat (Beck et al. 2001). Despite this attempt at formalising a definition to evaluate the nursery-role concept several latter studies have suggested alternate approaches (Dahlgren et al. 2006, Nagelkerken et al. 2015, Sheaves et al. 2015). Dahlgren et al. (2006) suggested that important nursery habitats may be missed by focusing on a per unit area basis. They suggested the term “effective juvenile habitat”, which would include habitats critical to sustaining the adult population by contributing a greater than average number of individuals, despite the size of the area (Dahlgren et al. 2006). Despite these attempts at formalising a definition for nursery habitats few

studies have estimated connectivity between estuaries, relative contributions among estuaries, and how this may change as fish age and among cohorts (i.e. through time).

A general criticism has been that earlier approaches viewed nurseries as static habitat units (Nagelkerken et al. 2015) or focused on the contribution made to adult fish stocks via ontogenetic migrations (Sheaves et al. 2015). Nagelkerken et al. (2015) indicate that identifying the relative contribution of juvenile habitats to the adult population is only part of the process of determining the importance of nursery habitats and that there should also be a focus on recognising the mosaic of potential contributing habitats and their linkages. Home range sizes, ontogenetic habitat shifts and primary migration routes across different spatiotemporal scales all need to be identified (Nagelkerken et al. 2015). Obtaining such information over a range of life history stages is difficult. Although I recognise that resource dynamics and ecological and ecophysiological factors (sensu Sheaves et al. 2015) are important in valuing nursery habitats, the focus of my study is on connectivity and population dynamics.

Even within guilds and species there can be variable use of estuaries. Partial migration, where populations comprise both resident and migratory individuals, has been found in all major vertebrate and some invertebrate groups, as well as in different ecosystems (Brodersen et al. 2008, Chapman et al. 2011a). Partial migration may influence population dynamics and the persistence of species largely through potential effects on predation and food availability. Flexibility in life history strategies related to partial migration may be a bet-hedging strategy to ensure survival of the species through both good and bad times. Fish may, for example, migrate to find increased food resources and maximise survival, as well as avoid adverse environmental conditions (Gillanders et al. 2015).

Knowledge of why partial migration occurs is scarce (Brodersen et al. 2014). Genetic and environmental factors have been considered with migration generally thought to be a result of the combined genotype and environment on individual migratory decisions (Brodersen et al. 2014). A genetic model describing whether individuals are migratory or resident has been proposed, namely the threshold model, which is viewed as an important starting point for evolutionary processes (Pulido 2011). The phenotypic and physiological characteristics associated with migration are considered threshold traits. How such traits are expressed depends on the environment, individual growth and development, and genetics (Kitano et al. 2012, Pearse et al. 2014).

Determining population structure and connectivity

Most fish species have a dispersive larval stage prior to settling to benthic habitats where they spend variable amounts of time. Understanding connectivity of fish between larval and juvenile, or juvenile and adult habitats is important for assessing population structure (Brazner et al. 2004, Kerr et al. 2009). Connectivity is defined as the exchange/movement of fish among areas. Generally, populations have previously been considered predominantly open (Caley et al. 1996, Cowen et al.

2000), but more recent research has suggested that populations may be more closed than initially thought (Jones et al. 1999, Cowen et al. 2000, Almany et al. 2007).

Identifying and tracking the natal origins of fish and movements between juvenile and adult populations aids in determining how closed populations may be. Conventional tagging methods, for example, external tags, and some internal tags have been used to estimate movement and connectivity among environments (for a review of methods see Gillanders et al. 2003). These methods are used to determine the proportion of the fish population that has moved from the point of tagging to the point of recapture (e.g. Gillanders et al. 2001). However, gathering such data is challenging due to the difficulties with marking large numbers of larvae or juveniles in order to obtain reasonable recapture rates due to high mortality rates at early life history stages. A further challenge is the small size of early life history stages that are not amenable to traditional marking methods. As a result the degree of connectivity between juvenile and adult fish populations is still relatively unknown in many areas and for many species (Gillanders 2002a). Moreover, information on the range of movements throughout their life histories or environments that fish have passed through between tagging and recapture is often not available. Thus, conventional tagging methods generally do not provide detailed information on fish movement and connectivity among different populations.

Acoustic tags, radio tags and associated satellite telemetry (e.g. pop up satellite telemetry), which rely on tags emitting signals, are widely used in studies of adult fish and have been used to investigate movement. For example, movements of bluefin tuna (*Thunnus thynnus*) based on data from pop-up satellite archival tags revealed complex population structure (Cermeno et al. 2015). Similarly, implantable archival tags in bluefin tuna across multiple years demonstrated repeatable patterns of aggregation areas, changing with environmental conditions (Walli et al. 2009). While these tags provide much useful information, they are generally still too large to use on juvenile estuarine fish. In addition, satellite telemetry has limited application to benthic fish species that do not surface, making the recovery of tag information difficult. Further approaches are therefore required. One such approach may be the use of otolith chemistry.

Otolith chemistry for determining population structure and movements of fish

Otoliths are part of the auditory-equilibrium organ (inner ear) found in the cranium of teleost fish (Popper and Lu 2000). The inner ear is comprised of three pairs of otoliths, including the sagitta, lapillus and asteriscus. The sagitta is the largest in most species and is the most commonly used in otolith chemistry studies (Campana 1999, Elsdon et al. 2008). Calcium carbonate (approximately 99% aragonite) is the major component of the otolith, with 1% organic and other elements being incorporated via the endolymph of the inner ear. Calcium carbonate is deposited incrementally and incorporated onto the growing surface of the otolith on daily and annual time scales. The newly deposited carbonate contains a suite of environmentally and dietary derived chemicals (i.e. minor and

trace elements and isotopes) (Campana 1999, Elsdon et al. 2008). Because the structural and chemical composition of otoliths does not alter after deposition, and the otolith grows through an individual fish's life time, it is able to record the entire growth and chemical life history of a fish (Campana 1999). However, as well as the physiology of the fish, abiotic factors (e.g. temperature, salinity, ambient elemental concentrations in the water) can affect the elemental composition of otoliths (Elsdon et al. 2008). Several studies suggest that the dominant contribution of elements to otoliths is from the water, rather than from the diet (Walther and Thorrold 2006, Webb et al. 2012, Doubleday et al. 2013). As there is evidence that environmental factors influence otolith chemistry, over the past few decades, otolith chemistry has been used to understand life history of fish including population structure (e.g. Fowler et al. 2005, Steer et al. 2009), connectivity among populations (e.g. Thorrold et al. 2001, Gillanders 2002a, Reis-Santos et al. 2013b) and environmental conditions that fish have experienced (e.g. Shima and Swearer 2009, Gillanders and Munro 2012).

Generally, a combination of elements expressed as ratios to calcium (Ca) (e.g. Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca) have been used as elemental signatures or tags. These elemental tags can vary significantly through time and among habitats (e.g. Thorrold et al. 1998a, Swearer et al. 2003, Reis-Santos et al. 2012). It is therefore important to know how elemental tags change through time (e.g. among years). If significant differences occur through time then it may be necessary to establish a spatially and temporally explicit reference set of elemental tags in order to track individuals back to their respective cohorts when assessing connectivity.

Otolith chemistry can also be used to determine partial migration within a species. Profiles of Ba:Ca concentrations in the otoliths of estuarine black bream have been used to classify fish as migratory or resident (Elsdon and Gillanders 2005, Gillanders et al. 2015). First, a relationship between otolith Ba:Ca and salinity was determined; this relationship was then used to infer fresh or saltwater habitat use of fish. Migratory patterns typical of resident fish, and migrants with either irregular or cyclical patterns of movement were found (Elsdon and Gillanders 2005). Thus, otolith chemistry also allows the assessment of whether different contingents (migratory vs resident for example) occur within an estuary.

Objectives

The overall aim of this study was to assess connectivity and partial migration within black bream (*Acanthopagrus butcheri*) populations in South Australia. The key objectives were to:

- (i) Determine spatial and temporal variation in otolith elemental signatures to assess their usefulness for determining connectivity of black bream populations;
- (ii) Retrospectively trace subadult fish from the Fleurieu Peninsula and Kangaroo Island, South Australia, to their juvenile regions to gain an understanding of movements between areas; and

- (iii) Determine the proportion of migrant and resident life history types within estuarine populations of black bream on Kangaroo Island and if these different contingents reflect hybrid fish.

Notes on chapter style

Chapters 2 to 4 of this thesis are written in a style suitable for publication in scientific journals. As such, they present original data and can be read independently. I have attempted to maintain a logical flow of ideas throughout the thesis, but there may be some repetition across chapters. Tables and figures are embedded within each chapter, and references are collated and listed at the end of the thesis. A brief overview of Chapters 2 to 5 is provided below.

Chapter 2

Black bream recruit to estuaries, but little is known of their movements among estuaries or whether subadults return to the estuaries they spent their juvenile lives in. Prior to assessing connectivity of fish among estuaries or between estuaries and coastal habitats using otolith chemistry it is necessary to determine the temporal and spatial scale at which there may be variation in elemental chemistry of otoliths. We used otolith chemistry of juvenile fish to determine differences among estuaries and groups of estuaries and how this varied across a five year period. These elemental tags can then be used to assess connectivity among estuaries or between estuaries and open coastal populations.

Chapter 3

An understanding of recruitment and connectivity patterns of estuarine fish is important for informing management and conservation efforts. In this chapter, I use the juvenile otolith elemental tags determined in Chapter 2 as baseline data. To ensure good classification success I focus on two broad regions, Fleurieu Peninsula and Kangaroo Island estuaries. I then sample 1 to 3⁺ year old subadult fish and retrospectively classify them to their juvenile regions. These data are used to determine whether fish move between these two broad regions and how this may change through time for different cohorts of fish and within a cohort as the fish ages. I also determine the degree to which fish remain in the same broad region as they originally recruited to as juveniles as an estimate of self-recruitment. An understanding of broader scale movements is also obtained.

Chapter 4

Many fish populations have coexisting migratory and resident life history types, which are often described as contingents (Secor 1999). The phenomenon is more broadly described as partial migration. Partial migration has previously been described for black bream in South Australian estuaries (Elsdon and Gillanders 2005, Gillanders et al. 2015). Here, I was interested in assessing population or estuary level partial migration patterns and determining whether the migratory

contingent may predominantly comprise a hybrid black bream-yellowfin bream complex. Otolith Ba:Ca profiles across otoliths were used to assign contingents (resident vs migrant) and to determine the proportion of time fish spent in freshwater, estuarine and marine environments.

Chapter 5

A brief General Discussion of the preceding chapters, how they are integrated and directions for future research are provided in this final chapter.

Below I provide information on the study area and species.

Study area – Fleurieu Peninsula and Kangaroo Island

Kangaroo Island is Australia's third largest island, spanning 145 km from east to west and around 55 km from north to south (Fig. 1.3). The island forms a natural protective barrier for the Gulf of St Vincent from the Southern Ocean. The closest point to mainland Australia is Cape Jervis on the Fleurieu Peninsula where the island is separated by Backstairs Passage, a 14 km wide strait (Fig. 1.3). Isolated from the mainland by post-glacial sea level increases approximately 9500 years ago (Hope et al. 1977), the island is presently bounded along the north west and north east coasts by 200 to 300 m vertical cliffs of tectonically elevated, igneous and metamorphosed indurate rock (Hope et al. 1977). Typical of an insular island, Kangaroo Island has a cool climate, discrete winter rainfall, and mild conditions experienced during both summer and winter (Tyler et al. 1979).

European settlement has had an impact on the region's natural environment, particularly over the last 50 years where large changes have occurred through the clearing of tracts of land for agricultural use (Baxter 1995). This combined with the introduction of fertilizers and increased runoff, has had a detrimental impact on the natural streams and in particular, on the fish species (Hammer 2004). Streams on Kangaroo Island are characterized by numerous shallow lagoons and inland sinks caused by old sand dune build-ups, which have progressively formed barriers to freshwater flow to the sea through time (Hope et al. 1977). Flow regimes at the mouths of these streams along the coast are strongly dominated by tide and wave action.

Characteristics of Kangaroo Island and Southern Fleurieu estuaries

Most of the estuaries on Southern Fleurieu and Kangaroo Island are wave-dominated coastal creeks (Figs 1.4, 1.5, Table 1.1) (Gillanders et al. 2008), which become closed to the sea through the formation of sand bars at their mouths. Such isolation occurring either seasonally or for prolonged periods can make some areas of the estuary hypersaline through evaporation during summer months (Heap et al. 2004).

The sand or barrier bars which periodically close off these streams are a consequence of erosion of the surrounding coast line where eroded sediments are transported by longshore currents and redeposited into bays and stream mouths by wave action. Prolonged or partial closure of the estuaries by barrier sands is often accompanied by reduction of freshwater flows during summer months and leads to variations in salinity of the water within (Gillanders et al. 2008). Consequently, the lower reaches of streams are usually saline for part of the year (summer) and subsequently flushed by freshwater discharges that break through or clear the barriers during winter.

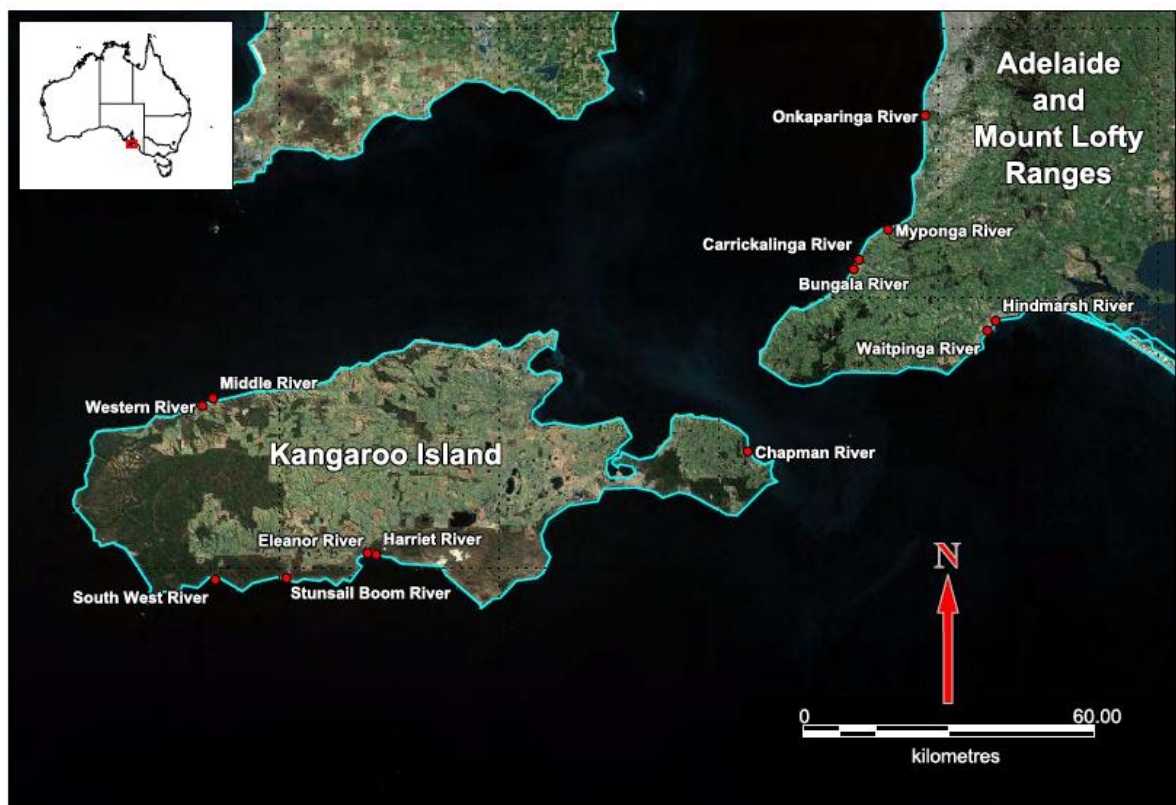


Figure 1.3. Map of estuaries on the Fleurieu Peninsula and Kangaroo Island. The inset panel shows the sampling region in relation to mainland Australia.



Figure 1.4. Estuaries of Kangaroo Island and the Fleurieu Peninsula. (a) Onkaparinga River, (b) Western River, (c) South West River, (d) Eleanor River, (e) Middle River, (f) Hindmarsh River, (g) Chapman River and (h) Western River.



Figure 1.5. Estuaries of Kangaroo Island and the Fleurieu Peninsula. (a) Western River, (b) Eleanor River, (c) Harriet River, (d) Chapman River, (e) Harriet River and (f) Bungala River.

Table 1.1. Summary of the estuaries on Kangaroo Island and the southern Fleurieu Peninsula, South Australia sampled for the study. Shown are estuaries and broad regions (Fleurieu Peninsula, FP; Kangaroo Island, KI), size, key habitats, condition/surrounding land use and type of estuary. Some estuary information is taken from Gillanders et al. (2008). Information was also obtained from Ozcoasts: http://www.ozcoasts.gov.au/search_data/estuary_search.jsp

Estuary	Region	Size	Habitats	Condition/ land use	Type
Bungala Creek	FP	Estuary 0.66 km long, 10-30 m wide	Sand/silt, limited seagrass	Residential, recreational park, light industrial, agriculture: grazing	Enters ocean between sand dunes to beach
Carrickalinga Creek	FP	Estuary 0.39 km long, 5-30 m wide	Vegetation close to edge, limited seagrass	Residential, recreational park, agriculture: grazing	Surrounded by sand dunes undergoing revegetation
Chapman River	KI	Estuary: 2.4 km long (including lagoon, 3.94 km long), water area: 0.96 km ² (includes lagoon), estuary width 0.59 km, perimeter 15.52 km	Intertidal flats, seagrass, saltmarsh, paperbark swamp, sand/silt	Largely unmodified; agriculture, conservation	Wave-dominated, coastal creek & Lashmar lagoon
Eleanor River	KI	River: ~3.14 km Estuary: 0.01 km ²	Seagrass, sand/silt	Largely unmodified, agriculture, native vegetation	Wave dominated, coastal creek
Harriet River	KI	River: ~17.94 km Estuary: 0.01 km ²	Vegetation close to edge, limited seagrass, intertidal flats	Largely unmodified, agriculture, native vegetation	Wave dominated, coastal creek
Hindmarsh	FP	River: 22.9 km long; estuary length 2.27 km;	Limited seagrass,	Modified, agriculture: grazing, residential	River-dominated; wave-dominated delta

River		entrance width 0.31 km, water area 0.24 km ² , perimeter 4.99 km	sand/silt		
Middle River	KI	Estuary: 2.48 km long, 10-40 m wide	Seagrass vegetation up to water edge, sand/silt	Largely unmodified, agriculture: grazing, some conservation areas	Wave-dominated coastal creek; significant damming in upper catchment
Myponga River	FP	Estuary 1.22 km long 10-30 m wide, perimeter 2.66 km, water area 0.12 km ²	Sand/silt decomposing seagrass	Extensively modified (reservoir with concrete dam); agricultural, residential	Wave-dominated, coastal creek
Onkaparinga River	FP	Estuary 11.02 km long, 5- 60 m wide, perimeter 22.87 km, water area 1.14 km ²	Saltmarsh, mangrove intertidal flats, sand/silt, seagrass	Extensively modified, agriculture, residential, recreation park	River-dominated, Wave-dominated delta
South West River	KI	Estuary length 0.24 km, entrance width 0.11 km, perimeter 0.75 km	Rocky reef, intertidal flats, seagrass, sand/ silt	Largely unmodified, agriculture, mostly native vegetation and conservation area	Wave dominated, coastal creek
Stunsail Boom River	KI	Estuary 0.30 km ² , river ~4.49 km	Intertidal flats, saltmarsh, tidal sand banks, rocky reef, seagrass	Largely unmodified, agriculture: grazing, mostly native vegetation and conservation area	Wave dominated, coastal creek
Western River	KI	River: 7.9 km long, estuary: 0.95 km long, 1-10m wide	Seagrass, saltmarsh, vegetation up to waters edge, sand/silt	Largely unmodified; agriculture: grazing, conservation area	Wave-dominated, coastal creek
Waitpinga River	FP	Estuary 0.01 km ² , river ~1.55 km	Seagrass, sand/silt	Largely unmodified, agriculture, coastal vegetation and conservation area	Wave dominated, coastal creek

Fishes of Kangaroo Island

The coastal and estuarine waters of Kangaroo Island provide important habitat for a range of species of fish, many of recreational and commercial significance (Rowntree 2004, Gillanders et al. 2008). One of the key species found within estuaries is black bream, *Acanthopagrus butcheri* (Munro 1949).

Study species – *Acanthopagrus butcheri*

Species of the family Sparidae are distributed through the Atlantic, Indian and Pacific Oceans, and are generally found throughout tropical waters in areas where there is an extensive shallow continental shelf (Nelson 2006, Gomon et al. 2008). Generally, sparids are slow growing, long-lived (Buxton 1993), and in some waters form the basis of large commercial and recreational fisheries (Gomon et al. 2008). Ten sparids from six genera occur in Australian waters; five species belong to the genus *Acanthopagrus* and one species to each of five other genera (Bray et al. 2012).

Black bream (*Acanthopagrus butcheri*) are distributed in temperate waters along most of the southern coast of Australia (including Tasmania), where they are relatively abundant in the brackish waters of estuaries and rivers (Fig. 1.6) (Norriss et al. 2002). They are found southwards from Shark Bay in Western Australia continuing eastwards along the southern coast of Australia to Myall Lake in New South Wales (Gomon et al. 2008); their distribution is not continuous across this range. Populations of black bream in South Australia are found from the Victorian border to Port Lincoln and around Flinders and Kangaroo Islands (Munro 1949). Within South Australian waters black bream are most common in the Coorong Lagoon, and in estuaries of the Gulf St Vincent and Kangaroo Island (Norriss et al. 2002, Elsdon and Gillanders 2005, Gillanders et al. 2008).

Black bream is a slow growing and long-lived fish (up to 30 years), and can reach around 40 cm in length at 20 years of age (Morison et al. 1998, Norriss et al. 2002). Males reach maturity at around 17 cm and females at around 15 cm (Norriss et al. 2002). Hybridisation of black bream with yellowfin bream (*Acanthopagrus australis*) has occurred where the two species overlap in southern New South Wales (Norriss et al. 2002, Roberts et al. 2009), but hybrids are not known to occur in South Australia (Fig. 1.6).

Black bream is one of the most sought after recreational species in southern Australia, and supports commercial fisheries in Victoria, Western Australia and South Australia (Lenanton and Potter 1987, Ferguson and Ye 2008). Although black bream are an important recreational fishery within South Australia (Ferguson and Ye 2008), they are not usually targeted (Jones 2009).

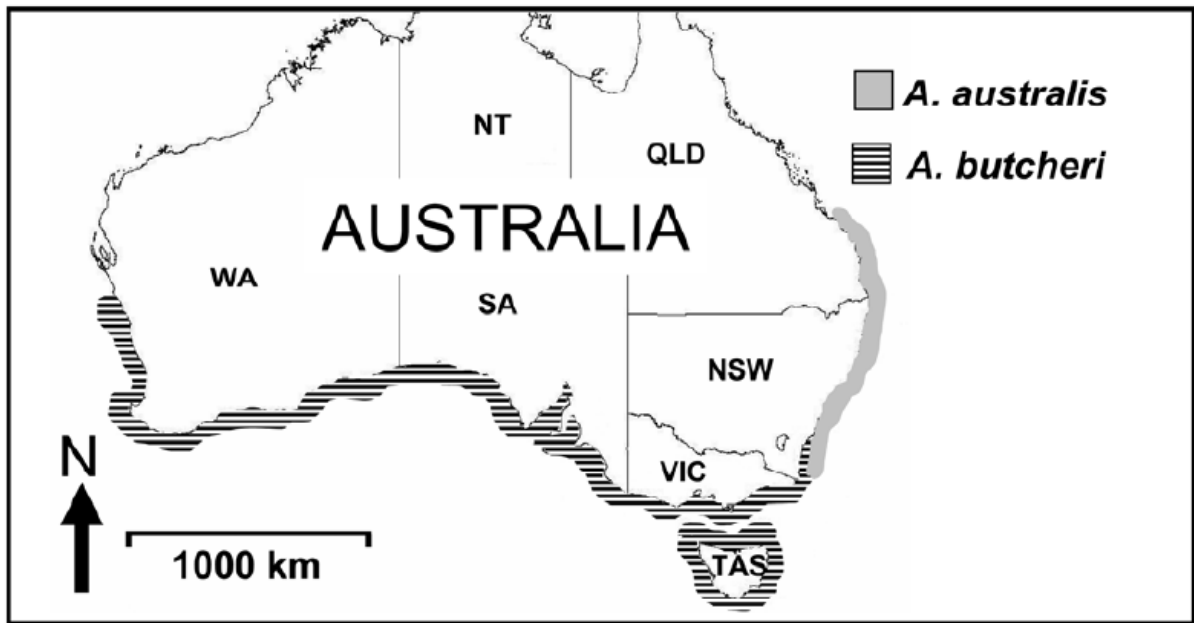


Figure 1.6. The distribution of black bream (*Acanthopagrus butcheri*) and yellowfin bream (*Acanthopagrus australis*) in Australian waters. From Roberts (2010).



Carrickalinga Estuary

Chapter 2 – Statement of authorship

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Principal Author

Name of Principal Author (Candidate)	Ana Judith Giraldo		
Contribution to the Paper	In this chapter, Ana Judith Giraldo collected the specimens, performed the otolith chemistry analyses, analysed the data and wrote the manuscript.		
Overall percentage (%)	80%		
Signature		Date	14 July 2015

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

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Travis Elsdon		
Contribution to the Paper	Travis Elsdon collected samples from two of the 5 years prior to me commencing my PhD, had input in the intellectual development of the research, assisted with chemical analysis methods and provided suggestions, comments and feedback on the manuscript. The research was funded via an ARC Linkage project grant to Bronwyn Gillanders and Travis Elsdon.		
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Name of Co-Author	Bronwyn Gillanders		
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Contribution to the Paper	Bronwyn Gillanders assisted with intellectual development and provided suggestions, comments and feedback on manuscript drafts. The research was funded via an ARC Linkage project grant to Bronwyn Gillanders and Travis Elsdon.		
Signature		Date	10 July 2015

Chapter 2. Temporal and spatial variability in otolith elemental composition: a precursor for determining population connectivity

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Abstract

The elemental concentration of otoliths provides a unique opportunity to discriminate among fish that live in different habitats, and to estimate population structure. We examined spatial and temporal patterns in otolith chemistry of juvenile 0⁺ black bream (*Acanthopagrus butcheri*), to discriminate stocks of fish living in different estuaries. Fish were sampled in up to 12 estuaries of Kangaroo Island (7 estuaries) and the Fleurieu Peninsula (5 estuaries) each year over a 5 year period (2007–2011). Otoliths were examined to determine if individual estuaries or groups of estuaries differed in chemical concentrations (tags), and to examine inter-annual variation in chemical tags. Tags differed among individual estuaries and in some cases adjacent estuaries shared similar chemical tags. Differences in chemical tags were detected among all estuaries in two of the year comparisons. Similarities in otolith tags were detected for five of the seven estuaries in 2010 and 2011. Grouping estuaries with similar chemical tags enhanced the classification accuracy of fish to estuaries and therefore the ability to discriminate stock structure based on otolith chemistry. Temporal differences in chemical tags were detected among years for several estuaries; however, there were no clear trends in the differences between years. The results highlight that black bream from different estuaries or groups of estuaries have unique chemical tags that can be used to trace cohorts of fish. Such differences can be used to estimate connectivity, population movements, and the function of estuaries as nursery areas for this species in subsequent years.

Introduction

Description of a species population structure is a central goal in ecology and underpins sustainable management and conservation (Hixon et al. 2002). Understanding the population structure of fish is also necessary to determine connectivity among habitats and areas (Hanski 1999, Cowen et al. 2000). It was originally thought that populations of fish were predominantly open and highly connected (Caley et al. 1996, Jones et al. 1999, Cowen et al. 2000). Fish within individual habitats and estuaries are now viewed as having populations comprised of both open and closed life histories (Swearer et al. 2002, Jones et al. 2005, Almany et al. 2009), with some estuaries supplying a larger proportion of recruits than others and therefore subsidising populations (Gillanders 2002a).

Deciphering links between populations of juvenile and adult fish requires accurate knowledge of the stock structure of juvenile fish within habitats, such as estuaries. Juvenile fish are inherently difficult to mark using conventional tags, and therefore research has focused on the use of natural tags, such as chemicals in otoliths, for estimating population structure (Campana 2005, Gillanders 2005b). Otoliths are paired calcium carbonate (CaCO_3) structures within the inner ear of fish, primarily used for hearing and balance (Popper and Lu 2000). The ability of otoliths to indicate fish stock structure and connectivity among estuaries is based on their accretion of new carbonate and protein material onto the outside surface on a daily basis (Campana 1999). Incorporated within the otolith material are elements and isotopes from the surrounding water (and to a lesser extent food, Webb et al. 2012) that are preserved and not subject to metabolic absorption, hence otoliths accurately record a chemical chronology over the fish's life time. When fish living in different environments incorporate different chemical concentrations, those chemicals can act as estuary-specific tags (e.g. Gillanders and Kingsford 2002, Reis-Santos et al. 2012). Therefore, the chemicals in otoliths can be used to assess the stock structure of fish, and the function of estuaries as nursery areas for species.

If all sub-populations of fish have the same chemical tag, then otolith chemistry is inappropriate to determine stock structure and connectivity (Elsdon et al. 2008). If baseline chemical tags are specific to individual estuaries, fish stocks can be described at scales that may closely match management of fish within different areas and habitats (Jones et al. 2005). Where several estuaries have fish with similar tags, fish from those estuaries can be considered indistinguishable and fish stocks ascribed to groups of estuaries rather than individual estuaries (Gillanders 2002a, Elsdon et al. 2008). Doing so requires knowledge of the spatial and temporal extent of different tags within populations, and a way to group fish from estuaries that have similar chemical tags.

Spatial and temporal variation in otolith chemical tags has been investigated in a number of papers (e.g. Schaffler et al. 2009, Tanner et al. 2011, Reis-Santos et al. 2012). Published studies of spatial and temporal variation in otolith chemistry primarily aims to establish baselines of chemical data

for either stock discrimination (Patterson et al. 2004, Dorval et al. 2005) or further connectivity research (Thorrold et al. 2001, Rooker et al. 2003). Spatial differences in chemical tags have been assessed on scales of metres and km (e.g. Gillanders and Kingsford 2000, Ruttenberg et al. 2008) to thousands of km (e.g. Secor and Zdanowicz 1998, Thorrold et al. 1998a, Walther and Thorrold 2008). Variation in chemical tags appears, not surprisingly, to be system dependent, and is likely influenced by the environment, physicochemical processes, and potentially sampling or analytical differences among studies. The degree of temporal stability in otolith chemical tags has been assessed within years, such as seasonal trends (Hamer et al. 2003), and across multiple years (e.g. Gillanders 2002b, Reis-Santos et al. 2012). Rarely has yearly variation in chemical tags been minimal enough to suggest that individual estuaries consistently produce fish with reliable and predictable chemical tags. The complexities of spatial and temporal variation in tags suggest a general model for predicting variation may not exist. However, based on geological and climatic influences on water chemistry (Barnett-Johnson et al. 2008, Walther and Thorrold 2009, Reis-Santos et al. 2013a), it may be expected that fish from closer watersheds would possess tags that are more similar than those further apart.

The objective of this study was to examine the spatial and temporal stability of chemical tags in juvenile black bream (*Acanthopagrus butcheri*, Munro 1948) within estuaries. If estuaries have reliably different chemical tags over multiple years, they may be used as baselines for estimating connectivity and self-recruitment. Specifically, we compared chemical tags among fish collected from 12 estuaries to establish spatial differences. These chemical tags were compared among young-of-year (0^+) fish over five consecutive years to determine the long-term stability of tags. We also tested whether grouping estuaries with similar chemical tags would affect the classification accuracies of those groups and minimise misclassifications. These tags can then be used to assess connectivity among populations (Chapter 4).

Materials and Methods

Sample collection

Young-of-year (0^+) black bream, *Acanthopagrus butcheri*, were collected from 12 estuaries on the Fleurieu Peninsula and Kangaroo Island (South Australia) to determine spatial and temporal trends in otolith chemistry (Fig. 2.1). Each estuary was sampled throughout the study using a 5 or 20-m seine net, during the Austral summer (November–March) of five consecutive years (2007–2011 inclusive). Collection was done along the entire length of estuaries, and estuaries were not subsampled at multiple sites to assess within estuary variation (e.g. Gillanders 2005b) because they were generally small in length (2.5 ± 1.5 km, mean \pm SEM; range 0.39–11.02 km; see also Table 1.1) and approximately 5–30 m in width. While all estuaries were sampled annually, fish were not

always found in each estuary (Table 2.1). Fish were handled in accordance with the Adelaide University ethics permits in the field and return to the laboratory where all fish were measured for standard length (SL, mm) and weight (W, g) (Table 2.2).

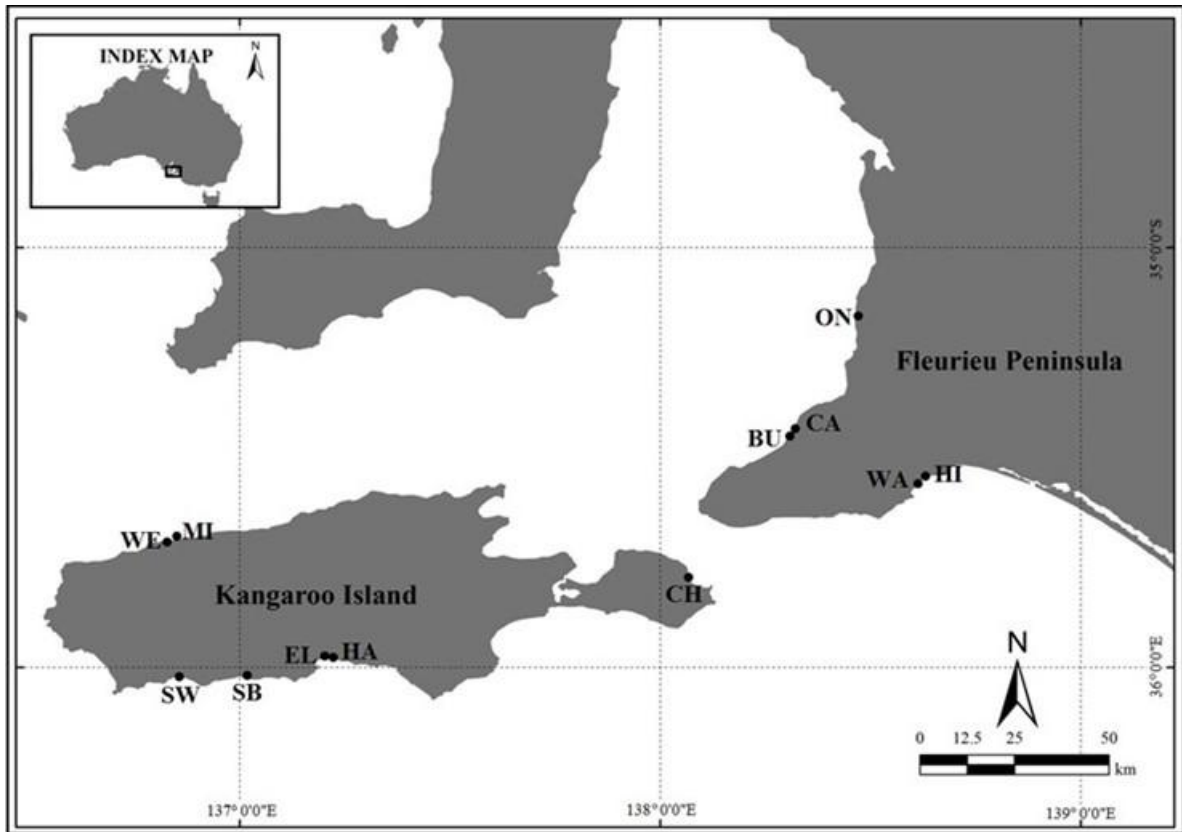


Figure 2.1. Map of the sampling locations for South Australian *Acanthopagrus butcheri* on the Fleurieu Peninsula and Kangaroo Island. The inset panel shows the sampling region in relation to mainland Australia. Estuary name codes are: ON - Onkaparinga River; CA - Carrickalinga River; BU - Bungala River; HI - Hindmarsh River; WA - Waitpinga River; MI - Middle River; WE - Western River; SW - South West River; SB - Stunsail Boom River; EL - Eleanor River; HA - Harriet River; CH - Chapman River.

- See Table 1.1 for particular information of each catchment.

Table 2.1. Sample sizes of fish obtained from each estuary for each year of sampling. Note that Stunsail Boom was not sampled during 2007 and 2008. NF indicates that the estuary was sampled, but no fish were obtained. NS indicates that the estuary was not sampled in that year.

Estuary	Code	2007	2008	2009	2010	2011
Bungala	BU	15	15	20	10	2
Carrickalinga	CA	NF	13	21	14	NF
Chapman	CH	15	15	20	9	23
Eleanor	EL	15	15	21	3	8
Harriet	HA	15	15	13	5	NF
Hindmarsh	HI	NF	15	20	17	7
Middle	MI	15	15	9	22	NF
Onkaparinga	ON	NF	15	19	7	22
South West	SW	15	15	21	1	NF
Stunsail Boom	SB	NS	NS	24	12	4
Waitpinga	WA	15	13	22	NF	6
Western	WE	15	15	24	11	16

Otolith preparation

The sagittal otoliths were removed from fish, cleaned of adhering material with ultrapure water, and allowed to air dry in microcentrifuge tubes. One otolith from each fish was embedded in EpoFix resin (Struers) that had been spiked with indium (In) at 30 ppm to allow discrimination between otolith material and resin during analysis (Elsdon and Gillanders 2002, Munro et al. 2008). Otoliths were sectioned transversely through the centre section (core) using a low-speed saw (Buehler Isomet). Sections were polished to 250 to 300 μm using lapping film and mounted onto glass microscope slides with indium-spiked thermoplastic glue (CrystalBond 509). Slides were cleaned in an ultrasonic cleaner for five minutes to remove surface impurities, dried in a laminar flow cabinet for 24-h and stored individually in sealed plastic bags until analysis.

Otolith chemical analysis

Concentrations of trace elements in otoliths were determined using a New Wave UP-214 high-performance (Nd:YAG) ultraviolet laser connected to an Agilent 7500 cs inductively coupled plasma-mass spectrometer (ICP-MS). The laser operating conditions were: frequency, 5Hz;

ablation spot size, 30µm; laser power, 75%; beam energy, ~0.08-0.12 mJ; carrier gas, Ar (0.87 L min⁻¹). The operating conditions of the ICP-MS were: optional gas, He (57.5%); dwell time, ⁴³Ca (50 ms), ⁸⁸Sr, ¹³⁸Ba, ²⁴Mg, ⁵⁵Mn, ⁷Li and ⁶⁶Zn (200 ms) and ¹¹⁵In (100 ms). Ablations occurred inside a sealed chamber with the sample gas extracted to the ICP-MS via a smoothing manifold in the presence of argon and helium gas. The chamber was purged after each ablation to remove background gas from previous ablations. Background concentrations of elements within the chamber were measured for 25-s before each ablation to allow for correction of sample concentrations.

Otoliths were analysed using a 30 µm spot on the outside edge to quantify chemical concentration in material laid down shortly before capture, which was representative of the early juvenile part of the life history. Otoliths were analysed in a number of sampling sessions; within each session, otoliths were randomised. A reference standard (National Institute of Standards and Technology, NIST612) was analysed after every 12 otolith ablations to correct for machine drift (Ludden et al. 1995). Detection limits were calculated as 3 standard deviations above the blank background counts, and were < 0.0005 mol·mol⁻¹ for Sr, Ba, Mg, Mn, Li and Zn and < 0.004 mol·mol⁻¹ for Ca. All otolith values were above detection limits for the five years, except for Zn that was below detection in some years. Analytical accuracy determined from the concentrations of the NIST standards and averaged across all samples was 100% for Ca and Sr; 101% for Ba, Mg, Mn and Li, and 103% for Zn (>100% indicates recovery is greater than expected) . Background counts were subtracted from sample counts, standardised to NIST 612 and converted to concentrations (ppm) using Glitter software (<http://www.glitter-gemoc.com>). These data were exported to Excel and converted to molar concentrations and expressed as ratios to ⁴³Ca to account for fluctuations in the ablation yield (Ludden et al. 1995).

Statistical analyses

Otolith chemical tags were compared among estuaries and years using statistical techniques including Permutational MANOVA (PERMANOVA), and Canonical Analysis of Principal Coordinates (CAP) (Anderson 2001, Anderson and Willis 2003). Data were log (*x*+1) transformed before Euclidean distance matrices were obtained. Permutations were done on the raw data with 4999 permutations per test. *Post-hoc* comparisons involving pair-wise tests were used to determine which estuaries or years differed. Similarity percentage analysis (SIMPER) was used to determine the percent contribution of each element to the separation between estuaries and years.

Canonical analysis of principal coordinates was used to determine the success of allocating fish to their capture locations. This procedure uses a jackknife or ‘leave one out’ approach. We expected 1/*N* estuaries, where *N* is the number of groups or estuaries, to be successfully allocated by chance. Estuaries were subsequently grouped if *post-hoc* PERMANOVA pairwise tests suggested that they

showed no significant differences or where CAP allocations suggested a number of fish from both estuaries were incorrectly allocated. Subsequent CAP analyses only used groupings of estuaries if the percent correctly allocated increased by an amount greater than that expected by chance alone given the reduction in number of groups.

Results

Fish were collected from between eight and 12 estuaries each year. Only four estuaries had fish collected in all five years (Bungala, Chapman, Eleanor, and Western) with most other estuaries having fish collected for four of the five years (Table 2.1). Despite extensive sampling within all estuaries, in some years sample sizes were low. Only 2 fish were collected from Myponga estuary in a single year, therefore it was not considered further. Juvenile 0⁺ black bream varied in standard length from 20 to 121 mm (Table 2.2). Although fish were collected at similar times each year, differences in length of fish were found among estuaries and years (Table 2.3).

Table 2.2. Mean standard length (SL ± SE, mm) for fish sampled from each year and estuary for *Acanthopagrus butcheri*. Refer to Table 2.1 for sample sizes. NF indicates that the estuary was sampled, but no fish were obtained. NS indicates that the estuary was not sampled in that year.

Estuary	2007	2008	2009	2010	2011
Bungala	53.89 ± 0.82	41.61 ± 1.10	35.26 ± 1.14	41.85 ± 1.78	57.73 ± 1.56
Carrickalinga	NF	61.11 ± 7.20	61.67 ± 3.26	44.54 ± 1.15	NF
Chapman	60.47 ± 0.85	44.35 ± 2.57	26.68 ± 16.62	32.78 ± 2.74	41.20 ± 1.46
Eleanor	55.18 ± 1.40	49.58 ± 1.39	50.81 ± 2.45	40.15 ± 3.62	37.42 ± 3.05
Harriet	69.82 ± 4.09	72.68 ± 3.75	67.96 ± 4.08	55.52 ± 3.06	NF
Hindmarsh	NF	39.26 ± 0.97	39.97 ± 2.03	46.42 ± 1.75	34.58 ± 1.05
Middle	55.71 ± 1.76	52.25 ± 1.68	50.82 ± 1.43	47.02 ± 1.65	NF
Onkaparinga	NF	49.91 ± 1.37	54.54 ± 1.93	37.62 ± 3.58	41.42 ± 1.75
South West	49.38 ± 2.66	65.44 ± 2.19	74.30 ± 2.20	57.19 ± 3.03	NF
Stunsail Boom	NS	NS	57.33 ± 3.00	55.88 ± 2.53	56.81 ± 0.63
Waitpinga	55.19 ± 0.90	46.51 ± 1.00	58.18 ± 2.06	NF	44.11 ± 0.92
Western	63.30 ± 1.52	74.29 ± 4.25	60.83 ± 1.91	40.68 ± 1.69	40.89 ± 1.41

Table 2.3. Permutational ANOVAs comparing fish standard length among years (Temporal tests) and among estuaries (Spatial tests). Data were $\log(x + 1)$ transformed.

Source	<i>df</i>	MS	<i>P</i>
Temporal tests			
Among years			
Year	4	0.596	< 0.001
Estuary	11	0.836	< 0.001
Y × E	34	0.328	< 0.001
Residual	664		
Spatial tests			
2007			
Estuary	7	0.160	< 0.001
Residual	112		
2008			
Estuary	10	0.639	< 0.001
Residual	150		
2009			
Estuary	11	1.280	< 0.001
Residual	222		
2010			
Estuary	10	0.150	< 0.001
Residual	100		
2011			
Estuary	7	0.120	< 0.001
Residual	80		

Temporal differences

Temporal variation in elemental tags was compared over 5 years for all the estuaries. A significant estuary \times year interaction was found for the multielement otolith signature, as well as all element:Ca ratios, suggesting that although there was spatial and temporal variation, it was not consistent among estuaries or years (Fig. 2.2, Table 2.4). Comparisons of the multielement signature between years for each estuary found that two between year comparisons differed for all estuaries (2007 differed from 2010, and 2009 differed from 2011). Otolith tags were similar between 2010 and 2011 for five of the seven estuaries (exceptions: Bungala, which also had a low sample size in 2011, and Chapman). All other between year comparisons found that 1 to 2 estuaries did not vary between years. Middle River estuary showed variation among all years, whereas the Hindmarsh River estuary only varied for three of the five between year comparisons. Fish were not found in this estuary each year; hence only 6 of 10 comparisons were possible. These results suggest that an overall elemental signature cannot be used for each estuary therefore further spatial comparisons focus on individual years.

Between year differences in otolith composition were largely due to differences in Mn:Ca and Zn:Ca, although Mg:Ca, Li:Ca and Ba:Ca were sometimes important (SIMPER). Mn:Ca contributed between 40 and 59% and Zn:Ca between 11 and 22% of the differences. The contribution of Mg:Ca was more variable (0-22%), as was that of Li:Ca (0-14%) and Ba:Ca (0-10%).

Spatial differences

Multielemental signatures of otoliths varied among most estuaries in all years (2007-2011; Table 2.4). Significant spatial differences were also found in the element:Ca ratios for all elements and years, with the exception of Zn:Ca in three years (2007, 2009 and 2011) and Mg:Ca in 2011 (Fig. 2.2, Table 2.4).

In 2007, 0⁺ black bream were collected from 8 of 12 estuaries. Of those 8 estuaries, all showed some overlap in elemental signatures with at least one other estuary (Fig. 2.3, Table 2.4). Overall, allocation success from the CAP analysis was 66%, but ranged from 47% (South West) to 93% (Waitpinga). Grouping of estuaries that showed similar elemental signatures based either on PERMANOVA post-hoc pairwise analyses or samples misallocated to another estuary from the CAP analysis increased the allocation success from 66 to 79% (when Middle and Western, and Southwest and Harriett were grouped) and 83% (when all four estuaries were pooled). The change in percent of fish correctly classified when the four estuaries were pooled was not significantly greater than that expected by chance alone given the decrease in number of groups (i.e. a change

from 6 to 4 groups would expect an improvement of greater than 8%). Waitpinga was distinguished from other estuaries along CAP axis 1 based on increased concentrations of Ba:Ca and Mn:Ca, and decreased concentrations of Li:Ca (Fig. 2.3). CAP axis 2 distinguished estuaries with increased concentrations of Sr:Ca from those with decreased concentrations of Li:Ca (Fig. 2.3).

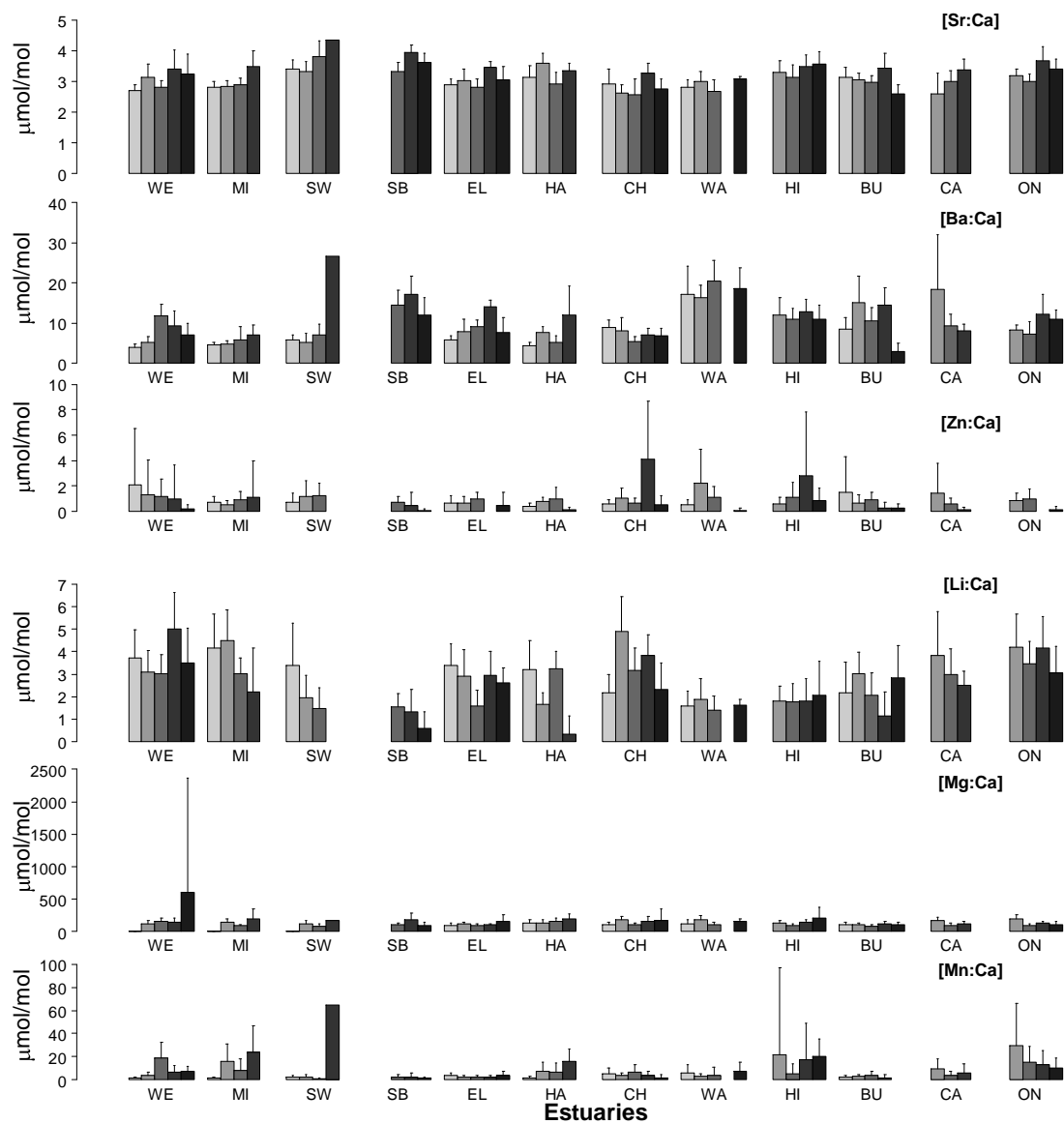


Figure 2.2. Mean (\pm SE) element:Ca ratios (Sr, Ba, Zn, Li, Mg, Mg) of juvenile *Acanthopagrus butcheri* collected in 5 years (2007 \square , 2008 \square , 2009 \blacksquare , 2010 \blacksquare , 2011 \blacksquare) from twelve estuaries of the Fleurieu Peninsula and Kangaroo Island, South Australia. The estuaries and their codes were: Western (WE), Middle (MI), South West (SW), Stunsail Boom (SB), Eleanor (EL), Harriet (HA), Chapman (CH), Waitpinga (WA), Hindmarsh (HI), Bungala (BU), Carrickalinga (CA) and Onkaparinga (ON).

Fish were collected from all 12 estuaries sampled in 2008. Elemental signatures were significantly different among estuaries, with four estuaries different to all others (Onkaparinga, Carrickalinga, Chapman and Middle) based on differences between group centroids (Fig. 2.3, Table 2.4). Fish were correctly allocated to the estuaries that they were collected from 56% of the time, which was significantly greater than by chance (8%), but considered too low for reallocation of unknown samples since almost half of all samples would be incorrectly allocated. There was a significant improvement in allocation success when several of the estuaries were grouped (an increase from 56 to 73% correctly allocated), although the individual estuary allocation success varied from 46 to 93%. Fish were separated along axis 1 by high levels of Ba:Ca in otoliths (negative values on CAP axis 1), whereas along axis 2 fish had high Sr:Ca (positive values on CAP axis 2) versus high Li:Ca and Mg:Ca (negative values on CAP axis 2) (Fig. 2.3).

In 2009, fish were again collected from all 12 estuaries, although sample sizes were low ($n=9$) in the Middle estuary. Significant differences among estuaries were found in the elemental signature, with the Western and the South West being significantly different from all other estuaries (Fig. 2.3, Table 2.4). Most other estuaries were similar to 2 to 3 other estuaries, with the exception of Hindmarsh which was similar to five other estuaries and Onkaparinga which had a similar elemental signature to the Middle. Fish were correctly allocated to their estuary of origin only 56% of the time, with only 10% correctly allocated for Hindmarsh, but greater than 80% for several estuaries. The percent correctly allocated increased to 76% when several estuaries were grouped (range 65-88% depending on estuary). Increased Ba:Ca ratios correlated with negative values along CAP axis 1 (Fig. 2.3). Increased Sr:Ca ratios correlated with positive values along CAP axis 2, whereas increased Li:Ca, Mn:Ca and Mg:Ca correlated with negative values along CAP axis 2 (Fig. 2.3).

In 2010 and 2011, fewer black bream were caught and a number of estuaries either had no fish (e.g. Waitpinga in 2010; Carrickalinga, Harriet, Middle and South West in 2011) or small numbers (Table 2.1). Canonical analyses of principal coordinates only used estuaries with greater than 10 fish. In 2010, the elemental signature of fish varied among estuaries (Fig. 2.3, Table 2.4). Most estuaries had distinct elemental signatures (exceptions: Carrickalinga and Western; Bungala and Stunsail Boom). Despite this, there was relatively poor allocation success (52%, range 30-64%). Grouping of the estuaries that overlapped in elemental signatures significantly improved the allocation success (from 52% to 66%). When the Middle estuary was combined with Carrickalinga and Western estuaries, the allocation success further increased to 78% (range 52-89% depending on estuary). Increased concentrations of Ba:Ca, and to a lesser extent Sr:Ca, correlated with negative scores along CAP axis 1 (Fig. 2.3). Negative scores along CAP axis 2 correlated with

increased concentrations of Li:Ca, whereas positive scores correlated with increased concentrations of Mn:Ca, Mg:Ca and Zn:Ca.

All three estuaries in which greater than 10 fish were collected in 2011 were significantly different to each other (Fig. 2.3, Table 2.4), 79% of fish were correctly allocated to the estuary that they were collected from. Along the CAP axis 1 increased concentrations of Zn:Ca correlated with positive scores and separated Chapman from other estuaries, whereas increased concentrations of Mn:Ca, Sr:Ca and Ba:Ca correlated with negative scores and separated Onkaparinga from the other estuaries (Fig. 2.3). CAP axis 2 separated fish with increased concentrations of Mg:Ca (positive scores) from those with increased concentrations of Ba:Ca (negative scores) (Fig. 2.3).

Table 2.4. Permutational MANOVA and ANOVAs comparing otolith elemental tags among years (Temporal tests) and among estuaries (Spatial test) for the multielement and individual element data. Data were log (x +1) transformed.

Source	<i>df</i>	Multi		Li:Ca		Mg:Ca		Mn:Ca		Zn:Ca		Sr:Ca		Ba:Ca	
		MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>	MS	<i>P</i>
Temporal tests															
Among years															
Year	4	14.044	<0.001	1.401	<0.001	2.669	<0.001	4.959	<0.001	2.446	<0.001	0.190	<0.001	2.379	<0.001
Estuary	11	16.996	<0.001	2.967	<0.001	0.635	0.003	7.990	<0.001	0.600	0.001	0.134	<0.001	4.669	<0.001
Y × E	34	6.813	<0.001	0.720	<0.001	0.504	0.002	4.340	<0.001	0.514	<0.001	0.026	0.001	0.710	<0.001
Residual	664	1.154		0.116		0.154		0.617		0.176		0.008		0.083	

Table2.4
continued

Spatial tests

2007 juveniles

Estuary	7	5.846	<0.001	0.887	<0.001	0.315	0.009	1.830	<0.001	0.267	0.138	0.052	<0.001	2.495	<0.001
Residual	112	0.759		0.104		0.111		0.324		0.171		0.005		0.044	

2008 juveniles

Estuary	10	11.327	<0.001	1.203	<0.001	0.644	<0.001	6.945	<0.001	0.343	0.017	0.084	<0.001	2.108	<0.001
Residual	150	1.098		0.078		0.100		0.632		0.156		0.011		0.121	

2009 juveniles

Estuary	11	13.624	<0.001	1.277	<0.001	0.966	<0.001	8.412	<0.001	0.148	0.249	0.131	<0.001	2.692	<0.001
Residual	222	0.923		0.076		0.090		0.568		0.116		0.007		0.007	

2010 juveniles

Estuary	10	10.589	<0.001	1.877	<0.001	0.311	0.043	5.994	<0.001	1.418	0.002	0.020	0.013	0.969	<0.001
Residual	100	1.916		0.235		0.150		1.049		0.396		0.008		0.077	

2011 juveniles

Estuary	7	8.474	<0.001	0.674	0.002	0.827	0.141	5.461	<0.001	0.223	0.058	0.067	<0.001	1.222	<0.001
Residual	80	1.499		0.166		0.501		0.595		0.110		0.009		0.119	

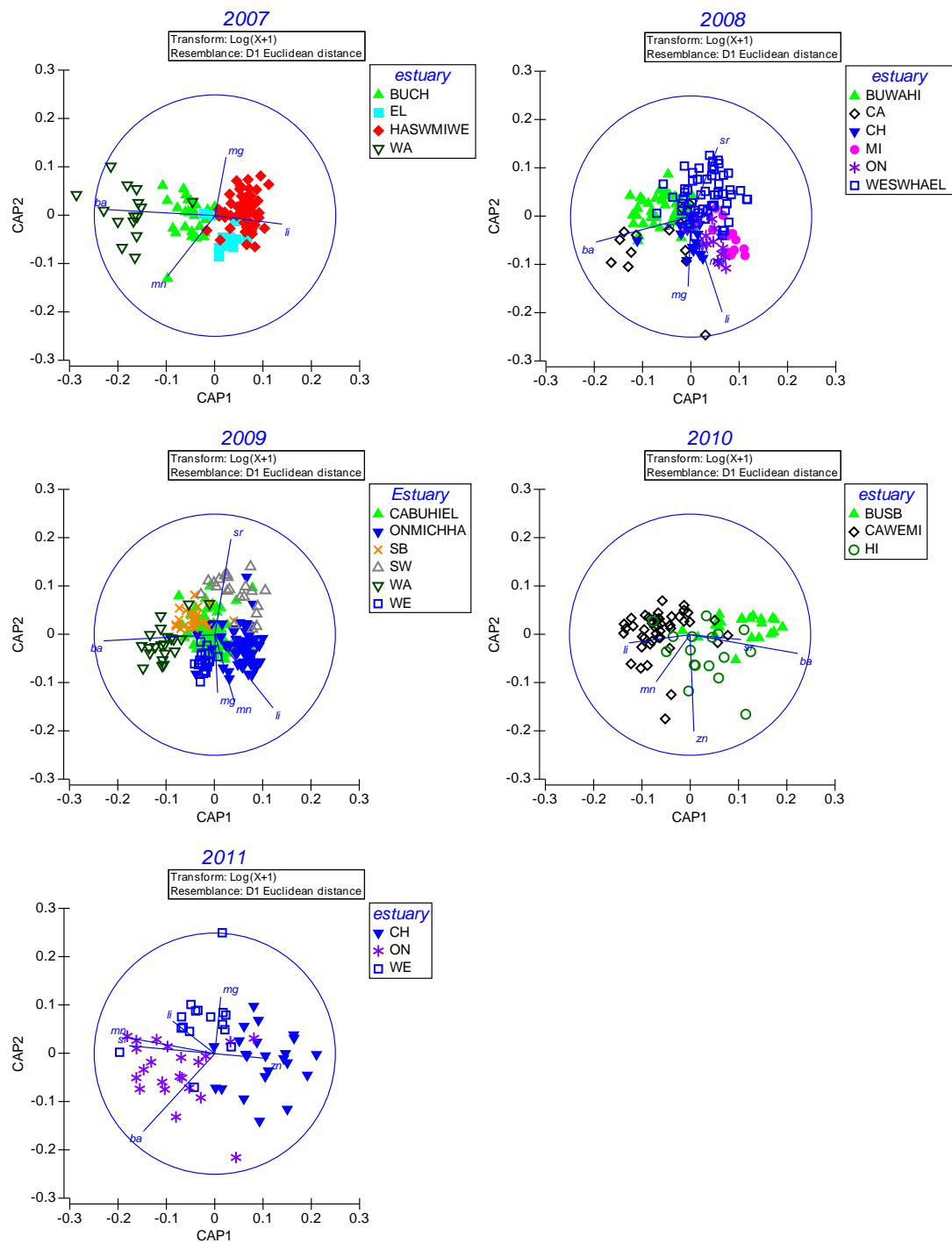


Figure 2.3. Canonical analysis of principal coordinates (CAP) plots showing spatial differences in Multielemental otolith tags of juvenile (0^+) black bream collected from estuaries on the Fleurieu Peninsula and Kangaroo Island, South Australia during 5 years of sampling. Codes represent estuaries that were grouped together for classification purposes. For example, in 2007, BUCH represents Bungala and Chapman, HASWMIWE represents Harriett, Southwest, Middle and Western estuaries. See Table 2.1 for all estuary codes.

Discussion

Results show that black bream inhabiting different estuaries, or groups of estuaries, have different chemical tags. Tags differed among years for individual estuaries, which highlights the importance in obtaining chemical tags from individual cohorts if tags of juveniles are to be used for connectivity estimates. The distinct chemical tags of black bream suggested that ecological processes and water chemistry associated with estuaries altered chemical properties of otoliths. Indeed, chemical tags of black bream are known to be affected by temperature, salinity, and ambient water chemistry (Elsdon and Gillanders 2004), some or all of which differ among the estuaries sampled (Elsdon et al. 2008).

Black bream are an ideal species for estimating connectivity among estuaries. Adults spawn in estuaries and eggs hatch after 48-h, with larvae settling within the same estuary (Chaplin et al. 1998). In South Australia, juvenile black bream spend summer within estuaries closed off by sandbars. In winter, when water levels rise, the sandbars erode (Gillanders et al. 2008) and black bream may move from the estuary into the marine environment. The fish return to estuaries in subsequent years to spawn. The unique natural chemical tags of black bream caught in estuaries can be used to describe the mixing of fish in coastal waters and their return to estuaries. Thus, otolith chemistry of black bream can address questions on population dynamics, connectivity and nursery role of estuaries for this species (Beck et al. 2001).

Temporal and spatial variation in otolith chemistry

A significant interaction was found for the multielement otolith signature, suggesting temporal and spatial differences; however, this variation was not consistent among estuaries or years. Temporal trends of fish elemental tags were compared among twelve estuaries over 5 consecutive years (2007-2011) and inter-annual differences in individual element concentrations and the multielement tags were detected. The temporal differences in tags among years suggests that baseline tags need to be established for individual years so that fish older than 1 year can be traced back accurately (Campana 2005, Elsdon et al. 2008). This is particularly important for connectivity studies that relate chemical tags of adults back to putative juvenile habitats (Thorrold et al. 1998a).

Temporal dynamics of otolith chemistry have been investigated both within a single year and among years (see Gillanders 2002b, for a review). Within year variation, i.e. seasonal differences, would be anticipated given that changes in precipitation and environmental processes would affect chemical concentrations in estuaries. It may be expected that individual estuaries would have similar chemical concentrations over consecutive years, however, this may depend on variation in environmental processes, such as river flows. The temporal variation detected in the

current study was found during a period of extreme drought in southern Australia in 2007 and 2008 with some winter rains in 2009. During this time there was a reduced impact of land-use on estuarine chemistry and environmental properties (Elsdon et al. 2009). Given that otolith chemistry is likely to reflect environmental change, temporal differences in otolith chemistry among years were not anticipated to be large. Indeed the elements that varied the most among years do not appear to be those influenced by land-processes (i.e. Sr), but instead those under control of physiology or redox conditions (e.g. Mn, Zn).

Studies investigating temporal differences in otolith chemistry among years have reported significant differences for Ba and Mn (Elsdon and Gillanders 2006b, Schaffler and Winkelman 2008, Reis-Santos et al. 2012). The regulation of Ba within otoliths is largely due to ambient chemistry (Elsdon and Gillanders 2004, de Vries et al. 2005) and ambient concentrations are related to ecosystem processes such as salinity (Dorval et al. 2005, Elsdon and Gillanders 2005), freshwater flows that are high in particulate matter (Li and Chan 1979, Gergel et al. 1999), and upwelling (Elsdon et al. 2008). It is therefore logical that estuaries that experience differences in flow and Ba inputs over time, such as those in the current study, would also display differences in otolith Ba from fish collected in different years. Other elements are likely to differ over time due to desorption from sediments. Differences in Mn among years most likely reflected changes in sediment oxidation and reduction potentials, which can alter the portions of Mn held as Mn oxides (MnO_2) versus Mn^{2+} (Du Laing et al. 2009). Changes in redox conditions affect ambient water pH and a decrease in pH can cause the desorption of other elements, such as Zn, Cd, Cu, Pb, Ni, and Fe (Salomons et al. 1987, Du Laing et al. 2009). In the current study, Mn and Zn were both dynamic over time and among estuaries, and changes in metal behaviour and bioavailability with redox potentials in estuaries may explain the dynamic patterns detected in fish otoliths. Conversely, Sr, which is not greatly affected by redox potentials because it is primarily controlled by geological inputs (Palmer and Edmond 1992, Basu et al. 2001, Limburg 2004) and somewhat by fish physiology (Gallahar and Kingsford 1992) appeared more stable over years within the individual estuaries sampled.

Spatial differences in chemical tags were detected among groups of estuaries over distances of 100s km, such as differences between Waitpinga and Bungala on the Fleurieu Peninsula (FP), and between Onkaparinga (FP) and South West on Kangaroo Island. Not all estuaries differed in their tags, and these were either adjacent estuaries or estuaries that share adjacent watersheds with common geology (e.g. north vs southward flowing estuaries of Kangaroo Island). Fish could be classified to individual estuaries with average accuracies of 73% (2008) to 79% (2007 and 2011) based on six element to calcium ratios, and grouping similar estuaries (see *Grouping of estuaries and applications for describing stocks*). The results indicate that black bream from different

estuaries can be discriminated and their estuary of origin determined. In some cases, however, only groups of estuaries could be discriminated.

Spatial and temporal variation in otolith chemistry can alter the scales at which stock structure and connectivity to later cohorts are described. When individual estuaries have different tags, fine scale description of stocks, and subsequently connectivity, can be estimated. However, if groups of estuaries have similar chemical tags, then descriptions of stocks have to be related to those groups (Elsdon et al. 2008). Previous research has shown chemical tags in fish otoliths vary both within and among estuaries (Gillanders 2005a, Tanner et al. 2012) with differences detected on a spatial scale as small as a 100 meters (Gillanders 2002b) and up to 1000s of km (Gillanders and Kingsford 2003, Walther and Thorrold 2008). The degree of spatial structure in chemical tags is likely to be influenced by fish movement as well as variability in environmental parameters, such as water chemistry, temperature, and salinity (Elsdon and Gillanders 2004). In the current study, the estuaries were small and the potential for mixing of waters and fish movement within estuaries were too great to warrant within estuary investigations of otolith tags. The similarity of some adjacent estuaries, also suggests that similar environmental processes may affect estuaries that were geographically close, a pattern previously detected in water quality of a subset of the examined estuaries (Elsdon et al. 2009).

Grouping of estuaries and applications for describing stocks

Determining stock structure of fish using otolith elemental tags has become an important tool in fish ecology, and is a precursor to describing connectivity among juvenile and adult populations. The approach can be used when conventional tagging is not applicable (i.e. in young-of-year fish). However, questions remain on how to deal with estuaries that have similar chemical tags, in terms of classifying and describing stock structure. The current study highlights how grouping estuaries that have fish with similar chemical tags can increase classification accuracies and better describe the stock structure of juvenile fish using otolith chemistry. Such groupings would lead to more accurate descriptions of connectivity based on otolith chemistry, but reduced spatial resolution (Elsdon et al. 2008).

The chemical grouping of estuaries increased average classification success compared to ungrouped estuaries, and improved classification of estuaries that shared similar chemical tags. In all years, except 2011 when estuaries were not grouped, the classification accuracies were greatly improved by grouping estuaries. This is best seen in 2010, when overall classification accuracy increased from 52 to 78%. Logically, classifying a set number of fish to fewer groups could increase the classification accuracy of those groups, but this remains a balance between improvements in classification success and improvements through chance alone. This may be

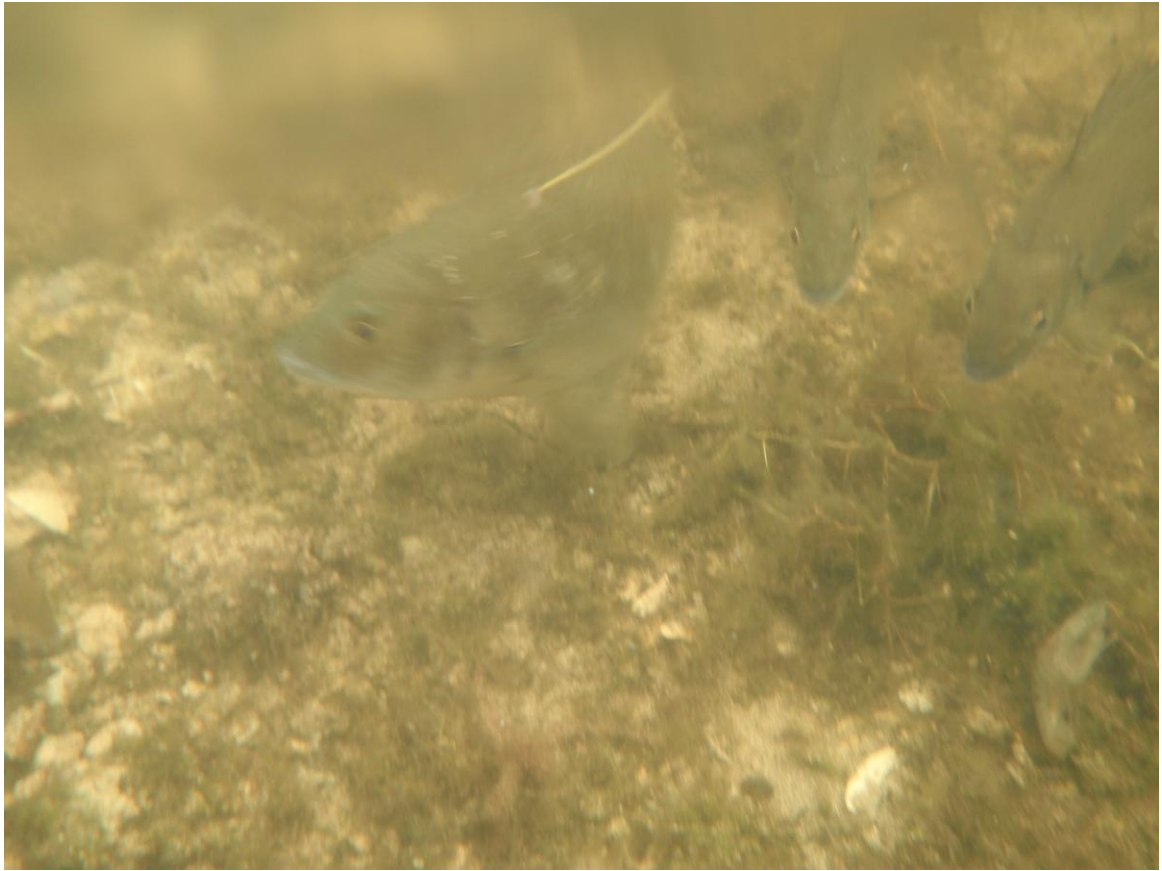
particularly relevant when groupings are based on geographical proximity, such as species with continuous distribution (Clarke et al. 2009). The data suggested that the grouping of estuaries with similar chemical tags would increase confidence in correctly assigning fish to estuaries or areas, and reduce the chance of misclassifying fish and inaccurately describing connectivity of older fish.

Estuaries with similar fish chemical tags were often geographically close to one another, and share adjacent watersheds, but this was not always the case. Bungala and Hindmarsh had similar chemical tags in fish for both 2008 and 2009, and these estuaries flow from glacial and fluvio-glacial deposits on either side of the Fleurieu Peninsula, whereas the adjacent Carrickalinga estuary (to Bungala) drains watersheds dominated by quartz and siltstones. Estuaries from non-adjacent areas of Kangaroo Island also had similar tags. South West and Harriet were more similar than the other estuaries, but differed substantially from Stunsail Boom, despite the Stunsail Boom watershed lying between these two estuaries. The watersheds of Eleanor and Harriet have deposits of tertiary sandstone along the estuary, but have less calcarenite and calcareous aeolianite compared to the South West. In both of the described examples, geology rather than geographical closeness may have influenced chemical tags of fish (Barnett-Johnson et al. 2008), and therefore the ability to discriminate among fish from different estuaries. The pooling of areas due to geographic proximity has been done previously (Clarke et al. 2009). However, where differences in chemical tags do not align with geographic proximity of estuaries, other estuary groupings may need to be considered.

Few studies have used statistical techniques to formally group estuaries with similar chemical tags (Schaffler et al. 2009) and assessed the effect of grouping estuaries on classification success. This study has shown how, using techniques to group estuaries that contain fish with similar chemical tags can strengthen interpretations and reduce errors in classification of stocks as well as future connectivity and recruitment estimates. Approaches for determining differences among estuaries in chemical tags can include statistical tests (such as MANOVA), classification statistics (Discriminant Function Analysis; DFA or quadratic DFA), and clustering techniques (Clarke et al. 2009). These statistics (with the exception of clustering) are widely used among studies examining spatial and temporal variation in elemental tags (e.g. Thorrold et al. 1998b, Gillanders and Kingsford 2003, Rooker et al. 2003, Schaffler et al. 2009).

Conclusion

This study highlights spatial and temporal patterns in otolith chemistry of juvenile black bream, an estuarine species and the application of appropriately grouping fish from estuaries with similar tags to enhance stock interpretations. Knowledge of spatial and temporal grouping of juvenile black bream within estuaries allows these cohorts to be tracked when the fish leave the estuaries and return in future years. Therefore, natural chemical tags in fish otoliths can be used to describe the mixing and movements of black bream among estuaries and to determine population dynamics and nursery areas for this species.



Black bream in its natural environment

Chapter 3 – Statement of authorship

Title of Paper	Temporal patterns of connectivity between juvenile and subadult areas inferred from otolith elemental fingerprints
Publication Status	<input type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication <input type="checkbox"/> Submitted for Publication <input checked="" type="checkbox"/> Publication Style
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Principal Author

Name of Principal Author (Candidate)	Ana Judith Giraldo		
Contribution to the Paper	In this chapter, Ana Judith Giraldo collected the specimens, performed the otolith chemistry analyses, analysed the data and wrote the manuscript		
Overall percentage (%)	80%		
Signature		Date	14 July 2015

Co-Author Contributions

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

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Christopher Izzo		
Contribution to the Paper	Christopher Izzo assisted with intellectual development, had input in the development of the chemical analysis protocols and provided suggestions, comments and feedback on manuscript drafts.		
Signature		Date	10 July 2015

Name of Co-Author	Bronwyn Gillanders		
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Contribution to the Paper	Bronwyn Gillanders assisted with intellectual development and provided suggestions, comments and feedback on manuscript drafts. The research was funded via an ARC Linkage project grant to Bronwyn Gillanders and Travis Elsdon.		
Signature		Date	10 July 2015

Chapter 3. Temporal patterns of connectivity between juvenile and subadult areas inferred from otolith elemental fingerprints

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Abstract

Understanding temporal patterns of recruitment and connectivity of estuarine fish populations is fundamental to elucidating population dynamics and informing effective management and conservation efforts. Estuarine associated species utilise sheltered habitats for growth and feeding. Estuaries are also subject to fluctuating environmental conditions, and as such, estuaries become temporally variable sinks or sources of juvenile recruits. Otolith chemistry provides a tool to reconstruct origins and to quantify connectivity between juvenile and adult components of fish populations. This study used laser ablation (LA) inductively coupled plasma-mass spectrometry (ICP-MS) to quantify the trace element composition of black bream (*Acanthopagrus butcheri*) otoliths collected from 12 estuaries on Kangaroo Island (KI) and Fleurieu Peninsula (FP) in South Australia, during the Austral summer of 2007 to 2010 (Chapter 2). Differences in otolith element concentration of young-of-year black bream were found among broad areas representing estuaries from each of KI and FP. Overall classification success to the two broad regions for these juvenile cohorts ranged from 70 to 84% depending on the region and year. Through LA-ICP-MS analysis of the juvenile region of subadult (1 to 3 year old) black bream, we were able to estimate the proportion of juvenile fish that recruited from these two regions through time as well as temporal patterns of connectivity between KI and FP. Our results suggest variability among cohorts of the same age, among age classes within a cohort and between regions in terms of self-recruitment. These results highlight the variable nature of connectivity among populations and recruitment to adult populations suggesting that conservation efforts will be more complex than protecting a single region.

Introduction

For many species of marine teleosts, estuarine ecosystems provide vital nursery habitats for the juvenile stage (Beck et al. 2001, Able 2005). Juvenile fish may utilise nursery habitats for extended periods before moving to adjacent estuarine or coastal areas. Some estuaries are likely to act as sources or sinks of juveniles to the adult population (McNeill et al. 1992), with some estuaries contributing significantly more juveniles to the next generation than others (Gillanders 2002a, Reis-Santos et al. 2013b). This is likely due to different estuaries or regions providing different levels of refuge and food, enhancing the growth and survival of the juvenile cohort (Nicholson et al. 2008). Identifying those source estuaries or regions of juveniles to the adult population and considering connectivity (interchange of individuals) among source and sink regions is fundamental to developing sustainable management strategies for estuarine dependent fish, as well as identifying ecologically important habitats (Gillanders 2002a). Temporal fluctuations in local environmental conditions (e.g. ambient temperature and salinity, freshwater inflows) may result in inter-annual variation in the proportion of recruits that are contributed to the population and the relative importance of estuaries as juvenile habitats (Fodrie and Levin 2008, Reis-Santos et al. 2012, Reis-Santos et al. 2013a). Therefore, it is also important to assess changes in recruitment and connectivity through time to better understand the relative importance of individual estuaries and, or regions to the long-term replenishment of populations.

Otolith chemistry provides a quantitative tool for identifying the natal and, or juvenile regions of fish and assessing the contribution of natal and, or juvenile sites to the adult population (Gillanders 2002a). Otoliths are metabolically inert calcium carbonate structures that develop continuously throughout the lives of fish (Campana 1999, Elsdon et al. 2008). Incremental layers of calcium carbonate are deposited on daily and annual time scales, incorporating primarily environmentally derived elements as they accrete (Campana 1999, Campana and Thorrold 2001). Analysing portions of the otolith that correspond to specific life history stages (i.e. the otolith core) can be used to delineate larval and, or juvenile origins of fish (Thorrold et al. 1998b, Walther and Thorrold 2010, Tanner et al. 2012) and to assess connectivity between putative juvenile regions and the adult population (Gillanders 2005b, Chittaro et al. 2009, Hamer et al. 2011).

Multielement otolith signatures have been used to differentiate young-of-year (0^+ year old) black bream (*Acanthopagrus butcheri*) from different estuaries in South Australia (Chapter 2), suggesting that individual juvenile and adult fish may be assigned to a particular recruitment area based on the analysis of the juvenile portion of the otolith (Gillanders and Kingsford 2000, Gillanders 2002a). Black bream are an endemic southern Australian sparid that primarily inhabits estuaries and coastal lakes (Hindell et al. 2008). In the Austral spring/summer mature ($> 3+$ years

of age) black bream can make excursions into low salinity and into fresh water waters to spawn, with juveniles recruiting to estuarine nursery habitats (Sarre and Potter 1999). Recruitment of juvenile black bream into estuarine nurseries varies temporally and spatially (Morison et al. 1998, Nicholson et al. 2008, Jenkins et al. 2010), with year class strength linked to ambient water temperature and salinities (Partridge and Jenkins 2002, Hassell et al. 2008). Given that black bream are an important recreationally and commercially targeted species throughout their range (Norris et al. 2002, Ferguson et al. 2013), there is a need to identify nursery regions that act as recruitment sources or sinks, facilitating quantification of connectivity between the juvenile and adult component of the population.

Naturally occurring temporal variations in local environmental conditions, which influences black bream recruitment (Nicholson et al. 2008), also alter water chemistry and in turn otolith chemistry (Elsdon and Gillanders 2006b), such that significant inter-annual differences in otolith signatures exist within individual estuaries (Chapter 2). Indeed, there are few examples of consistent otolith element signatures for single locations through time (Gillanders 2002b, Elsdon and Gillanders 2006b, Reis-Santos et al. 2012). Significant inter-annual variation of multielement signatures necessitates that the retrospective assignment of juveniles and adults be undertaken on an annual basis (Tanner et al. 2013). This study aimed to assess temporal and age-related patterns of recruitment of black bream in South Australia based on the quantification of multielement otolith signatures. By determining the juvenile sources of black bream recruits, this study was also able to assess patterns of inter-annual and age-related connectivity within South Australia.

Materials and methods

Sample collection

Black bream were collected from 12 estuaries on Kangaroo Island (KI) and the Fleurieu Peninsula (FP) during the Austral summers (November to March) of 2007 to 2010 (Fig. 3.1, Table 3.1). Where possible, samples of 15 to 20 juvenile black bream (< 136 mm standard length, SL) were caught using either 5- or 20-m seine nets. These estuaries were targeted as they are known to support high abundances of young-of-year black bream (Elsdon and Gillanders 2005); however, not all the estuaries had juvenile fish at the time of sampling. Upon capture, all fish were handled in accordance with Adelaide University ethics permits in the field and returned to the laboratory. In the laboratory SL (range: 44.2–136 mm; average \pm standard error: 72.86 ± 3.53 mm) and body weights (BW, g) (range: 5–24 g; average \pm standard error: 4.65 ± 2.26 g) were recorded for each fish. In addition, the paired sagittal otoliths were removed for analyses.

Subadults (136–170 mm SL, 1-3⁺ years of age) were also collected by beach seine, as well as by line-baited hook, between 2008 and 2011 to match cohorts for which estuarine signatures were available (i.e. recruits collected between 2007 and 2010) (Table 3.1). They were treated similar to juvenile black bream.

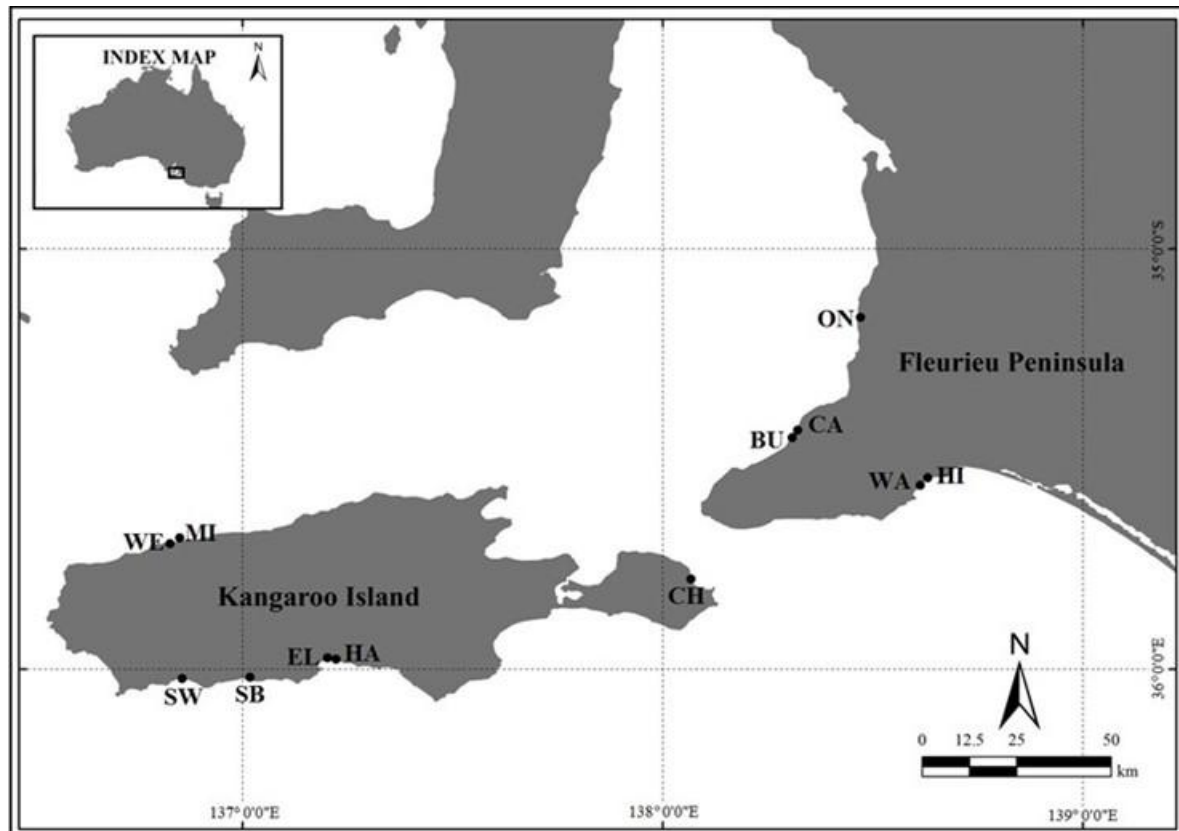


Figure 3.1. Sampling locations for *Acanthopagrus butcheri* on the Fleurieu Peninsula and Kangaroo Island in South Australia. The inset map shows the sampling locations in relation to mainland Australia. Individual estuary codes are: ON - Onkaparinga River; CA - Carrickalinga River; BU - Bungala River; HI - Hindmarsh River; WA - Waitpinga River; MI - Middle River; WE - Western River; SW - South West River; SB – Stunsail Boom River; EL - Eleanor River; CH - Chapman River.

Otolith preparation

Sagittal otoliths were rinsed in ultrapure water to remove any adhering tissue and air dried in microcentrifuge tubes. One otolith from each fish was embedded in clear setting resin spiked with indium (In) to allow discrimination between otolith material and resin during later chemical analysis (Munro et al. 2008). Transverse sections (approximately 300 µm thick) were made through the otolith using a low-speed saw (Buehler). Sections were polished using progressively

finer grades of lapping film and mounted onto glass microscope slides with In-spiked thermoplastic glue. Slides were ultrasonically cleaned in ultrapure water and dried in a laminar flow cabinet, before being stored individually in sealed plastic bags.

Age estimation and assignment of annual recruitment cohort

Estimation of fish age was done by counting growth increments in sectioned otoliths under a stereomicroscope at $\times 10$ -magnification with transmitted light. Annual increment periodicity has been validated in black bream (Morison et al. 1998), and annuli were defined as paired opaque and translucent bands. Based on estimated ages, an individual's year of birth was calculated relative to the year of capture (Sarre and Potter 2000), and fish were assigned to one of four recruitment cohort years (2007–2010) (Table 3.1). In total, 662 young-of-year fish were identified (Table 3.1), and used as baseline data for elemental analysis. Fish aged 1-3+ were then used to retrospectively trace their juvenile region of origin. All fish 4+ and older were discarded as they did not match a baseline dataset for juveniles.

Multielemental otolith analyses

Otolith element concentrations were quantified using a New Wave UP-214 high-performance (Nd:YAG) ultraviolet laser connected to an Agilent 7500cs Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). The laser operating conditions were: frequency, 5Hz; ablation spot size, 30 μ m; laser power, 75%; beam energy, ~ 0.08 -0.12 mJ; carrier gas, Ar (0.87 L min⁻¹). The operating conditions of the ICP-MS were: optional gas, He (57.5%); dwell time, ⁴³Ca (50 ms), ⁸⁸Sr, ¹³⁸Ba, ²⁴Mg, ⁵⁵Mn, (200 ms) and ¹¹⁵In (100 ms). Ablations occurred inside a sealed chamber with the sample gas extracted to the ICP-MS via a smoothing manifold in the presence of argon and helium gas. Background concentrations of elements within the chamber were measured for 25-s before each ablation to allow for correction of sample concentrations.

Table 3.1. Number of fish sampled from four cohorts of *Acanthopagrus butcheri* from Kangaroo Island (KI) and the Fleurieu Peninsula (FP). Numbers in parentheses indicate the years that the cohorts and age classes represent. Samples from unknown (Ukn) estuaries/regions indicate the numbers of black bream that fell outside of the bounds of the 95% confidence ellipses of the PCAs that represents a combined KI and FP juvenile multi-element otolith signature (refer to Fig. 3.2).

	Cohort 1 (2007)				Cohort 2 (2008)				Cohort 3 (2009)			Cohort 4 (2010)		
	Age classes (sampling years)													
Region	0 ⁺ yrs	1 ⁺ yrs	2 ⁺ yrs	3 ⁺ yrs	0 ⁺ yrs	1 ⁺ yrs	2 ⁺ yrs	3 ⁺ yrs	0 ⁺ yrs	1 ⁺ yrs	2 ⁺ yrs	0 ⁺ yrs	1 ⁺ yrs	
(n)	(2007)	(2008)	(2009)	(2010)	(2008)	(2009)	(2010)	(2011)	(2009)	(2010)	(2011)	(2010)	(2011)	
KI	90	47	19	12	90	27	44	19	124	46	25	67	34	
Ukn		2	6	4			24	37		2	5		5	
Total	90	49	25	16	90	27	68	56	124	48	30	67	39	
FP	30	39	16		70	24	2	3	107	31	2	44	5	
Ukn		4	1	1		3	1	3		3	2		1	
Total	30	43	17	1	70	27	3	6	107	34	4	44	6	

For young-of-year black bream, a 30 μm spot in the otolith core was used to quantify the concentration of elements laid down during settlement, which was assumed to represent a juvenile signal (Gillanders and Kingsford 1996) (see also Chapter 2). Otoliths were analysed in a number of sampling sessions and otoliths were randomised across sessions. A reference standard (National Institute of Standards and Technology, NIST 612) was analysed after approximately every 12 otolith ablations to correct for machine drift (Ludden et al. 1995). Detection limits were calculated as 3 standard deviations above the blank background counts that were run during analyses, and were $< 0.0005 \text{ mol}\cdot\text{mol}^{-1}$ for Sr, Ba, Mg and Mn, and $< 0.004 \text{ mol}\cdot\text{mol}^{-1}$ for Ca. All element values were above detection limits. Analytical accuracy was calculated from repeated measures of the elemental concentrations of the NIST 612 standards and averaged across all samples. Analytical accuracy was 100% for Ca and Sr, and 101% for Ba, Mg and Mn. Background counts were subtracted from sample counts, standardised to the NIST 612 standard and converted to concentrations (in ppm) using Glitter software (Griffin et al. 2008). Elemental concentration data were exported and processed in Excel (Microsoft) to express the elemental concentration data as ratios to Ca thereby accounting for fluctuations in the ablation yield (Munro et al. 2008).

Otolith element concentrations of subadult black bream were analysed using transect profiles along an axis of growth, from the core to the most recently developed increment at the otolith edge. Transect analyses and data processing were conducted using the same methodology and experimental conditions as the spot analyses; with the exception that elemental profile data were smoothed using a 7-point running median and a 7-point running mean to reduce instrumental noise along the transects (Sinclair et al. 1998). The ablation path of otolith elemental profiles was then viewed at $\times 5$ -magnification on a compound microscope with reflected light. Using a LEICA DFC 320 digital camera and Image Pro Plus software, the length of the ablation path that corresponded to the juvenile portion of the elemental profile was measured (in μm). This portion of the elemental profile was then averaged to provide a juvenile elemental signature. Only opaque zones were considered in these analyses, as this represents the summer growth period of the black bream and corresponds to the timing of sampling (Sarre and Potter 2000, Elsdon and Gillanders 2006a).

Statistical analyses

Juvenile otolith signatures were based on the multielement otolith chemistry data of young-of-year black bream (Chapter 2). The juvenile signatures from the various estuaries for each of four recruitment cohorts (2007–2010) were grouped into Kangaroo Island (KI) and Fleurieu Peninsula (FP) estuaries and used as baseline data in which to calculate the proportion of subadult fish that originally recruited to each broad region and therefore the potential movements of fish (Fig. 3.1). It was necessary to group young-of-year fish into two broad groups rather than analyse by individual

estuaries because although there were significant differences among estuaries (Chapter 2), there was relatively poor discriminatory power based on multielement signatures (total CAP ranging from 40 to 64%; Chapter 2). Each cohort and year were analysed separately.

Elemental data were $\log(x+1)$ transformed before being fit to a Euclidean distance matrix in Primer v7.0 (Clarke and Gorley 2006). Single-factor Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001) was used to assess spatial differences between regions, namely Kangaroo Island (KI) and Fleurieu Peninsula (FP). Canonical analyses of principal coordinates (CAP, Anderson and Willis 2003) were employed to test the efficacy of multielement signatures to discriminate among regions using a leave one out approach. All analyses were done using 4999 permutations of the raw data.

The multielement otolith data from the young-of-year black bream from both KI and FP were analysed using Principal Components Analysis (PCA) and displayed in 2-dimensions with 95% confidence ellipses around the data. The multielement otolith data of subadult black bream (1-3⁺ fish) were also analysed using PCA and superimposed on the young-of-year data. Subadult fish that did not fall within the 95% confidence ellipse based on the young-of-year fish (i.e. the juvenile signature) were assumed to have recruited from an estuary or region that was not represented in the sample and were therefore removed from further analyses (Hamer et al. 2003, Reis-Santos et al. 2012). In total, 104 fish did not fall within the bounds of the 95% confidence ellipses of the PCA (Table 3.1, Fig. 3.2). For these analyses subadult fish were matched to their correct cohort and data were analysed by year.

The remaining subadult black bream were then assigned to their juvenile region using CAP analyses implementing the “add new samples” function. The numbers of fish classified as either recruiting from KI or FP were then combined with the counts of fish identified as recruiting from unknown juvenile regions (i.e. outliers) in order to determine the relative contributions of recruits through time. The locations of capture of individual subadult fish were contrasted against their estimated juvenile or recruitment region to better understand connectivity of populations through time.

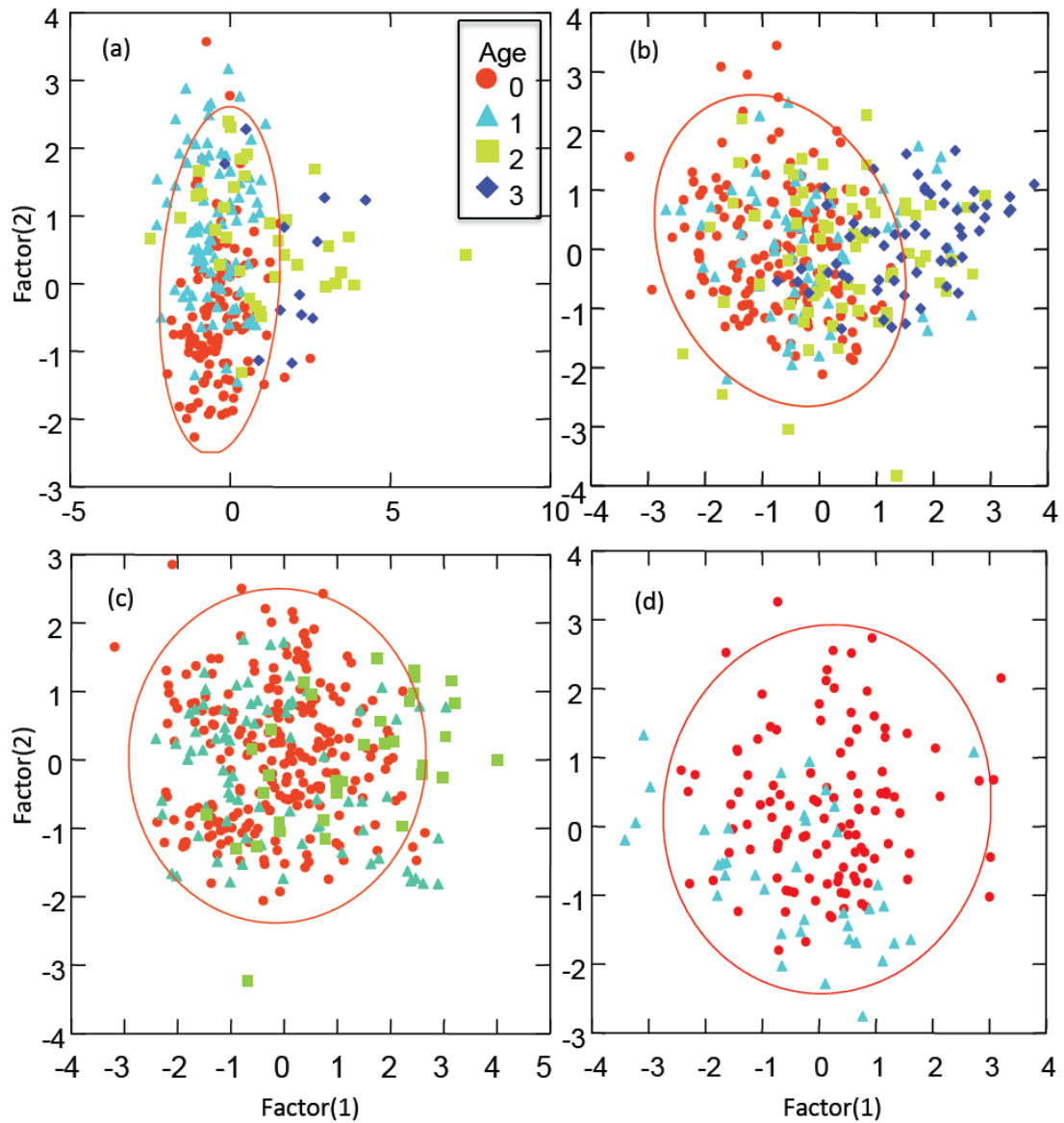


Figure 3.2. Canonical variate plots of the multielemental otolith juvenile signatures of *Acanthopagrus butcheri* from Kangaroo Island and the Fleurieu Peninsula, South Australia. The ellipses represent the 95% confidence interval around the juvenile otolith chemistry values (●) for the four recruitment cohorts: (a) 2007; (b) 2008; (c) 2009; and (d) 2010. Overlaid are the otolith chemistry data for the subadult black bream that were retrospectively traced to recruitment regions. Each age class was traced to its actual recruitment cohort.

Results

Juvenile otolith signatures

For each recruitment year (2007 to 2010), the regional multielement juvenile otolith signatures differed significantly between KI and FP ($P < 0.05$) (Table 3.2). Juvenile signatures provided a reasonable degree of classification success back to the two regions (CAP > 70%), although some

variation was seen among years and between regions (Table 3.2). Given this level of classification success we then used these regional differences to trace subadult fish back to the region that they likely recruited to as juveniles.

Table 3.2. PERMANOVA tests of spatial differences in juvenile (young-of-year) *Acanthopagrus butcheri* otolith chemistry from Kangaroo Island (KI) and the Fleurieu Peninsula (FP) in South Australia. Also shown is the percent of juvenile fish correctly classified to Kangaroo Island or Fleurieu Peninsula using canonical analysis of principal coordinates.

cohort	Recruitment Source	df	MS	F	P	% correct		
						Total	KI	FP
2007	Region	1	11.928	18.724	< 0.005	84	88	73
	Residual	118	0.637					
2008	Region	1	19.711	15.092	< 0.005	82	86	77
	Residual	158	1.306					
2009	Region	1	5.988	4.784	< 0.005	80	79	80
	Residual	229	1.252					
2010	Region	1	7.645	4.300	< 0.050	70	70	70
	Residual	109	1.778					

Temporal contributions of recruits

The multielement signature of the juvenile region of subadult fish generally fell within the 95% confidence ellipses of the juvenile baseline data (Fig. 3.2). However, for all years there were some subadult multielemental signatures that fell outside the juvenile baseline data (Fig. 3.2, Table 3.3). The proportion of subadults falling outside the juvenile baseline data generally increased as the cohort aged (e.g. 2007 and 2008 cohorts). Between 10 and 63% depending on cohort and age of fish fell outside the juvenile baseline data suggesting that these fish came from an area not characterised as part of this study. This assumes that these areas would differ in elemental signatures to Kangaroo Island or the Fleurieu Peninsula.

For the 2007 and 2008 recruitment cohorts, similar proportions of recruits from KI and FP contributed to the 1⁺ and 2⁺ age classes (Table 3.3), with variable contributions from unknown estuaries (range 10-33%). Kangaroo Island and unknown estuaries provided the greatest contribution of 3⁺ age class recruits to the 2007 and 2008 cohorts, respectively (Table 3.3), with few recruits from FP. A consistent pattern of contributions of recruits was seen for the 2009 and 2010 cohorts, whereby KI provided the greatest proportion of recruits in all age classes (> 63%), with lesser contributions from FP and only a small contribution from unknown regions (Table 3.3).

Temporal patterns of connectivity

Relating the locations of capture of subadults to estimated juvenile regions for individual fish enabled the assessment of patterns of connectivity among the regions sampled (Table 3.4). Considering proportions of fish from different age classes provided a means of understanding age-related patterns of connectivity among the putative juvenile regions. Black bream caught in KI showed highly variable patterns of inter-annual and age-related self-recruitment (Table 3.4). For some age classes, self-recruits made up approximately 80% of the fish caught (Table 3.4). Contributions of recruits from FP and unknown estuaries also varied among annual cohorts and age classes, with recruits from FP contributing from 0 to 58% suggesting some movement between FP and KI. Contributions from unknown regions to KI ranged from 0 to 66% (Table 3.4).

For FP, similar proportions of self-recruits and fish originating from KI were generally found (Table 3.4), with some exceptions (e.g. KI contributed 82% of the 1⁺ year recruits in the 2009 cohort). Numbers of recruits from unknown estuaries varied among age cohorts (range 6–50%), although only the 3⁺ age fish from the 2007 recruitment cohort originated from an unknown estuary (Table 3.4). Sample sizes were generally quite small for most age classes of fish caught in FP (exceptions: 1⁺ year fish from 2007, 2008 and 2009 recruitment cohorts).

Table 3.3. Temporal patterns of the actual and estimated percentage of *Acanthopagrus butcheri* from Kangaroo Island (KI) and Fleurieu Peninsula (FP). Actual contributions represent the relative proportions of young-of-year black bream caught within estuaries on KI and FP which were used as baseline data. The estimated contributions are of subadult fish traced back to their juvenile region based on multielement otolith signatures. Samples from unknown (Ukn) regions indicate those black bream that fell outside of the bounds of the 95% confidence ellipses for juvenile fish based on the PCA (refer to Table 3.1).

Recruitment cohort		Percentage (%) contribution			
		Actual	Estimated		
cohort	Juvenile region	YOY	1⁺ yrs	2⁺ yrs	3⁺ yrs
2007	Sampling year		(2008)	(2009)	(2010)
	KI	75	50	40	75
	FP	25	40	45	0
	Ukn		10	15	25
	Fish assigned (n)	120	92	42	17
2008	Sampling year		(2009)	(2010)	(2011)
	KI	56	40	35	28
	FP	44	37	32	9
	Ukn		23	33	63
	Fish assigned (n)	160	54	71	62
2009	Sampling year		(2010)	(2011)	
	KI	46	63	84	
	FP	54	30	11	
	Ukn		7	5	
	Fish assigned (n)	231	82	34	
2010	Sampling year		(2011)		
	KI	60	74		
	FP	40	13		
	Ukn		13		
	Fish assigned (n)	111	45		

Table 3.4. Temporal patterns of age-related connectivity of *Acanthopagrus butcheri* between Kangaroo Island (KI) and Fleurieu Peninsula (FP) in South Australia. Recruitment was determined by relating sampling regions of individual black bream to their estimated juvenile regions (assigned based on multielement otolith signatures). Bold values indicate self-recruitment. Samples from unknown (Ukn) regions indicate those black bream that fell outside the bounds of the 95% confidence ellipses based on the PCA (refer to Table 3.1).

Recruitment cohort		Percentage (%) recruits sampled in each region					
		KI			FP		
	Juvenile Region	1+yrs	2+yrs	3+yrs	1+yrs	2+yrs	3+yrs
2007	Sampling year	(2008)	(2009)	(2010)	(2008)	(2009)	(2010)
	KI	86	27	75	52	53	0
	FP	10	49	0	38	41	0
	Ukn	4	24	25	9	6	100
	Fish assigned (n)	49	25	16	43	17	1
2008	Sampling year	(2009)	(2010)	(2011)	(2009)	(2010)	(2011)
	KI	43	40	24	41	17	5
	FP	57	25	10	48	50	45
	Ukn	0	35	66	11	33	50
	Fish assigned (n)	27	68	56	27	3	6
2009	Sampling year	(2010)	(2011)		(2010)	(2011)	
	KI	82	49		82	50	
	FP	14	35		9	0	
	Ukn	4	17		9	50	
	Fish assigned (n)	48	30		34	4	
2010	Sampling year	(2011)			(2011)		
	KI	29			37		
	FP	58			47		
	Ukn	13			16		
	Fish assigned (n)	39			6		

Discussion

Natural chemical tags in otoliths have been used to answer questions about connectivity between juvenile source areas and adult populations (Gillanders 2002a, Chittaro et al. 2009, Reis-Santos et al. 2013b). Based on young-of-year otoliths this study developed four annual elemental signatures

for two key juvenile black bream regions in South Australia. These juvenile chemical signatures were region-specific, providing a high degree of spatial discrimination and hence, could be used to understand recruitment dynamics and track the connectivity of black bream between Kangaroo Island (KI) and the Fleurieu Peninsula (FP) in South Australia. Classification accuracies differed among recruitment years, with some years having better classification success than others.

The elemental composition of otoliths is influenced by a range of abiotic (environmental) and biotic (physiological) factors (reviewed in Campana 1999, Campana and Thorrold 2001, Elsdon et al. 2008), hence elements may show significant temporal differences among years (e.g. Chittaro et al. 2009, Clarke et al. 2009) (Chapter 2) and seasons (e.g. Kalish 1991, Reis-Santos et al. 2012). In highly dynamic estuarine ecosystems, fluctuations in water chemistry may occur at weekly, daily or tidal time scales, which in turn may result in fluctuations in concentrations of specific elements in otoliths, limiting their use as natural tags (Elsdon and Gillanders 2006b, Chang et al. 2012). For otolith element chemistry to function as an effective natural tag, the elemental signature needs to remain stable between the time of assimilation and when fish are tracked to their respective juvenile area (Campana 1999, Campana et al. 2000). Considerable temporal variations may supersede spatial differences, resulting in the erroneous estimation of the contributions of recruits from estuarine nurseries (Gillanders 2002a, Reis-Santos et al. 2012). Temporal variation in elemental signatures also means that fish may need tracking back to their respective recruitment cohort. For practical fisheries management and conservation purposes, integrating fine scale temporal variation into an annual signal is logistically advantageous as it facilitates the matching of juvenile portions of adult otoliths to juvenile signatures to assess recruitment and connectivity (Reis-Santos et al. 2012).

Four annual juvenile regional otolith elemental signatures were developed which facilitated the retrospective assignment of subadult black bream to their juvenile region. Patterns of recruitment varied between regions and among annual cohorts. As a result no one region appeared to act as a primary source of black bream recruits to the South Australian population. Grouping estuaries into regions prevented the identification of individual estuaries that may have contributed increased numbers of subadults to the population. Similar numbers of young-of-year fish were collected from both regions, with the exception of the 2007 cohort where the majority of juveniles were collected from KI, potentially as a result of increased sampling in this region.

Subadult fish collected from KI and FP showed variable contributions from each region for different sampling years and age classes. Temporally variable recruitment is a consistent feature throughout the distribution of black bream (Morison et al. 1998, Nicholson et al. 2008, Jenkins et al. 2010), with recruitment success dependent on local environmental conditions (Partridge and Jenkins 2002, Hassell et al. 2008). The estuaries in our study were generally small in size, with limited freshwater inflows and are sporadically closed to the adjacent ocean (Kench 1999). At the

time of sampling for juveniles in the Austral summer, most estuaries were closed to the marine environment by sands bars (exception: Onkaparinga estuary), and salinities were generally high (ranging from 20 to 51‰). These environmental conditions (high salinities and warm water temperatures) are generally conducive to the survival and hatching success of black bream eggs (Haddy and Pankhurst 2000, Partridge and Jenkins 2002, Hassell et al. 2008). However, based on numbers of young-of-year fish encountered at the time of sampling, there were obvious inter-annual differences in the recruitment success among the estuaries (A.J. Giraldo, personal observation). Although a range of estuaries were sampled using similar methods, we did not quantify abundance of recruits within estuaries.

When the estimated juvenile regions of subadult black bream were related to their locations of capture, we were able to assess patterns of connectivity between KI and FP. These data suggest that there were significant levels of connectivity between mainland South Australia (FP) and Kangaroo Island. In general, the older age classes showed the highest degree of population connectivity, with few self-recruits captured indicating that older fish were capable of moving greater distances. Otolith element profiles have detected increased movements with increasing age in a range of diadromous species (e.g. Secor and Piccoli 1996, Chang et al. 2004, Morales-Nin et al. 2012). However, analogous profile analyses in black bream did not detect increasing migratory movements with age, but rather found that within an estuarine population contingents of fish undergo migrations between freshwater and marine habitats (Elsdon and Gillanders 2005). Fish from the migratory contingent were found to first move between 0 and 5⁺ years for a population of black bream from the River Murray estuary (Gillanders et al. 2015).

Fish were also sourced from regions external to the study area (up to 63% of 3⁺ year fish from the 2008 recruitment cohort). This implies that black bream are capable of undergoing movements in excess of 150 km between mainland FP and KI. Indeed, the migratory fish from Gillanders et al. (2015) may have moved from the River Murray estuary to other regions, although these fish movements may also reflect movement within the Murray River estuary. Previous tagging studies suggest that while black bream utilise large areas of an estuary, movements between adjacent estuaries are generally restricted to 10s of km (Hindell et al. 2008, Sakabe and Lyle 2010), although these studies were undertaken in Victoria and Tasmania. Similarly, previous tagging observations of black bream in Kangaroo Island estuaries have usually found that most fish were recaptured within the estuary in which they were tagged (A.J. Giraldo, personal observation).

Molecular analyses of the south-eastern and south-western Australian distributions of black bream are generally consistent with the available tagging data, inferring low levels of genetic connectivity among adjacent estuaries (Yap et al. 2000, BurrIDGE and Versace 2007). Furthermore, as these estuaries are open for relatively short periods of time, there is little opportunity for black bream to

leave their juvenile estuaries and move between estuaries. Despite this, there are clearly some fish which move between regions.

This study focused on broad regional scale differences in connectivity and recruitment dynamics (i.e. between KI and FP), as there were too many individual estuaries (n=12) to yield high discriminatory power (i.e. allocation success >65%). The geographic groupings used here effectively combined the estuaries into a mainland South Australia (FP) and KI region. It was assumed that the combined elemental signatures of the estuaries accurately represented each geographic region, as samples from as many possible source estuaries within KI and FP where black bream were known to occur were obtained (Gillanders et al. 2008). Other studies have used alternate criteria to group estuaries, including geographic proximity, similarities in elemental signatures, and levels of anthropogenic loadings (Gillanders and Kingsford 2002, Hamer et al. 2003, Chittaro et al. 2009). Estuaries have been pooled due to geographic proximity (Clarke et al. 2009), but when elemental signatures do not align with geographic proximity of estuaries, due to other features of estuaries differing, other approaches to grouping estuaries may be used. Grouping estuaries that had similar elemental signatures increased average classification success, and thus, potentially increases accuracy in estimating connectivity of older fish (Chapter 2). Additional elements or isotopes could also facilitate differentiation at finer spatial scales (e.g. estuaries).

A requirement for the use of otolith element composition as natural tags is that all possible groups (i.e. estuaries or sub-populations) contributing to the larger regional or population signal have been characterised (Campana 1999, Campana et al. 2000, Woods et al. 2010). While efforts were made to obtain young-of-year samples from all potential source estuaries that might contribute black bream in South Australia, a component of the subadult black bream examined here were classified as originating from unknown nursery estuaries. Our approach of only using subadult fish whose otolith elemental signatures overlapped their respective juvenile cohort of fish reduces the chances of incorrectly assigning fish to regional areas that they may not have come from. This approach has also been used in several other studies (e.g. Reis-Santos et al. 2013b).

These unknown fish may have originated from one of two potential sources. First, from nursery estuaries located on KI or FP that were not sampled, for example on Kangaroo Island there are several smaller estuaries that could potentially provide black bream recruits. Hence, the actual contributions of recruits from these regions may be greater than what was indicated here. Alternatively, these unknown fish may have originated from nursery estuaries located externally to KI and FP. In South Australia the Lower Lakes and Coorong region at the terminal end of Murray-Darling river system is an important nursery habitat for black bream (Ferguson et al. 2013). While this study did not include fish from the Coorong region, the period of sampling in part coincided with the decadal drought that reduced the water levels in the Lower Lakes (at times below sea level) and closed the mouth of the River Murray to the ocean for extended periods (Kingsford et al.

2011, Wedderburn et al. 2012); thus potentially limiting the ability of black bream to leave the system. There are large expanses of wave dominated coast between the Murray Mouth and the Victorian border with few fish likely to be sourced from this region. Similarly, although we sampled fish from the Fleurieu Peninsula, there are few areas where juvenile black bream recruit further west.

Hence, the most likely source of unknown fish was from the Coorong and Lower Lakes system. It was assumed that for many sampling years, there would be little to no connectivity between the Lower Lakes and the adjacent marine habitat. In the Austral spring of 2010, significant rainfall and increased flows of the River Murray led to the sustained opening of the River Murray Mouth, reducing the salinity of the estuary (Ye et al. 2011). This induced a range of environmental changes in the Lower Lakes and elicited considerable responses from the local fish assemblages, including the southward range extension of the black bream (Ye et al. 2011). Interestingly, the bulk (85%) of the unknown fish immigrating into the KI and FP regions were caught in 2010 and 2011 (following the end of the drought), suggesting that they may have come from this region.

Conclusion

Understanding the spatiotemporal patterns of fish population connectivity is paramount for establishing spatially explicit management plans as measures should ideally be dictated by the dispersal and connectivity of species. The development of four annual juvenile regional signatures facilitated the retrospective assignment of subadult black bream to their juvenile region. The collation of the individual estuaries into several regional signals prevented the identification of primary estuary areas based on otolith chemistry. However, considerable variation in estimated contributions of recruits among years and between age classes suggests that inter-annual environmental variation is a key driver in the recruitment success of black bream populations. The connectivity of juvenile cohorts of black bream between Kangaroo Island and the Fleurieu Peninsula indicates extensive movements and marks the potential for homogeneity within the local population structure. Future molecular research is required to better explore the patterns of connectivity observed here, with assessments of population structure and connectivity being addressed across the entire distribution of the species.

Chapter 4 – Statement of authorship

Title of Paper	Partial migration in black bream from different estuaries on Kangaroo Island
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Principal Author

Name of Principal Author (Candidate)	Ana Judith Giraldo		
Contribution to the Paper	In this chapter, Ana Judith Giraldo collected the specimens, performed the otolith chemistry analyses, analysed the data and wrote the manuscript		
Overall percentage (%)	80%		
Signature		Date	14 July 2015

Co-Author Contributions

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

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Chris Izzo		
Contribution to the Paper	Christopher Izzo assisted with intellectual development, had input in the development of the chemical analysis protocols and provided suggestions, comments and feedback on manuscript drafts.		
Signature		Date	10 July 2015

Name of Co-Author	Bronwyn Gillanders		
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Contribution to the Paper	Bronwyn Gillanders assisted with intellectual development and provided suggestions, comments and feedback on manuscript drafts. The research was funded via an ARC Linkage project grant to Bronwyn Gillanders and Travis Elsdon.		
Signature		Date	10 July 2015

Chapter 4. Partial migration in black bream from different estuaries on Kangaroo Island: do migratory fish represent a hybrid complex?

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Abstract

Determining fish movement and the coexisting migratory and resident life history types is difficult. Partial migration was evaluated in six estuarine populations of black bream (*Acanthopagrus butcheri*) from Kangaroo Island, South Australia, using otolith Ba:Ca ratios. Profiles of Ba:Ca across otoliths were used to broadly determine fish habitat use (freshwater, estuarine, or marine) and assign euryhaline or stenohaline status. In addition, we evaluated whether euryhaline fish may represent hybrid fish between black bream and yellowfin bream (*Acanthopagrus australis*) using an eight locus microsatellite library. Partial migration was observed in all six estuarine populations, although for most estuaries there were greater numbers of euryhaline fish than stenohaline fish. Hybrid fish were found in five of the six estuaries and comprised both euryhaline and stenohaline fish. Fish showed contrasts in life history profiles of euryhaline and stenohaline nature. Both euryhaline and stenohaline fish spent most time in marine waters, but euryhaline fish also moved to estuarine and freshwaters. Similar patterns were also seen for hybrid fish. The data highlight the different behaviours of adult individuals and show that hybrid bream had reached estuaries previously thought to be inhabited by pure black bream. Complex salinity habitat use may allow populations to persist under adverse environmental conditions and in systems subject to change.

Introduction

Migration determines the distribution and abundance of animals and is integral for ecological and evolutionary processes. In addition, an understanding of migration also aids species conservation and management. Migration has been well documented in some taxa, particularly those making long distance migrations (Chapman et al. 2011a). Although migrations of some fish taxa have been well studied, our understanding of fish migrations for many species is still limited as individuals are difficult to track throughout their life history (Chapman et al. 2012a). Within species, migration behaviours may vary between populations and among individuals within a population (Green and Wroblewski 2000, Secor et al. 2001, Kent Smedbol and Wroblewski 2002). This within population variation in behaviour where some members of the population migrate and others remain resident is known as a partial migration (Chapman et al. 2012a). In fish this may also reflect differences in salinity of environments inhabited by fish.

For fish, partial migration was initially observed in anadromous species (e.g. salmonids) (Chapman et al. 2012b). More recent examples suggest that partial migration is widespread among many types of fishes and across a range of habitats and spatial scales (Chapman et al. 2012b). Within estuarine species, partial migration has been documented in white perch (*Morone americana*), where part of the population inhabit freshwater natal habitats and the other portion migrates into brackish waters (Kerr et al. 2009, Kerr et al. 2010a, Kerr and Secor 2012). The occurrence of migratory and resident life history types has also been observed for estuarine populations of black bream (*Acanthopagrus butcheri*) (Elsdon and Gillanders 2005, Gillanders et al. 2015), but may reflect euryhaline (migratory) or stenohaline (nominally termed resident fish but these fish may also reflect migrants moving in marine waters depending on the method used to assess migratory vs resident status). Partial migration may allow species to persist in systems where there is considerable environmental variability (Kerr and Secor 2012). One strategy may be more favourable under a certain types of environmental conditions, whereas the other strategy may be more favourable under different environmental conditions (e.g. Gillanders et al. 2015). Partial migration therefore may be seen as a population-level bet hedging strategy.

Many studies suggest that partial migration occurs for a given population, but do not assess what factors promote partial migration or the ecological and evolutionary consequences (Chapman et al. 2012b). Although advantages of partial migration have been suggested as increased survival through reduced predation risk and, or increased food availability, reasons for partial migration are unknown in many species. Several studies have investigated migratory and resident phenotypes of fish (e.g. Chapman et al. 2011b, Skov et al. 2011), with some suggestion that body size differs between contingents (Chapman et al. 2012b). The ecological reasons for this difference have not been widely examined, but may include predation risk-growth trade-offs, competitive release, and resource polymorphisms, such as trophic differences (Chapman et al. 2012b). Environmental

influences have been suggested as more important than genetic determinants (Kerr and Secor 2010, Skov et al. 2010, Chapman et al. 2012b).

While a suite of research methods are available to delineate partial migration of fish (e.g. conventional tags, acoustic telemetry), the application of these methods to determine patterns of movement within populations is difficult as they are time and cost inefficient, limited to the oldest cohorts of the population, and cannot assess movements throughout the entire life history of the fish. Otoliths, the calcified structures found in the inner ear of fish, may provide an alternate approach as they incorporate naturally occurring elements from the environment into the most recently formed growing layer (Campana 1999, Campana and Thorrold 2001, Elsdon and Gillanders 2003). This elemental incorporation occurs throughout the life history of the fish and the elements are not usually subject to resorption. As otoliths also form growth increments, the chemical information can be matched to the growth increments of the fish and the environment that they have inhabited throughout their life estimated (Campana and Thorrold 2001, Elsdon and Gillanders 2003, Elsdon et al. 2008). Thus, retrospective analysis of past habitat use in fish can be obtained from otoliths and used to indicate whether fish have experienced euryhaline (potential migrants) or stenohaline (potential residents) conditions.

Trace elements and stable isotopes in otoliths have been used to estimate salinity environments of fish (Elsdon and Gillanders 2005, Hamer et al. 2006, Miller 2011). Ba:Ca is often significantly greater in freshwater and decreases in estuarine and marine waters. It has therefore been used to determine movements and environmental histories (e.g. salinity) of fish. Elsdon and Gillanders (2005) used Ba:Ca concentrations in the otoliths of black bream to classify fish as migratory or resident. They first demonstrated a relation between otolith Ba:Ca and salinity, and then used this relation to infer fresh or saltwater habitat use of fish. Interestingly, this study identified partial migration for fish from the same estuary, although their sample sizes were relatively small. Sr:Ca ratios in otoliths have also been used to investigate partial migration in an estuarine species (Kraus and Secor 2004a). Similarly, other studies have used oxygen isotopes to identify fish that remain resident within freshwater habitats and migrants to higher salinities (Kerr and Secor 2012). Thus, complex migratory behaviours have been ascertained through examination of otolith chemistry.

The aims of this study were to determine the extent of partial migration, or the proportions of euryhaline (migratory) and stenohaline (potentially resident) life history types, within estuarine populations of black bream. In addition, we were interested in determining whether fish showing euryhaline life history types may represent hybrids between black and yellowfin bream. Several studies of black bream have investigated patterns of gene flow among estuaries suggesting a significant relationship with geographic isolation at least in southeast Australia (Burrige et al. 2004). A lack of gene flow among estuaries in Western Australia was also found (Chaplin et al. 1998). Along the east coast of Australia black bream is sympatric with yellowfin

bream (*Acanthopagrus australis*) and although hybridisation is known to occur (Roberts et al. 2009, Roberts et al. 2010b, Roberts et al. 2011), yellowfin bream are panmictic (Roberts and Ayre 2010). As black bream are primarily estuarine associated and yellowfin bream are coastal, hybridisation of the two species may explain contingent structure within black bream. Such a scenario is plausible given that estuarine populations of bream in New South Wales were primarily composed of hybrids and there have been suggestions that introgressed individuals of *Acanthopagrus* spp. are more widespread than previously thought (Roberts et al. 2011).

The specific aims were to use Ba:Ca ratios in otoliths of black bream to characterise the stenohaline (potentially resident) versus euryhaline (migratory) fish in six estuaries on Kangaroo Island, and to determine the proportion of time fish spent in freshwater, estuarine and marine waters. In addition, we also determined if euryhaline fish, as determined from Ba:Ca ratios in otoliths, represent hybridisation between black bream (*Acanthopagrus butcheri*) and yellowfin bream (*Acanthopagrus australis*), since black bream are generally regarded as resident in estuaries.

Materials and Methods

Study species

Black bream (*Acanthopagrus butcheri*) is an endemic, euryhaline fish distributed in the saline and brackish waters of Australia's southern coast, but has a disjunct distribution within estuaries ranging from central New South Wales to Western Australia (Roberts et al. 2009, Roberts and Ayre 2010) (see Fig. 1.4). Yellowfin bream (*Acanthopagrus australis*) is a migratory marine fish with a wide distribution along Australia's east coast, overlapping with the range of black bream (Roberts et al. 2009, Roberts and Ayre 2010, Roberts et al. 2011). Hybridisation between black bream and yellowfin bream occurs in south-eastern Australia, but has not been recorded from South Australia (Roberts et al. 2010b).

Acanthopagrus butcheri is a commercially and recreationally important species, and is farmed in dams throughout south-western Australia. In Western Australia, adult black bream (>3 year old) appear to move from the ocean to the estuaries in winter (May to August) during heavy freshwater discharge, returning to the natal estuary once the discharge has declined (Norriss et al. 2002). The movement patterns of black bream in South Australia are complex with adults spawning in estuaries and rivers, juveniles being found in both estuarine and marine waters and adults occurring in freshwater, estuarine and marine waters (Gillanders et al. 2008). Fish from a single estuary show a range of movement patterns, which can vary over the lifespan of individual fish (Elsdon and Gillanders 2005, Gillanders et al. 2008, Gillanders et al. 2015).

Sampling

Adult black bream were collected by beach seine and line-baited hook from six estuaries on Kangaroo Island, South Australia (Table 4.1, Fig. 4.1). More than 50 fish were collected from each estuary, where possible, such that samples were representative of estuarine populations. Most of the fish were sampled in the austral summers (November to March) of 2008, 2009, 2010 and 2011 (some fish were collected in August or September 2008 and September 2009; n=31). The sampling focused on the rivers/estuaries in which black bream are known to occur (Gillanders et al. 2008). During the Austral summers of sampling, all estuaries were closed to the open ocean by sandbars. At each sampling site salinity and temperature were recorded using a WP-84 conductivity–salinity meter. Once caught, fish were handled in accordance with Adelaide University ethics permits in the field and returned to the laboratory, where standard length (SL, mm) and body weight (BW, g) were recorded (Table 4.1).

Table 4.1. Estuaries (and estuary code) sampled on Kangaroo Island, South Australia, showing numbers of fish collected (total fish), numbers of hybrid fish, standard length, body weight and maximum age of adult black bream, *Acanthopagrus butcheri*, collected for analysis.

Estuary	Code	Coordinates	Total fish	Hybrid fish	Standard length (range, mm)	Body weight (range, g)	Maximum age (years)
Western River	WE	35° 42' 03 S 136° 49' 37 E	39	1	99-332	27-640	18
South West River	SW	36° 01' 17 S 136°51' 20 E	45	0	97-279	31-215	9
Eleanor River	EL	35° 58' 20 S 137° 12' 08 E	50	9	103-214	37-317	16
Harriet River	HA	35° 58' 30 S 137° 10' 18 E	51	10	100-283	34-205	14
Chapman River	CH	35° 47' 05 S 138° 04' 03 E	50	1	113-332	50-395	28
Middle River	MI	35° 41' 13 S	50	6	101-220	31-259	16

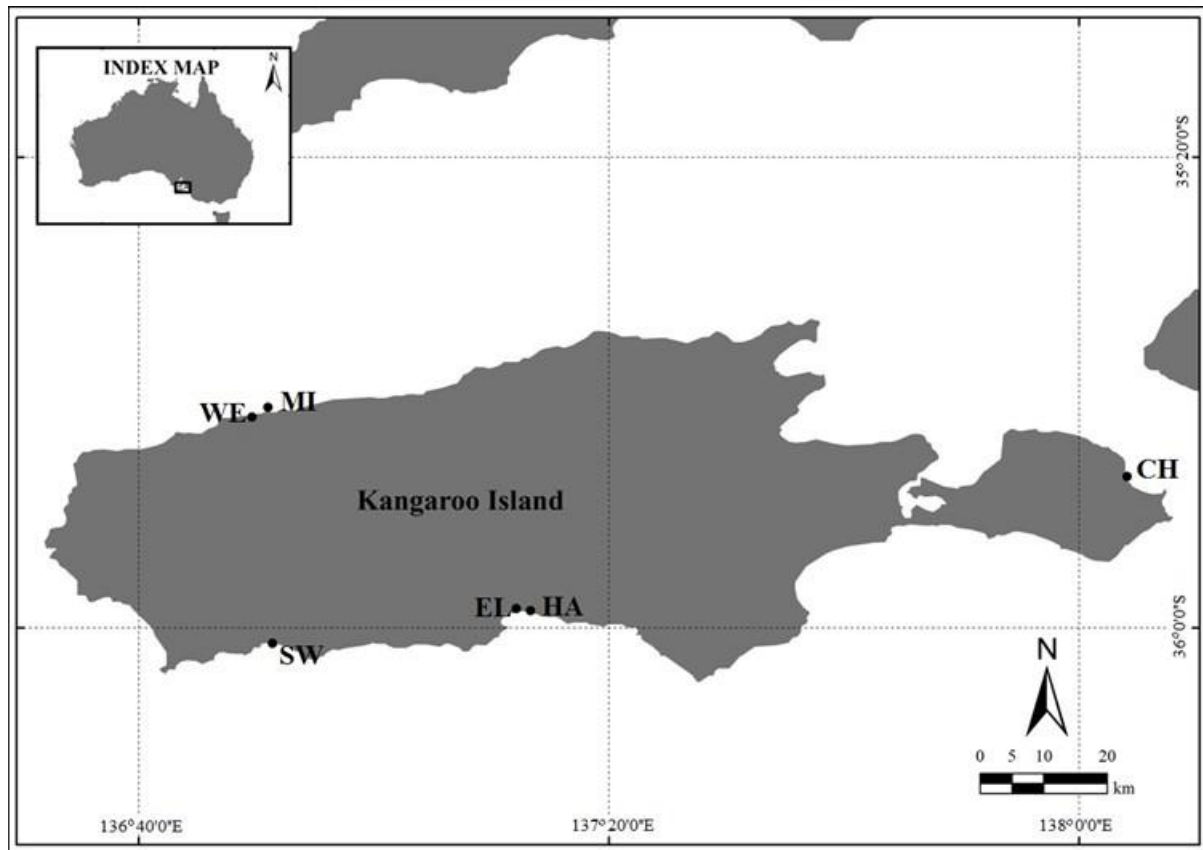


Figure 4.1. Kangaroo Island, showing estuaries in which adult black bream were collected: MI, Middle River; WE, Western River; SW, South West River; EL, Eleanor River; HA, Harriet River and CH, Chapman River.

Otolith preparation

Sagittal otoliths were removed from the fish and cleaned of adhering tissues with ultrapure water. Otoliths were then placed in vials and left to air dry in a laminar flow cabinet for 24-h. One otolith from each fish was embedded in a clear resin that had been spiked with indium at 30 ppm to enable discrimination between otolith material and resin during analysis. Embedded otoliths were then sectioned transversely through the core using a low-speed diamond saw, lubricated with ultrapure water. Sections were polished to a thickness of 250 to 300 μm using progressively finer grades of lapping film and mounted onto glass microscope slides using EpoFix resin. The slides were cleaned by sonicating in ultrapure water to remove surface impurities, and dried in a laminar flow cabinet for 24-h, before being stored individually in sealed plastic bags until analysis. Thin sections of otoliths were viewed under a stereo-microscope with transmitted light to estimate age

based on counts of growth (opaque zone) increments. Growth increments have been validated as forming on an annual basis in black bream (Morison et al. 1998, Sarre and Potter 2000).

Analysis of environmental history of fish

Otolith Ba:Ca ratios have previously been shown to be a good indicator of past environmental history of black bream from South Australian waters (Elsdon and Gillanders 2005). Concentrations of otolith barium (^{138}Ba) and calcium (^{43}Ca) were determined using a New Wave UP-214 high-performance (Nd:YAG) ultraviolet laser connected to an Agilent 7500cs inductively coupled plasma-mass spectrometer (ICP-MS). Barium concentrations were standardised to ^{43}Ca (e.g. Ba:Ca), as Ba is likely to substitute for Ca in the otolith matrix (Campana 1999). The laser operating conditions were: 5 Hz frequency; 75% laser power; $\sim 0.08\text{--}0.12$ mJ beam energy; with a ~ 0.87 L $\cdot\text{min}^{-1}$ flow of argon carrier gas. Ablations occurred inside a sealed chamber with the sample gas extracted to the ICP-MS via a smoothing manifold in the presence of argon and helium gas. The chamber was purged for 20-s after each ablation to remove background gas from previous ablations. Background concentrations of elements within the chamber were measured for 25 s before each ablation to allow for correction of sample concentrations.

The laser ablation ICP-MS was operated in time resolved mode and a 30 μm wide transect ablation path was used. Otoliths were analysed from the core to the most recently developed increment at the otolith edge at an approximate ablation rate of 1.3 $\mu\text{m}\cdot\text{s}^{-1}$. A reference standard (National Institute of Standards and Technology, NIST612) was analysed after every 12 transects to correct for machine drift. Background counts were subtracted from sample counts and standardised to the NIST 612 standard, which normalises the data to a known ^{43}Ca internal standard. Raw count data were then converted to elemental concentrations (in ppm) using the Glitter software program (<http://www.glitter-gemoc.com>).

Elemental concentration data were further processed in Excel (Microsoft) and expressed as ratios to calcium to account for fluctuations in the ablation yield (Munro et al. 2008). To determine trends in Ba:Ca across otolith profiles, data were smoothed to reduce the noise along transects, using a 7-point running median and a 7-point running mean (Sinclair et al. 1998).

Determining environmental histories of individual fish

Otolith Ba:Ca profiles for all individual fish ($n=285$) were plotted and examined in order to assess environmental histories of fish in six estuaries on Kangaroo Island. Movements between different habitats (e.g. marine, estuarine and freshwater) were categorised based on an inverse otolith Ba:Ca-salinity linear relationship calculated for all sampling sites on Kangaroo Island. The last 30 μm of the otolith Ba:Ca profile was used for this relationship as these data were assumed to be

representative of the salinity environment at the time of capture. The resulting otolith Ba:Ca-salinity relationship was:

$$\text{OTOLITH Ba:Ca} = -0.1591 \times \text{SALINITY} + 16.836, \quad r^2=0.5 \text{ (n=285)}$$

The percent of time that individual fish spent in each salinity environment (habitat) was calculated based on the proportion of the otolith profile that exceeded specified Ba:Ca thresholds. When Ba:Ca was $\leq 12 \mu\text{mol}\cdot\text{mol}^{-1}$ black bream were classified as inhabiting a marine environment (salinity $>30\text{‰}$). Conversely, otolith Ba:Ca concentrations $\geq 16.7 \mu\text{mol}\cdot\text{mol}^{-1}$ indicated that black bream inhabited a fresh water environment (salinity $<5\text{‰}$). Ba:Ca concentrations between 12 and $16.7 \mu\text{mol}\cdot\text{mol}^{-1}$ were assumed to represent a transitional estuarine signal (salinities ranging from 5 to 20‰).

Individual black bream were then defined as being either stenohaline (potentially resident) or euryhaline (migrant) based on the Ba:Ca profiles. Black bream were classified as being *resident* if their Ba:Ca profiles did not change between the freshwater and marine (including estuarine) salinity habitats (e.g. a solely marine resident, or a solely freshwater resident, Fig. 4.2). Alternatively, black bream were considered *migrant* (euryhaline) if their Ba:Ca otolith profiles crossed multiple potential salinity environments at least once (e.g. from marine to estuarine, or from estuarine to fresh or vice versa) (Fig. 4.2).

Microsatellite analyses

Tissue samples of all fish were analysed to determine if any black bream may represent hybrid fish, and therefore whether hybridisation explained the migratory contingent. Hybrid bream (*Acanthopagrus* spp.) were identified based on an eight-locus microsatellite genotype (Roberts et al. 2009). The eight microsatellite loci were developed for *Acanthopagrus schlegelii* (Jeong et al. 2003) and *Acanthopagrus butcheri* (Yap et al. 2000). Details of the microsatellite loci and PCR conditions are described in Roberts et al. (2009).

All samples were tested for evidence of mixed ancestry using assignment tests. Hybrid assignment tests were undertaken in STRUCTURE v2.0 (Falush et al. 2003) using an admixture model to calculate q_i , the mean posterior proportion of ancestry ($\pm 95\%$ CIs) (Roberts et al. 2009, Roberts et al. 2010b). Any individual with a q -value between 0.05 and 0.95 was classified as a hybrid bream (Roberts et al. 2009, Roberts et al. 2010b). Outside these values fish were classified as either pure *A. australis* ($q_i < 0.05$) or pure *A. butcheri* ($q_i > 0.95$) (see Roberts et al. 2009, for further details).

Statistical analysis

The percent of time that black bream spent in each of the three salinity environments was $\log(x + 1)$ transformed before being fit to a Euclidean distance matrix. Differences in percent time among

estuaries and between contingents (i.e. migrant vs resident fish) were assessed using a two factor (estuary and contingent) permutational analysis of variance (ANOVA). Permutations were done on the raw data with 4999 permutations per test. *Post-hoc* pairwise analyses were conducted when significant differences were detected among factors.

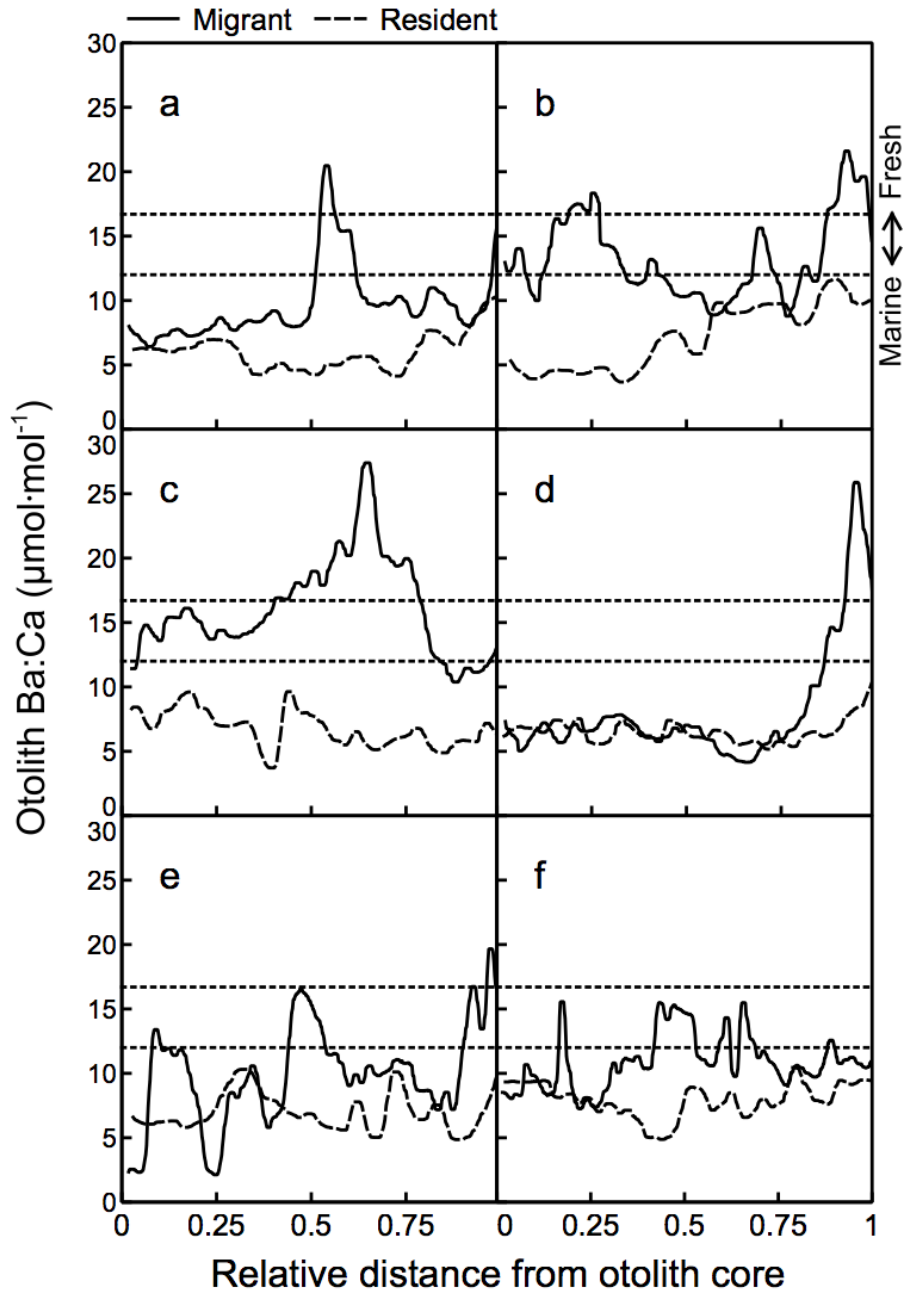


Figure 4.2.

Representative resident and migratory otolith Ba:Ca profiles of black bream from six estuaries on Kangaroo Island, South Australia. Profiles are plotted on a relative scale from 0 (otolith core) to 1 (otolith edge). Estuaries are: (a) Western River; (b) South West River; (c) Eleanor River; (d) Harriet River; (e) Chapman River and (f) Middle River. The dashed horizontal lines represent the range for estuarine waters; below these dashed lines fish were characterised as inhabiting marine waters and above these lines fish were characterised as inhabiting freshwater environments. The standard length (in mm) of fish were: WE, 177 (M), 175 (R); SW, 135 (M), 123 (R); EL, 129 (M), 139 (R); HA, 136 (M), 122 (R); CH, 195 (M & R) and MI, 220 (M), 155 (R), where M=migrant (solid line) and R=resident (dashed line) fish. Estuary codes are in Table 4.1. Estimated ages of adult fish were between 4 and 13 years.

Results

Otolith Ba:Ca profiles showed a wide range of patterns including those characterised as stenohaline (resident) and euryhaline (migrant) within each estuary (Fig. 4.2, Table 4.2), thereby suggesting partial migration. For most estuaries there were greater numbers of euryhaline fish than stenohaline (exception: Middle River) (Table 4.2). Across all estuaries sampled, there were 106 (37%) stenohaline and 179 (63%) euryhaline fish (Table 4.2). Of the 285 fish sampled, 27 (9.5%) were hybrids between black bream and yellowfin bream (Table 4.1). Hybrid fish comprised both stenohaline (37%) and euryhaline (63%) fish (Table 4.3) and were found in all estuaries except South West (Table 4.1, 4.3). Western and Chapman estuaries only had a single hybrid fish (2-2.5% of fish sampled), whereas Harriet estuary had 10 hybrid fish (20% of all fish sampled).

Table 4.2. Environmental histories of stenohaline and euryhaline black bream (including pure and hybrid fish) from six estuaries in Kangaroo Island, South Australia (see Table 4.1 for estuary codes used) showing percent time spent in each environment (\pm SE). Sample sizes are also shown (n).

Estuary	Pattern	n	Time spent in relative habitat		
			% Fresh	% Marine	% Estuarine
WE	<i>Stenohaline</i>	9	0.00 \pm 0.00	99.34 \pm 0.66	0.66 \pm 0.66
	<i>Euryhaline</i>	30	4.71 \pm 1.81	83.51 \pm 3.71	11.79 \pm 2.24
SW	<i>Stenohaline</i>	10	0.00 \pm 0.00	99.17 \pm 0.66	0.83 \pm 0.66
	<i>Euryhaline</i>	35	5.05 \pm 1.32	79.60 \pm 2.36	15.35 \pm 1.59
EL	<i>Stenohaline</i>	8	0.00 \pm 0.00	100.00 \pm 0.00	0.00 \pm 0.00
	<i>Euryhaline</i>	42	8.96 \pm 2.20	66.74 \pm 4.24	24.31 \pm 2.90
HA	<i>Stenohaline</i>	24	0.00 \pm 0.00	99.36 \pm 0.40	0.64 \pm 0.40
	<i>Euryhaline</i>	27	11.70 \pm 4.42	70.21 \pm 6.00	18.09 \pm 3.55
CH	<i>Stenohaline</i>	18	0.00 \pm 0.00	98.42 \pm 0.78	1.58 \pm 0.78
	<i>Euryhaline</i>	32	4.37 \pm 1.10	81.74 \pm 2.62	13.89 \pm 1.77
MI	<i>Stenohaline</i>	37	0.00 \pm 0.00	99.60 \pm 0.25	0.40 \pm 0.25
	<i>Euryhaline</i>	13	0.46 \pm 0.32	91.68 \pm 2.37	7.86 \pm 2.19

Table 4.3. Percent of time spent in freshwater, estuarine and marine waters (\pm standard error) for hybrid stenohaline and euryhaline bream from six estuaries on Kangaroo Island, South Australia (see Table 4.1 for estuary codes used). Sample sizes are indicated (n). na indicates no hybrid fish for that estuary and contingent type.

Estuary	Hybrid Stenohaline: Time spent in habitat				Hybrid Euryhaline: Time spent in habitat			
	n	% Fresh	% Marine	% Estuarine	n	% Fresh	% Marine	% Estuarine
WE	0	na	na	na	1	23.7	10.8	65.6
SW	0	na	na	na	0	na	na	Na
EL	0	na	na	na	9	7.6 \pm 4.8	61.8 \pm 8.3	30.7 \pm 5.2
HA	5	0.0 \pm 0.0	100.0 \pm 0.0	0.0 \pm 0.0	5	7.3 \pm 6.3	81.2 \pm 5.8	11.6 \pm 4.1
CH	0	na	na	na	1	0.0	95.6	4.4
MI	5	0.0 \pm 0.0	100.0 \pm 0.0	0.0 \pm 0.0	1	0.0	99.1	0.9

Environmental histories

Stenohaline fish spent the majority of time in marine waters (98-100% depending on estuary) based on their Ba:Ca profiles (Fig. 4.2, Table 4.2). Stenohaline fish did not spend time in freshwater and only occasionally spent time in estuarine waters (<5% of time) (Table 4.2). Given these fish spent most of their time in marine waters this may indicate that stenohaline fish comprise both resident and migrant fish. Euryhaline fish also spent the majority of their time in marine waters (Fig. 4.2, Table 4.2). Euryhaline fish generally also spent time in freshwater (0.5-12%) and estuarine waters (8-25%), but this varied by estuary (Fig. 4.3). Euryhaline fish from Eleanor River spent the least amount of time in marine waters (66%), whereas those from Middle River spent 92% of their time in marine waters. The other estuaries had intermediate patterns. In addition to the variation in environmental histories observed between estuaries, there was also considerable variation within estuaries (Fig. 4.3, Table 4.2). The greatest variability was seen in Eleanor and Harriet Rivers and the least in Middle and Western Rivers. Euryhaline fish represent migratory fish.

A significant estuary \times contingent interaction was found for the amount of time spent in each of marine and estuarine waters (Table 4.4). Stenohaline fish always spent more time in marine waters than euryhaline fish, whereas euryhaline fish spent more time in estuarine waters than stenohaline fish. For each estuary there were always significant differences between the amount of time euryhaline and stenohaline fish spent in either marine or estuarine waters. Significant differences

were found between the amount of time euryhaline and stenohaline fish spent in freshwater, but this did not vary by estuary (Table 4.4).

Table 4.4. Permutational ANOVA of amount of time spent in marine, estuarine and freshwater habitats for black bream, *Acanthopagrus butcheri*, from six estuaries (Estuary, Est) on Kangaroo Island, South Australia, and for two contingents (Con, stenohaline and euryhaline).

Source	df	MS	F	P
Marine				
Estuary	5	734.260	2.418	0.036
Contingent	1	21680	71.401	0.001
Est × Con	5	777.760	2.562	0.023
Residual	273	303.630		
Estuarine				
Estuary	5	1.450	2.45	0.033
Contingent	1	252.180	431.240	0.001
Est × Con	5	1.411	2.412	0.030
Residual	273	0.585		
Freshwater				
Estuary	5	1.607	1.592	0.149
Contingent	1	50.372	49.897	0.001
Est × Con	5	1.607	1.592	0.167
Residual	273	1.010		

In addition to providing evidence of habitat occupancy, the otolith Ba:Ca profiles were examined for evidence of patterns of habitat change in individual fish. Among the 106 fish categorised as stenohaline, there were either no or irregular patterns of change as these individuals had only occupied marine habitat. Among the 179 fish categorised as euryhaline, there was evidence of between one and nine habitat changes throughout the fish's life. For the euryhaline fish, the patterns of habitat change varied between estuaries (Table 4.5). For example, 19 euryhaline fish from South West River occupied all three habitats (Table 4.5), contrasting with the euryhaline fish from Middle River where only 2 fish moved through the three habitats and the rest of the fish (n=11) moved between marine and estuarine water (Fig. 4.2; Table 4.5).

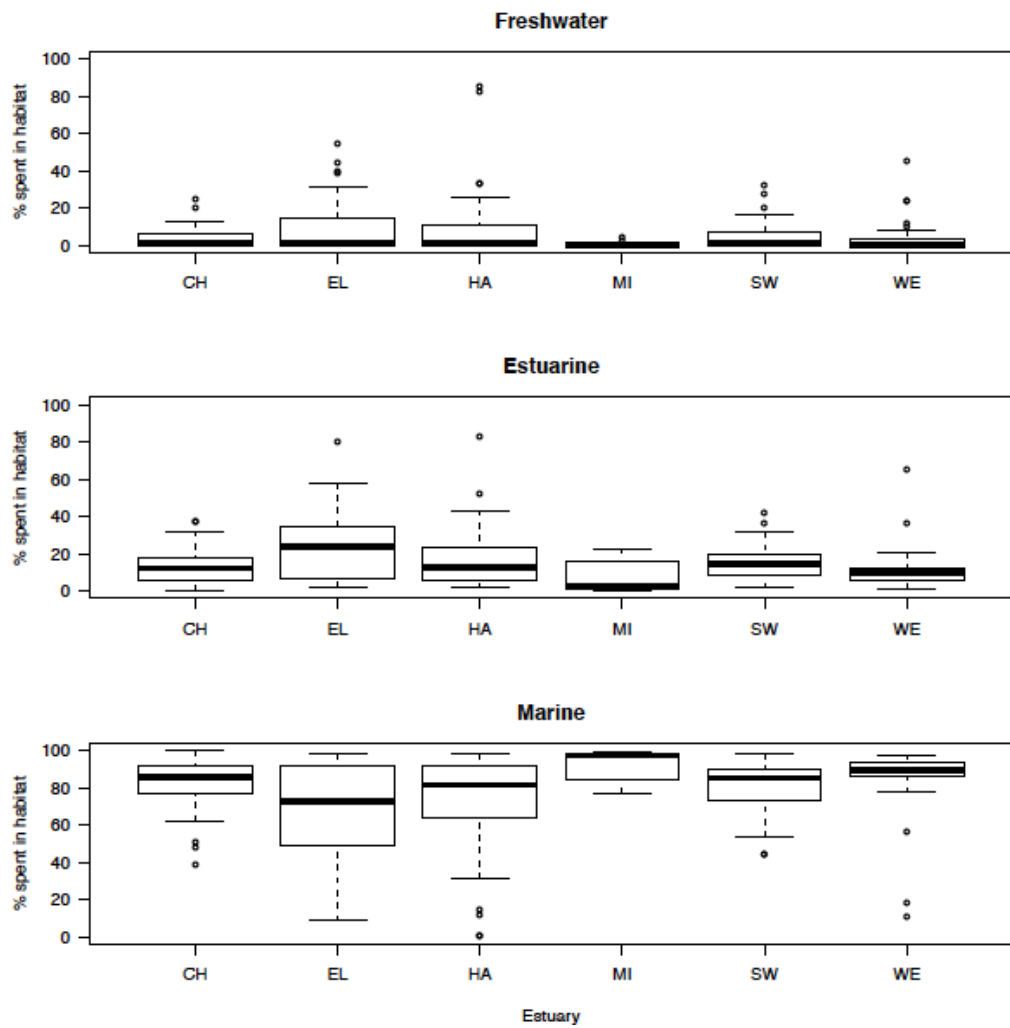


Figure 4.3. Box plots of proportion of time spent in fresh, estuarine and marine water for euryhaline black bream (*Acanthopagrus butcheri*) from six estuaries (CH, Chapman River; EL, Eleanor River; HA, Harriet River; MI, Middle River; SW, South West River; WE, Western River) on Kangaroo Island, South Australia. Shown are the median (solid horizontal line), lower and upper quartile (box), minimum and maximum values (within $1.5 \times$ the inter quartile range; whiskers) and outliers ($> 1.5 \times$ the inter quartile range).

Table 4.5. Euryhaline history of black bream, *Acanthopagrus butcheri* (with hybrids in brackets) from six estuaries of Kangaroo Island, South Australia. Shown is the number of fish having lived in a marine plus estuarine environment and in three environments (marine, estuarine and fresh) for all fish. Occupancy of an environment was deduced from Ba:Ca of otoliths. Refer to Table 4.1 for estuary codes.

Estuary	Marine + Estuarine	Marine + Estuarine + Fresh
WE	17	13 (1)
SW	16	19
EL	21 (3)	21 (6)
HA	16 (2)	11 (3)
CH	18 (1)	14
MI	11 (1)	2

Hybrid fish and environmental histories

The stenohaline hybrid fish were only found in two estuaries, Harriet and Middle Rivers, and in contrast to most of the non-hybrid stenohaline fish, the otolith data indicated they did not spend time in estuarine water, thus, they spent 100% of their life histories in marine waters (Table 4.3). Euryhaline hybrid fish were found in five estuaries and habitat occupancy was similar to non-hybrid euryhaline fish. The single hybrid fish (~4% of hybrid fish sampled) from Western River had spent 24% of its time in freshwater, which was well above the mean seen for the non-hybrid euryhaline fish (Table 4.2, 4.3). Figure 4.4 shows the variation seen between a euryhaline and stenohaline hybrid fish from the Harriet River. Euryhaline hybrid fish from Harriet River and Eleanor River appeared to move between the three salinity habitats (Table 4.5), but still spent the majority of time in marine waters. Only a small proportion of time was spent in freshwater habitats (<10%, Table 4.3).

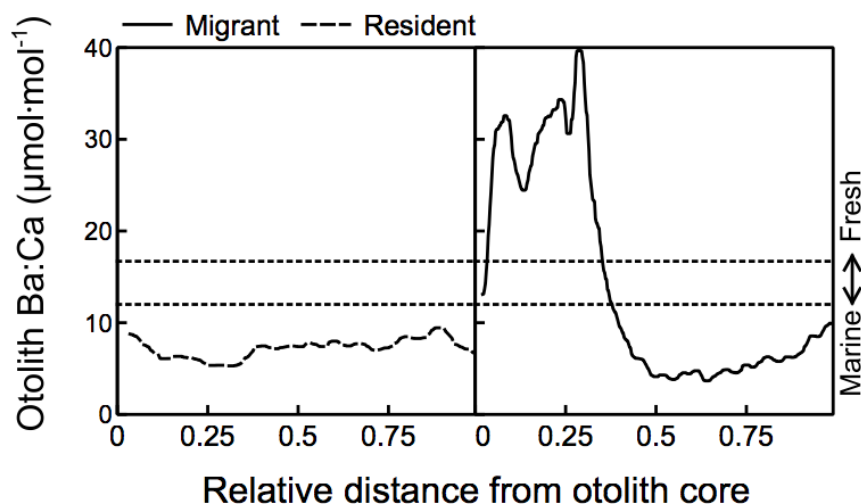


Figure 4.4. Representative stenohaline (indicated as resident in the figure) and euryhaline (indicated as migrant in the figure) otolith Ba:Ca profiles from individual hybrid bream from Harriet River (HA), Kangaroo Island, South Australia. Profiles are plotted on a relative scale from 0 (otolith core) to 1 (otolith edge). Two representative profiles are given: one for a fish that migrates between marine and freshwater (right), and one for a fish resident in marine water (left). Fish were 130 mm SL (resident fish) and 152 mm SL (migrant fish).

Discussion

Using Ba:Ca ratios of otoliths we were able to show that fish from six estuarine populations exhibited partial migration with both stenohaline and euryhaline fish occurring in each estuary. Otolith chemistry has previously been used to determine partial migration in fish species. Most studies have used Sr:Ca (e.g. Tzeng et al. 2003, Kerr et al. 2007, Kerr and Secor 2012) to identify migratory contingents, although Ba:Ca has also been used (Elsdon and Gillanders 2005, Gillanders et al. 2015). Indeed, the majority of individuals from five of the six estuaries were migratory (euryhaline) with individuals spending most of their lives in marine and estuarine environments. Thus, some individuals choose to migrate (euryhaline contingent) and others remained in estuaries (stenohaline contingent). Euryhaline and stenohaline contingents were not related to whether fish were hybrids or not. Hybrid fish were found in most estuaries, and these fish represented both contingent types. Hybridisation between black bream and yellowfin bream occurs on the east coast of Australia with hybrids reported as far south as the Gippsland Lakes in Victoria beyond the range of yellowfin bream (Roberts et al. 2009, Roberts and Ayre 2010, Roberts et al. 2010a, Roberts et al. 2010b, Roberts et al. 2011). Our study is the first report of hybrids from South Australian waters.

Ba:Ca in otoliths of black bream

Ba:Ca in otoliths was negatively correlated with salinity, which is similar to other studies (e.g. Elsdon and Gillanders 2005, Hamer et al. 2006). Using the relationship between otolith Ba:Ca and salinity for Kangaroo Island estuaries we were able to ascertain the environment that fish spent time in. Kangaroo Island estuaries are wave-dominated with limited freshwater input, especially in summer when sampling was done (salinity ranged between $26.25 \pm 1.01\text{‰}$ in Eleanor River and $42.97 \pm 1.94\text{‰}$ in Middle River (mean \pm SE)). These estuaries typically have higher salinity than seawater values during summer when rainfall is low and evaporation high (Gillanders et al. 2011). It is not surprising that fish spent virtually no time in freshwater and the majority of time in marine waters for both contingents. Stenohaline (resident) fish within estuaries would be expected to have largely marine signatures and only during winter when there is some freshwater input would we expect an estuarine signature. Euryhaline (migratory) fish generally showed evidence of inhabiting all three environments. Elsdon and Gillanders (2005) also found that resident fish were predominantly in marine waters and migratory fish were in all three environments. They found that some fish spent their entire life in freshwater which differs from our study, although they targeted freshwater systems in their sampling.

The salinity range in which the relationship between Ba:Ca and salinity was determined in our study was much less than that of Elsdon and Gillanders (2005). Our sampling was only undertaken in summer limiting estuarine sampling to when waters were largely marine in their salinities. In contrast, Elsdon and Gillanders (2005) sampled in both summer and winter and across a wider range of estuaries than just Kangaroo Island, sampling freshwater habitats by selecting two freshwater locations in each season. Our sampling occurred during a period of extensive drought further limiting the salinity range of our estuarine environments. Given conditions in the estuaries had likely changed between the two studies and the fact that our study was focused on Kangaroo Island estuaries we chose to determine our own Ba:Ca-salinity relationship.

Ba:Ca in otoliths may reflect differential bioavailability in freshwater and marine waters. Barium is 99% free Ba^{2+} in freshwater, but only 86% free from binding with other compounds (e.g. BaCl_2 , BaSO_4 , BaCO_3) in marine waters (Turner et al. 1981). Barium is also bound to sediment and estuaries may receive sediment inputs from runoff during winter periods (Li and Chan 1979). Barium can also vary considerably among locations largely due to differences in bedrock geology, fluvial input and differential release from sediments (Elsdon and Gillanders 2005). Given potential differences in barium associated with locations and salinity it is not surprising that there was variation in Ba:Ca in otoliths.

Variation in contingents among estuaries

Understanding movements of fish across estuaries is necessary for determining habitat use and connectivity. Variation in movements of fish among estuaries has previously been reconstructed using Sr:Ca, as there is often a positive relationship between salinity and otolith Sr (Secor 1999, Secor and Rooker 2000). However, varying relationships between environment and Sr:Ca ratios have been found (Elsdon and Gillanders 2002) and Sr in freshwater can be greater than that of marine waters (Kraus and Secor 2004b). Strontium isotopes have also been used to investigate movement between freshwater and marine waters (Kennedy et al. 2000, Kennedy et al. 2002, Huey et al. 2014), but greatest variation in strontium isotope ratios is usually found at low salinities (<5‰). Thus, both Sr:Ca and Sr isotopes are more useful for tracing movements in estuaries with lower salinities. Other tracers are required for estuaries which typically have marine salinities such as those found on Kangaroo Island, hence our use of Ba:Ca in otoliths. Ba:Ca has previously been used to understand variation in contingents among estuaries (Elsdon and Gillanders 2005, Hamer et al. 2006).

All estuaries showed evidence of both stenohaline and euryhaline contingents. A previous study on black bream using smaller sample sizes (between 2 and 8 fish per site and season) suggested evidence of both contingent types which they termed migratory and resident, but it was not clear whether both types were found in all estuaries sampled (Elsdon and Gillanders 2005). Both contingents were however found in the Glenelg River (Elsdon and Gillanders 2005). Around 63% of all fish in our study were euryhaline (equivalent to migratory), although this varied between 26% (Middle River) and 84% (Eleanor River) when investigated at the estuary level. A previous study of black bream in the Coorong found that 38% were migratory and 62% resident (n=173 fish); contingent type was not related to size, age or sex of fish as both contingents were generally found across all size, age and sex classes (Gillanders et al. 2015). Rainbow trout (*Oncorhynchus mykiss*) also show considerable variability in the proportion of fish which are anadromous, with the proportion decreasing with increasing drainage area of streams (McPhee et al. 2007). Similarly, the contribution of resident versus migratory juveniles of white perch (*Morone americana*) varied not only among estuaries, but also among years (Kerr and Secor 2012).

Euryhaline (migratory) fish made between 1 and 9 changes between environments, which is similar to the 2 to 6 changes made in an earlier study (Elsdon and Gillanders 2005). Such changes do not appear to be related to the length of the fish and were unlikely related to the age of the fish as patterns are largely irregular. Elsdon and Gillanders (2005) found that a few fish showed cyclical patterns and that this corresponded with annulus position potentially suggesting anadromous movements, but the majority of fish showed irregular patterns.

Are euryhaline fish hybrids?

Black bream typically complete their life cycle within estuaries, whereas yellowfin bream are coastal moving between estuarine and marine waters. Hybrids of the two species are well known from south-eastern Australian estuaries including as far south as Gippsland Lakes (Roberts et al. 2009, Roberts et al. 2010b, Roberts et al. 2011). We predicted that euryhaline fish may represent hybrids. Our prediction was not upheld as only 63% of hybrid fish were euryhaline, similar to the overall estimate for all black bream. Thus a reasonable proportion of hybrid fish were stenohaline (likely residents) within an estuary, and these fish occurred in two of the six estuaries we sampled, but had only occupied marine salinities. Given stenohaline hybrid fish were found, these likely represent second generation or later fish. All hybrid fish in three of the estuaries were euryhaline, although sample sizes for two of these estuaries were small with only one individual found. For the remaining estuary, Eleanor River, all nine hybrid fish were euryhaline potentially representing first generation fish.

Microsatellite genotyping identified hybrid *Acanthopagrus* spp. in five of the six estuaries sampled. Hybrids represented less than 10% of the total population, but ranged from no fish in Southwest River to almost 20% in Harriet River. Along the eastern coast of Australia greater than 90% of *Acanthopagrus* were hybrids within two lagoons closed to the ocean compared to 12 to 27% in estuaries that were either permanently or intermittently open to the ocean (Roberts et al. 2010b). *Acanthopagrus* hybrids occurred in 68% of 25 estuaries surveyed in south-eastern Australia (Roberts et al. 2009). Our study represents the most westerly position that hybrids have been detected. Thus, hybrid bream have reached estuaries which were previously thought to be inhabited by pure black bream. Hybridisation may introduce genetic variation and reduce inbreeding depression, but high levels can cause demographic and genetic swamping (Roberts et al. 2010b).

We did not investigate whether there were demographic differences between hybrid and pure black bream populations. Such an investigation would likely require additional hybrid fish from the estuaries sampled. A previous study in two eastern Australian estuaries found no differences in population structure, growth and maturity between hybrid and pure black bream leading to the suggestion that the two groups had similar life histories (Ochwada-Doyle et al. 2012). Differences in growth did however occur between the two estuaries (Ochwada-Doyle et al. 2012). No investigation of whether partial migration occurs in these populations was made.

Few studies have investigated if there is a genetic basis to partial migration in fishes. Instead studies usually examine whether migratory and resident fish show differences in population structure. For example, using microsatellite DNA, strong differentiation of freshwater resident populations of lampreys (*Lampetra* spp.) was found, which contrasted with anadromous

populations where little structure was found (Bracken et al. 2015). Several salmonid studies suggest that genetic diversity is higher in anadromous versus non-anadromous populations (see McPhee et al. 2007). Dodson et al. (2013), however, suggest that allopatric populations of anadromous and non-anadromous salmon may be genetically differentiated, but that it is unlikely that sympatric populations would be unless spawning sites were differentiated. Their review concludes that genetic divergence among alternative migratory phenotypes of salmonids is unlikely. Rapid advances in next generation sequencing may allow traits associated with alternative migratory strategies to be ascertained (Kitano et al. 2012).

Consequences of partial migration

Research into the consequences of partial migration has been less well studied despite potential ecological and evolutionary consequences and implications for conservation and management (Chapman et al. 2012b). From an ecological viewpoint an increasing proportion of euryhaline or migratory fish in a population leads to increased nutrient transport and trophic effects. The most widely studied effect is that of salmonids transporting nutrients from marine waters to freshwater ecosystems and the reverse pattern (Naiman et al. 2002). Top-down effects where migratory species affect their prey as well as potentially causing effects that cascade down the food chain are possible (Chapman et al. 2012b). Similarly, predators and competitors of partial migrants may also be affected by their movements since these may influence abundances (Chapman et al. 2012b). There has been little research assessing ecological consequences of partial migration and we made no attempt to assess this in the current study.

We assume that both euryhaline and stenohaline black bream spawn together. Whether differential selective pressures occur for the two contingents is unknown and may depend on whether migratory behaviour is genetically fixed or environmentally driven. Migratory tendency in several other species is a phenotypic trait driven by environment (e.g. white perch, Kerr and Secor 2012). Additional evolutionary consequences may arise if there is assortative mating between contingents. Partial migration may also be seen as a gradual evolution towards adoption of a completely resident or completely migratory movement type (Pulido 2011).

Significant challenges face migratory and euryhaline fish including energetic and metabolic requirements associated with movement, osmoregulatory challenges, and potentially increased predation risk, lack of food and decreased reproductive output (Sahashi and Morita 2013, Dennenmoser et al. 2014). The trade-off between benefits of moving and costs associated with moving often drives life history divergence. Environmental instability is thought to lead to partial migration as no one contingent type has a long-term fitness advantage (Olsen et al. 2006). Gillanders et al. (2015) suggested that fish may move to avoid deteriorating environmental conditions. They were able to show that while average growth of fish was similar between the two

contingents, there were substantial year-to-year differences in growth. In addition, resident fish growth declined through time in line with declining environmental conditions, whilst migratory fish growth increased slightly presumably reflecting their ability to find more favourable conditions (Gillanders et al. 2015).

Estuaries are highly productive systems used by a large range of organisms (Beck et al. 2001), but are also under increasing pressure from human activities (Edgar et al. 2000). Euryhaline contingents may move across a range of environments and be more susceptible to environmental impacts, however species exhibiting partial migration may also be buffered from environmental impacts since only part of the population may be affected. Barriers to movement would affect the euryhaline contingent over the stenohaline contingent. The importance of life history diversity should be considered in management plans since diversity can allow for population persistence. Species demonstrating partial migration may be more resistant to disturbance and have a greater capacity to rebuild following environmental and climatic changes (Chapman et al. 2012b). In addition, recreational and commercial fishing has potential to remove only part of a population which may have conservation implications. Thus, there have been suggestions that contingent structure should be incorporated into fishery management plans, although this likely requires higher resolution spatial surveys and sophisticated population models (Kerr et al. 2010a, Kerr et al. 2010b).

Conclusion

This study highlights the application of otolith Ba:Ca to investigate diversity of life history types and partial migration in fish. We were able to demonstrate that hybrid fish occurred in both euryhaline and stenohaline contingents and was therefore unlikely to explain why fish moved across different salinity environments. Further work is required to determine the likely benefits and costs of partial migration in black bream (and fish more generally). The ability of black bream to respond behaviourally to different conditions may be beneficial in a dynamic environment such as an estuary. Indeed, partial migration may be particularly beneficial to estuarine organisms where environmental variability occurs since it allows the persistence of populations when faced with increasing habitat degradation.

Chapter 5. General Discussion

Determining life history strategies and movement patterns of fish is important for defining the appropriate management units (i.e. stocks) for fisheries and conservation. Otolith chemistry has been widely applied to investigate the movements of fish at different life history stages as well as to discriminate among geographically and chemically dissimilar populations (e.g. Elsdon et al. 2008). In this thesis, I use otolith chemistry as a natural tag to explore life history strategies and movement patterns of black bream (*Acanthopagrus butcheri*, Munro 1949) populations on Kangaroo Island (KI) and the Fleurieu Peninsula (FP) in South Australia. Specifically, I assessed the spatial and temporal stability of elemental tags in the otoliths of juvenile (0^+ year of age) fish within estuaries (Chapter 2). Examining spatial differences at a range of scales (i.e. between estuaries and among regions), I was able to enhance classification accuracies (Chapter 2), which allowed the retrospective assignment of juvenile and subadult fish ($< 3^+$ years of age) to their putative juvenile areas. As such, these data formed the basis for investigations into inter-annual patterns of recruitment of juvenile black bream to the adult component of the population, both within and between KI and FP (Chapter 3). Inter-annual variations in elemental tags inferred a strong environmental control on otolith chemistry, which I explored by developing a salinity-element (Ba:Ca) relationship (Chapter 4). Using this salinity-Ba:Ca relationship, I was able to reconstruct the salinity environments of individual fish, by marking transitions between marine and freshwater environments, which helped reveal the diversity of migratory patterns in adult black bream. In doing so, I verified that partial migration (where coexisting groups of a population are both potentially both migratory and resident) was found in all of the estuarine populations of black bream examined (Chapter 4) based on their euryhaline versus stenohaline life histories. In addition, I found that the hybridisation between black bream and yellowfin bream did not explain the euryhaline component of the population as originally hypothesised. This chapter provides a general discussion of the main findings presented in this thesis. For expanded discussion of details made here and for further information the reader should consult the relevant data chapters (Chapters 2 to 4).

Spatial and temporal variability in elemental signatures of black bream

Identifying and tracking fish origins and the movements between juvenile and adult populations highlights mechanisms for the long-term survival of fish species (Gillanders 2002a). Estuaries are highly productive ecosystems, used by a large range of organisms (Beck et al. 2001), and for many species of fish, estuaries provide critical spawning and juvenile nursery habitats (Potter et al. 2015). Black bream are an endemic southern Australian sparid that primarily inhabits estuaries and coastal lakes, with some movement of fish between adjacent estuaries and inshore waters (Hindell 2007, Hindell et al. 2008). Black bream are considered to be 'estuarine-dependent' due to their ability to complete their entire or components of life cycle within estuaries (Potter and Hyndes

1999). In South Australia, the greatest abundances of black bream are found in the estuaries and adjacent waters on KI and the FP (Gillanders et al. 2008). Some of these estuaries are separated by up to 150 km from one another. This geographic separation among suitable estuarine habitats suggests that the species' southern Australian distribution is comprised of numerous isolated spawning populations within estuaries (Burrige et al. 2004, Burrige and Versace 2007). More recently, the successful recruitment of juvenile fish into the adult component of many black bream populations in southern Australia has markedly reduced (Nicholson et al. 2008), evident by considerable inter-annual variation in year class strength (Morison et al. 1998, Ferguson et al. 2013). While having implications for fisheries managers (Sarre and Potter 1999), the persistence of some populations post-recruitment failure suggests that there may be some degree of connectivity among adjacent estuaries (i.e. some estuarine populations are receiving contributions of recruits from other estuaries).

In Chapter 2, I explored the use of multielement otolith signatures as a means of discriminating among estuaries for juvenile black bream. Juveniles (0^+ year old fish) were sampled from a number ($n=12$) of estuaries on KI and FP over five years (from 2007 to 2011). Through the quantification of the multielement signature of the juvenile portion of these otoliths, I was able to detect significant temporal and spatial differences among estuaries. However, patterns of variation among estuaries were not consistent across sampling years. Although estuaries or groups of estuaries had unique chemical tags with the overall classification success ranging from 73 to 83%, the classification success for each of the individual estuaries or groups of estuaries was sometimes relatively low meaning that our ability to classify subadult fish would be relatively poor.

Fish with similar multielement tags enable the grouping of estuaries where there is poor initial classification success (Gillanders 2002a, Gillanders and Kingsford 2003). Given the variable classification success of juveniles to their known juvenile estuaries, I subsequently grouped estuaries that had 0^+ black bream with similar elemental tags, which increased classification accuracies (e.g. in 2008 classification increased from 56 to 73%). Estuaries have previously been grouped based on geographical proximity, such as species with continuous distributions (e.g. Clarke et al. 2009). Rarely do researchers investigate whether grouping of estuaries increases classification success by an amount greater than that expected by chance alone given the reduction in number of groups being considered. In Chapter 2, I grouped estuaries based on similarly from statistical analyses and only grouped estuaries if there was an increase in allocation success that was greater than that expected by chance alone. Several studies have suggested advanced statistical approaches for retrospectively assigning fish to larval or juvenile source populations (White et al. 2008, Mercier et al. 2011, Neubauer et al. 2013). Based on my findings in Chapter 2, I suggest that grouping estuaries based on *post-hoc* statistical analyses (i.e. pairwise comparisons and, or classification scores) provides an appropriate means of improving the discriminatory power of otolith chemistry.

Increasing the accuracy with which fish can be assigned to individual estuaries is required for accurate allocation of subadult fish to individual estuaries. I used trace elements (six element:Ca ratios), but future research could investigate the use of stable isotopes (e.g. oxygen, strontium) or integrating trace elements combined with otolith shape (e.g. Ferguson et al. 2011), genetics or tissue stable isotopes. As the number of estuaries that need to be distinguished increases, the number of variables used for distinguishing estuaries will also need to increase.

Connectivity between populations of black bream on the Fleurieu Peninsula and Kangaroo Island

Analysis of the otoliths of juvenile (0^+ year old) black bream confirmed that it was possible to discriminate among stocks of fish living in broad regions (Chapter 2). Hence, in Chapter 3 I used the juvenile otolith chemistry data as baseline signatures, to define spatially and temporally variable multielement signatures that permit the accurate classification of subadult black bream to their putative juvenile region. Several studies have established baseline elemental signatures for stock discrimination and assessment of connectivity (e.g. Thorrold et al. 2001, Gillanders 2002a, Reis-Santos et al. 2013b), but few investigate multiple year classes or how connectivity changes through time.

In order to accurately classify adult fish to the juvenile regions based on otolith chemistry, consideration of the year class is required if there is temporal variability in elemental signatures to ensure that the juvenile portion of the otoliths is temporally matched to the correct year class of elemental signatures (Gillanders 2005b). If these criteria are not met, then efforts to classify fish back to their juvenile regions may be confounded by potential inter-annual variability in elemental signatures. In Chapter 2, I demonstrated that there was significant temporal variation in the multielement composition of juvenile (0^+ year old) otoliths. Therefore, to overcome this potential temporal confounding factor, I developed estuary/region specific baseline element otolith signatures over a five year period (from 2007 to 2011). In this way I could temporally match the core region in the otoliths of subadult ($<3^+$ year old) black bream collected over four sampling years. For example, a 2^+ year old fish caught in 2010 was classified based on the 2008 baseline signatures (see Chapter 3). In doing so, I was able to estimate the proportion of juvenile fish that recruited from KI and FP through time and also estimated temporal patterns of connectivity between the two regions (Chapter 3).

For retrospective assignment of fish to estuaries, an assumption is made that fish from all potential putative juvenile estuaries have been sampled (Gillanders 2005b). Although we endeavoured to sample all estuaries in the vicinity of KI and FP, some may have been missed. Also, due to logistical limitations (i.e. monetary, time and inaccessibility), I was unable to obtain samples from estuaries further afield that may be recruitment areas for black bream in KI and FP. However,

using a 95% confidence ellipse fit to the multielemental composition of juvenile (0^+ year old) otoliths, I used a conservative approach to remove those potential subadult fish that originated from estuaries that were not sampled in the course of my candidature. Overall, these unknown areas contributed between 10 and 63% of subadult fish depending on the cohort and age of fish. However, there appeared to be a trend of increasing detection of fish from unknown regions with increasing age of fish, suggesting that the potential migratory range of black bream increases with age.

Estimation of the relative contributions of KI and FP to the recruitment of juvenile black bream (1 to 3^+ year olds) to the adult population varied among the four years surveyed. Overall, there was variability among cohorts of the same age, among age classes within a cohort and between regions in terms of self-recruitment (i.e. whether fish were caught in their region of origin) (Chapter 3). My results highlight the variable nature of connectivity among populations and recruitment to adult populations. They do, however, suggest that there were significant levels of connectivity between mainland South Australia (FP) and KI.

Inter-annual variation in local physicochemical environmental conditions may influence both the numbers of fish that initially recruit within an estuary and the subsequent movement and patterns of connectivity between regions for subadult fish. For example, many of the KI estuaries are relatively small often reaching hypersaline levels during summer and having their entrances closed by sandbars thereby restricting movement between the estuary and open ocean.

Partial migration of black bream on Kangaroo Island

Behavioural responses of fish due to environmental variability (e.g. estuarine eutrophication, climate change) can also alter fish habitat preferences and changes to distribution (i.e. range shifts) (Booth et al. 2011). Thus, environmental changes may influence the life history characteristics of black bream, and the ability to respond to those changes (Gillanders et al. 2011). However, as KI estuaries are largely unmodified, wave-dominated coastal creeks (Gillanders et al. 2008), isolation can make some areas of estuaries hypersaline through evaporation during summer months (Heap et al. 2004), affecting black bream population dynamics. Black bream from KI estuaries exhibited partial migration, whereby part of the population is stenohaline (potentially resident) and another part is euryhaline (migratory) and the proportion migrating varied by estuary (Chapter 4).

In Chapter 4, I demonstrated that Ba:Ca concentrations in otoliths of black bream were significantly related to ambient salinity. I described a relationship between otolith Ba:Ca and salinity specific to KI estuaries, which encompassed marine salinity values. I demonstrated that when Ba:Ca was $\leq 12 \mu\text{mol}\cdot\text{mol}^{-1}$, black bream could be classified as inhabiting a marine environment. Conversely, otolith Ba:Ca concentrations $\geq 16.7 \mu\text{mol}\cdot\text{mol}^{-1}$ indicated that black bream were in a freshwater environment. Ba:Ca concentrations between 12 and $16.7 \mu\text{mol}\cdot\text{mol}^{-1}$

were assumed to represent a transitional estuarine signal consistent with other studies in South Australian estuaries. I acknowledge that whilst these values represent fish inhabiting freshwater, estuarine and marine waters, they may not necessarily indicate that the fish have moved into these waters. The fish may actually be stationary and the water masses the fish inhabit may change, for example, in response to freshwater input. Given there were both stenohaline and euryhaline fish in the same estuaries based on otolith Ba:Ca this is suggestive that at least some fish are moving. At the very least these data demonstrate that black bream inhabit a range of salinities.

The resultant salinity-Ba:Ca relationship was then related to Ba:Ca profiles across otoliths of black bream to reconstruct the salinity histories of individual black bream throughout their entire lives. These salinity histories were also used to mark the number of transitions between marine, estuarine and freshwater habitats (Chapter 4). I observed partial migration among the individuals from all six estuarine populations sampled, although for most estuaries there were greater numbers of euryhaline than stenohaline black bream (Chapter 4). Within estuarine-dependent species, partial migration, has been observed in white perch populations, where part of the population inhabits freshwater environments as residents and the other portion migrates into higher salinities waters (Kerr et al. 2009, Kerr et al. 2010a, Kerr and Secor 2012). These unpredictable changes in migration patterns may allow species to persist in systems where physical processes such as estuarine mouth closure due to drought would otherwise limit or prevent species survival (Closs et al. 2003). Therefore, understanding partial migration within estuarine populations as well as the potential causative drivers of this phenomenon is crucial for fisheries and ecosystem management.

In Chapter 4 I used microsatellite genotyping to explore whether there was a genetic basis for the occurrence of partial migration within populations of black bream, namely that euryhaline fish represented hybrids between black and yellowfin bream. It is known that black bream generally completes its entire life cycle within estuaries spawning in the middle to upper reaches of the estuary (Haddy and Pankhurst 2000, Roberts et al. 2009), whereas yellowfin bream is highly mobile moving to entrances of estuaries or adjacent to estuaries for spawning (Roberts and Ayre 2010). Yellowfin bream uses estuaries as nursery habitat for juveniles, but then moves between coastal and estuarine waters (Roberts et al. 2009). Thus, there is potential for hybridisation and anthropogenic impacts have potentially increased the likelihood of hybridisation (Roberts et al. 2009).

Hybridisation between the two species is widespread along the east coast of Australia (Roberts et al. 2009, Roberts et al. 2010b, Roberts et al. 2011). Through microsatellite genotyping, I identified hybrid *Acanthopagrus* (*Acanthopagrus australis* × *Acanthopagrus butcheri*) individuals in five of the six estuaries sampled, with hybrids on KI being the most westerly range of detection. This may suggest that hybridisation and introgression between *Acanthopagrus* species has reached areas previously unknown, raising questions about the evolution and conservation of pure black bream in

South Australia. Further genetic analyses are required to understand the prevalence of hybridisation in *Acanthopagrus* throughout its southern Australian distribution.

I hypothesised that hybrid *Acanthopagrus* would most likely possess a euryhaline life history strategy and that this may explain partial migration in black bream. Reconstructions of the salinity histories of black bream based on Ba:Ca profiles indicated that partial migration was also found among the hybrids and that hybridisation likely did not explain partial migration in black bream populations. Overall, I found that 63% of hybrids possessed a euryhaline life history strategy, with patterns of salinity habitat occupancy similar to those of non-hybrid migrant fish (Chapter 4).

Thus, these findings suggest that there is not a genetic basis for the occurrence of partial migration in black bream. In addition, identification of stenohaline hybrid *Acanthopagrus* infers that these fish are at least second generation hybrids (i.e. they originated in the estuary that they have remained resident within as opposed to having migrated there). Roberts et al. (2010b) also reported that most hybrids from coastal lagoons in New South Wales were later generation hybrids which are likely multigenerational residents within lagoons. This provides indirect evidence of the viability of the hybrid *Acanthopagrus* (see also Roberts et al. 2010a). More information is required on the causes and consequences of partial migration in fish.

Future Research Directions

Throughout this thesis, I focused on using the elemental composition of otoliths to better understand the life history strategies and movement patterns of estuarine black bream. In general, I detected a large degree of temporal and spatial variation in the otolith element signatures, which if not appropriately accounted for, may confound interpretations of the otolith-derived connectivity data. However, results from this study highlight a number of specific areas that require future research.

- In Chapters 2 and 3, I showed that there was significant spatial differentiation among estuarine associated populations of black bream in South Australia. However, the findings of these Chapters also indicated that there was a high degree of inter-annual variability in elemental signatures. I suggest that molecular approaches to assessing the population structure of black bream in South Australia and more widely throughout the species' southern Australian distribution would provide a long-term (i.e. evolutionary) perspective of population structure. Previous genetic investigations of the population structure of black bream have focused on the western (Chaplin et al. 1998, Yap et al. 2000) and eastern part of Australia (Farrington et al. 2000, Burrige and Versace 2007). These studies were often limited to investigating the between estuary level of separation among populations (e.g. Chaplin et al. 1998). Next generation approaches where thousands of markers can be sequenced or analysing loci under selection might provide additional spatial resolution or

reveal relationships with environmental variables or life history traits (e.g. those associated with migratory or resident individuals).

- In Chapter 3, I described patterns of connectivity between the juvenile (0⁺ year old fish) and subadult component of black bream populations. However, there still remains a paucity of information on the habitat use of juvenile black bream, in particular, knowledge on the duration of residency of juveniles and timing of movements up and down the estuary/river and how these movements may relate to environmental changes. Otolith chemistry could help address this gap, and would likely be the most appropriate methodological approach given the logistical constraints of working with small fish (i.e. not conducive to artificial tagging). Element (transect) profile analyses of the otoliths of juvenile fish may be resolved to a daily time scale. Based on these analyses, insights into early life history movements may be gleaned. Additional information on movements of adult fish may be obtained from acoustic tags; such data may also help validate the indirect methods of otolith chemistry.
- In Chapter 4, I demonstrated that partial migration and patterns of habitat use by pure black bream and hybrid *Acanthopagrus* were similar implying that partial migration is not related to hybridisation. Further research is required to better understand the drivers and benefits/consequences of partial migration of fish in general. Resolving elemental profiles (on which characterisation of partial migration are based) against the date of increment formation will enable transitions between salinity environments to be related to corresponding environmental records. In such a way, it might be possible to assess whether there is an environmental basis for the movement of fish. However, it is likely that a range of direct and indirect effects acting upon individual fish may promote migration and, or residency, making the assessment of potential drivers difficult.

Conclusion

Otolith chemistry data have provided greater insight into patterns of connectivity between broad geographic regions and life history of black bream in South Australia. Spatial and temporal variability in otolith chemistry of juvenile fish allowed subadult fish to be retrospectively traced to their region of origin as juveniles. Considerable variation in estimated contribution among years and between age classes suggests that there may be environmental drivers that affect the early life history and juvenile recruitment that subsequently influences adult populations. Similarly, assessment of life history via the use of otolith Ba:Ca profiles suggested that partial migration was important for all estuarine black bream populations investigated and that this was not related to hybridisation with yellowfin bream. Results from this study highlight the importance of long-term data for investigating movement and life-history patterns of black bream populations. While

anthropogenic factors may result in degradation of important recruitment areas for juveniles, population dynamics and movement are key to understanding the relative sources of juveniles to the population and the degree of connectivity between adjacent populations through time and space.

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