Population structure of a predatory demersal fish (*Argyrosomus japonicus*, Sciaenidae) determined with natural tags and satellite telemetry

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Thomas Chaffey Barnes

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Cover image: Every anglers dream – photo courtesy of Michael Hammer

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Acknowledgements

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Abstract

Predatory demersal fish are declining on a global scale but in many cases there is a lack of ecological information to assist management agencies in reducing the decline. Information on population structure and connectivity is central to our ecological understanding, not just for fisheries biology but also more broadly for conservation and persistence of species. Natural tags and satellite telemetry can help address population structure questions. Various tags were used to investigate population structure and connectivity of the important mulloway (*Argyrosomus japonicus*) in southern Australia.

Fifteen microsatellite markers were developed to help determine the genetic population structure of mulloway. These markers were tested, and found to be useful, on other members of the family sciaenidae and hence can be used to investigate molecular structure in other sciaenids. Within mulloway, broad scale genetic structuring between South African and Australian mulloway was evident. Within Australia, four genetic populations across the range of mulloway were also evident. Biogeographic factors drove population structuring, as determined using decomposed pairwise regression, a rarely applied approach in the marine environment. Furthermore, our results form a growing body of evidence suggesting population structure is possible in the connected marine environment.

The various natural tags have different advantages and disadvantages, hence, by applying multiple approaches concurrently a greater scrutiny of population structure can be achieved. However, there is continuing debate on how best to integrate multiple approaches and thus condensing and producing simplified results ready for managing agency interpretation. Three approaches, namely, microsatellites, otolith chemistry and otolith shape were applied to the same mulloway samples. These three approaches are each affected by different intrinsic and extrinsic factors and are informative over different temporal scales. Thus, an overall integrated tag was obtained that provides a broader 'body of evidence' to population structure. The results also demonstrated that

integration of different data sets was feasible prior to statistical testing, facilitating a single overall result. However there were some slight differences in the results produced by the different approaches, which highlights the need for a thorough understanding of the influence of intrinsic and extrinsic factors on natural tags.

To properly interpret data from natural tags it is useful to understand factors contributing to variation in the tag. Otolith chemistry is a widely used natural tag, but differing responses to environmental parameters have been reported. Temperature and salinity were manipulated in a controlled laboratory setting along with intrinsic (population) differences and their effects on the otolith chemical tag investigated. A significant influence of genetic population on otolith chemistry was found and also evident was a response to temperature. This understanding of mulloway otolith chemistry will enable more accurate estimation of environmental patterns and supports the view that the otolith chemical tag is more complex than previously thought.

Satellite telemetry can be used to track fish movement while also logging environmental information. This technology has been used on pelagic and benthic predatory fish, but has not been used on demersal finfish. Whilst the natural tags can provide indirect evidence of movements, satellite telemetry has the ability to provide direct information on fish movements. Satellite telemetry was utilised on an isolated population of mulloway to provide information on the spatial scale of movements of mulloway; this information was related to movement in and out of marine parks. The results demonstrated the medium spatial scale of movements (up to 500 km) was similar to other predatory demersal fish and whilst undergoing these movements the fish interacted with marine park boundaries. Thus satellite telemetry was a useful approach for understanding demersal fish population dynamics.

Several approaches were successfully implemented to investigate population structure and movements of a large predatory fish, mulloway. A better understanding of population structure could assist management of some species. An enhanced understanding of predatory fish populations

and the associated scientific approaches used to study such populations is urgent, due to the worldwide decline.

Chapter 1: General introduction

Background

Numerous definitions of stock or population (hereafter referred to as population) exist. Most definitions indicate that populations should be self-replenishing, show similarities in life history and have definable boundaries, which is pertinent for management (Pawson and Jennings 1996; Begg et al. 1999; Begg and Waldman 1999; Secor 1999). Understanding population structure is vital to assess species response to anthropogenic and environmental changes (Begg et al. 1999; Ovenden et al. 2009; Rijnsdorp et al. 2009; Ciannelli et al. 2013). For example, spatially structured populations may demonstrate differences in growth, mortality, age and maturity (Pawson and Jennings 1996). Exploited populations may be sympatric (structured), source-sink metapopulations (complex structure) or panmictic (no structure) (Table 1), with each population structure type responding differently to extrinsic forces (e.g. loss of habitat) (Ciannelli et al. 2013).

Table 1: Types of populations based on their genetic and demographic characteristics (B: per capita birth rate, D: per capita death rate, E: per capita emigration rate, I: per capita immigration rate), with case studies (adapted from Ciannelli et al. (2013)).

Type/genetic	Demography	Ecotype/case study	Case study reference
Sympatric discrete populations/structured	B + D >> E + I	Demersal/*Theragra chalcogramma*	(Bailey et al. 1999)
Complex metapopulation/homogeneous and weakly structured	B + D $\geq$ E + I	Demersal/*Gadus morhua*	(Kelly et al. 2009)
Panmictic/homogeneous	B + D << E + I	Pelagic/*Clupea harengus*	(Slotte and Fiksen 2000)

There are numerous approaches, which include natural and artificial tags, used to answer questions on population structure of demersal fish. Each approach brings its own unique set of information in terms of ecological temporal scale and blend of intrinsic and extrinsic forces (Begg and Waldman 1999). Commonly used approaches include: molecular genetics, hard part (particularly otolith) trace

elemental chemistry, morphometrics (particularly otolith shape), artificial tags, parasite morphology and genetics, physiological traits and life history traits.

The natural tags

In the following section I describe and review the approaches or tags that I employ to investigate population structure and connectivity. These include molecular genetics, otolith chemistry and shape, satellite telemetry and holistic or an integrated approach. The review does not include all known approaches, rather just the cutting edge approaches utilised in this thesis.

Molecular genetics

Recent advances in technology have increased the utility of genetics as a tool to investigate fish population structure (Sunnucks 2000; Chevolot 2006; Selkoe 2006; Schwartz et al. 2007; Catalano et al. 2014; Alanis-Lobato et al. 2015). Traditional approaches such as protein electrophoresis lacked the resolution to find structuring of fish populations in the marine environment due to low variability (Campana and Casselman 1993; Ward and Grewe 1994). The low variation resulted in a lack of ability to detect weak structuring which is often the case in marine waters where high connectivity can occur (Carvalho and Hauser 1994; but see Andreev et al. 2015). However, the introduction of polymerase chain reactions and next generation sequencing facilitated the successful use of highly variable markers such as microsatellites (Carvalho and Hauser 1994; Ward and Grewe 1994; Jarne and Lagoda 1996; Sunnucks 2000; Van Oosterhout et al. 2006; DeBoo et al. 2012). The natural molecular tag provided by microsatellites is inherited by offspring from their parents and is mainly modified by intrinsic factors via mutations and genetic drift (Koizumi et al. 2006; Ovenden 2013; Agostini et al. 2015). Detectable genetic differences are typically only apparent after a population has been isolated for 100s of generations (Broderick et al. 2011). Hence, the molecular tag operates at long evolutionary timescales (Begg and Waldman 1999; Welch et al. 2015). Thus, when populations are genetically differentiated they are likely to be demographically independent which is central to fisheries and conservation management.

Understanding spatial structuring at the sub-population level can be achieved by assessing the contribution of genetic drift and gene flow. However, traditional spatial analysis such as isolation by distance does not provide such information (Koizumi et al. 2006; Junge et al. 2011). Population level analysis has been readily utilised on freshwater fish populations via decomposed pairwise regression which assesses the merits of gene flow and genetic drift (Koizumi et al. 2006; Huey et al. 2011; Junge et al. 2011; Harris et al. 2015), but this approach has not been widely used on demersal marine fish (but see Cunningham et al. 2009). Barriers to gene flow are more obvious in freshwater environments but are also evident in marine applications (e.g. the North American marine migration barrier - Cape Hatteras) (Lankford Jr and Targett 2001; Baker et al. 2007). Although subtle, marine migration barriers require consideration to thoroughly understand structure and connectivity patterns (Ovenden 2013).

Otolith chemistry

Otoliths are a "hard part" useful for delineating population level differences via variations in chemistry. Otoliths are composed of layers of material which reflects extrinsic and intrinsic factors, but are metabolically inert and hence form a stable natural tag over the fish's entire life (Campana 1999; Campana et al. 2000; Campana and Thorrold 2001; Gillanders 2002; Tanner et al. 2015). Traditionally used for ageing, in a manner similar to counting the growth increments on a cut tree trunk, break throughs in technology in the last 10 to 15 years have further opened their usefulness for fish biology. For example, instrumentation is now available to detect fine differences in trace elements, with precision adequate to sample fish life history; such as the larval phase and the outer edge representing the time of capture (Campana 1999). This ability to sample a chronological record of the fish's life from the otoliths provides unprecedented information (Campana and Thorrold 2001; Gillanders et al. 2003).

A variety of elements have been detected in the otolith but two main trace elements form the natural tag, namely strontium and barium, which are substituted for calcium, the main constituent

(Campana 1999; Doubleday et al. 2013). However, otolith trace elemental chemistry is not constrained to these elements (e.g. lead has been used to trace pollutants) (see Secor et al. 1995). The utility of strontium and barium comes from their predictable response to extrinsic factors; in particular to fluctuations in water chemistry which is linked to salinity (Elsdon and Gillanders 2003a; Elsdon and Gillanders 2005; Gillanders and Munro 2012). However the otolith incorporation of these elements is further modified by other extrinsic factors such as temperature (Kalish 1989; Radtke 1989; Limburg 1995; Thorrold et al. 1997; Elsdon and Gillanders 2002; Elsdon and Gillanders 2003b; Elsdon and Gillanders 2004; Martin et al. 2004; Martin and Wuenschel 2006) and possibly by intrinsic factors (Kalish 1989; Clarke et al. 2011; Sturrock et al. 2015). As trace elements are incorporated whilst a fish grows the population information provided by otolith chemistry is restricted to an ecological timescale (Campana and Neilson 1985) and depends on how the otolith is sampled (e.g. edge, core or entire life history) (Campana 1999; Morris et al. 2003; Elsdon et al. 2008). Nonetheless, when groups of conspecifics experience different environments and hence recording different amounts of key trace elements in the natural tag, they may allow populations to be differentiated.

Whilst otolith chemistry potentially forms a stable and informative natural tag there are some assumptions which require further empirical testing (Campana 1999; Thresher 1999; Morris et al. 2003; Elsdon et al. 2008). The concentrations of the key elements are mainly influenced by salinity and or water chemistry but modified by extrinsic and intrinsic factors; such as temperature (Elsdon and Gillanders 2002), diet (Milton and Chenery 2001) and genetic differences (Clarke et al. 2011). The contributions of these other factors need to be assessed to understand otolith chemistry (Thresher 1999). Furthermore, elemental tags are influenced by interspecific differences, as such validation for any given target species is desirable (Elsdon and Gillanders 2003b; Gillanders and Kingsford 2003; Hamer and Jenkins 2007).

Otolith shape

The shape of otoliths is another useful natural tag for delineating population structure of demersal fish (Monteiro et al. 2005; Ferguson et al. 2011; Leguá et al. 2013). Recent advances in microscopy and software have facilitated economical use of this approach for population studies (e.g. Libungan and Pálsson 2015). The shape of otoliths is species specific, but also varies at the intraspecific level geographically (Campana and Casselman 1993). The geographic variation is thought to be derived from a combination of intrinsic and extrinsic factors namely genetic and environmental (Campana and Casselman 1993; Begg and Waldman 1999; Vignon and Morat 2010). The various contributions of these differences were initially considered unimportant because both genetic and environmental differences are informative for delineating populations (Campana and Casselman 1993) but recently there has empirical testing of these contributions (Vignon 2012). As the otolith is metabolically inert the shape is stable and informative at the generational timescale (e.g. over the fish's lifetime) (Campana and Casselman 1993). However, as there is some genetic influence the ecological timescale could be slightly beyond an individual's lifetime.

The main assumption of otolith shape as a population delineator is the unknown contributions of genetic and environmental factors. As mentioned some authors initially suggested an understanding of these contributions to be unimportant as either factor can differentiate fish populations (Campana and Casselman 1993). However, a better understanding of the relative contributions of genetic and environmental forces will aid in understanding why conspecific otolith shape varies and hence strengthen otolith shape based management decisions (Vignon 2012).

Satellite telemetry

Various artificial tags have provided movement and environmental data on demersal fish that has been useful in delineating population structure and connectivity. Traditionally this has been from mark recapture approaches using conventional tags (Swearer et al. 2002), but more recently data storage tags (DSTs) (Metcalfe 2006). Whilst these approaches have been very informative they are

limited somewhat by the fact the individuals must be recaptured to acquire the data (Armsworthy et al. 2014). However, the recent (decadal) availability of pop-up satellite archival tags (PSAT) has eliminated the reliance on fisheries to gather data (Block et al. 2011). PSATs provide movement information such as net displacement between the capture location and tag pop-up location, with the pop-up location recorded via the ARGOS satellite network. As well as the fish net displacement, the PSAT can archive data on at large movement, water temperature and depth, and survival of the tagged individual. However, this technology has traditionally been mainly used on pelagic fish (Block et al. 1998), but with some recent interest in flatfish (Loher 2008; Peklova et al. 2012; Armsworthy et al. 2014; Roy et al. 2014). The net displacement information has been recorded for some individual fish over temporal periods of days up to two years (Musyl et al. 2011). As such the movement and connectivity information has been utilised on a wide variety of fish and other marine animals to support population structure (Le Port et al. 2012) and conservation objectives such as interactions with marine parks (Palumbi 2004).

Despite PSATs providing unprecedented movement and other ecological information on predatory pelagic fish and benthic flatfish, there have been no published studies on demersal finfish. Furthermore, fish of this habitat preference are hard to track by other means (e.g. spotter planes and GPS) (Sims et al. 2009; Peklova et al. 2012). Hence there is potential for PSATs to provide data on demersal fish, however, questions on the suitability of the approach remain unanswered. For example, it is unclear if tag retention rates will be reasonable given demersal fish will encounter underwater structure and thus could snag the device.

Holistic approach to population structure

It is now recognised that concurrent use of multiple population structure approaches forms a more robust investigation. The robustness is improved as individual approaches have different advantages (Begg and Waldman 1999; Selkoe et al. 2008; Smith and Campana 2010; Welch et al. 2015), hence the use of multiple approaches draws on the different strengths of these approaches. Furthermore,

the use of multiple approaches aids in catering for unique ecological aspects for any given study on demersal fish. Otolith approaches are often combined with molecular approaches (e.g. Tanner et al. 2014) but holistic population structure studies are not confined to these approaches (see review in Catalano et al. 2014). Combinations of otolith and molecular approaches are particularly informative because they blend different operational timeframes and extrinsic and intrinsic influences (Begg and Waldman 1999) and as such provide a "weight of evidence" (Welch et al. 2015). For example, if otolith and molecular methods are employed on the same individual fish the information provided has a long term information aspect (100s of generations from the molecular data) and short term information aspect (entire life history or recent life history depending on the type of otolith method utilised).

Currently there is no standard method to integrate data from multiple approaches (Smith and Campana 2010). Approaches have been integrated at various levels including between different studies on the same putative populations and within studies, where multiple approaches are applied concurrently. When approaches are combined at the inter-study level the framework provided initially is built on in subsequent studies (e.g. Roques et al. 2002; Marcogliese et al. 2003; Arkhipkin et al. 2009). When approaches are combined at the intra-study level the data sets from individual approaches are generally acquired and analysed concurrently (e.g. Welch et al. 2015), although not always on the same individuals. There is a lack of consistency on the integration of data and hence the ability of holistic studies to provide readily interpreted results varies (Smith and Campana 2010; Welch et al. 2015). Some studies have analysed samples from different approaches separately and then combined the results in a table or plot for comparison (e.g. Miller et al. 2005; Perrier et al. 2011; Welch et al. 2015), whilst others have combined data and tested for significant differences or allocation of individuals to collection site (e.g. Smith and Campana 2010; Tanner et al. 2014). To my knowledge, this has only been done for two independent data sets, therefore it is unclear on which is the best practice to integrate three or more data sets; whilst also keeping the results simple enough to facilitate interpretation by fisheries managers.

Study species

Sciaenids are diverse, with 90 genera and 280 species across their global range; they are typical of predatory demersal fish. They are typical because large bodied species from this widespread family are in decline (Chao et al. 2015), which is in line with the global decline of predatory demersal fish, which have been suggested to be 10% of the pre-industrialised fishing biomass (Myers and Worm 2003). The decline is likely from habitat destruction and extractive industries causing unsustainable mortalities in some regions. Large bodied sciaenids evolved with low natural mortality due to a lack of predation (Griffiths 1996) which meant their 'bet hedging' or 'periodic strategist', referring to their relatively late but high fecundity, was initially suitable (Winemiller and Rose 1992; Ferguson et al. 2014). However, recent interest in harvesting and manipulating marine resources means that reaching maturity late in life is no longer the best survival strategy. Further increasing their vulnerability is the predictable life history, such as entering near shore coastal environments such as estuaries at certain times (Chao et al. 2015), potentially increasing their mortality via fishing.

The life history traits of marine predatory demersal fish including sciaenids make it hard to estimate (pre-investigation) if conspecific populations from this family should be structured or not (Swearer et al. 2002; Andreev et al. 2015). For example, residing in the connected marine environment and a pelagic larval stage of reasonable duration suggests that species should be panmictic (Palumbi 1994; Fauvelot and Planes 2002; Miller et al. 2005). Alternatively, medium spatial scale migrations or range, estuary association and particular spawning sites suggest there may be some population structuring (Andreev et al. 2015).

Mulloway

Mulloway, *Argyrosomus japonicus* (sciaenidae), were chosen as the study species as they are a suitable species to test the utility of various tagging techniques. In addition, like many large bodied predatory demersal fish the species is in decline in many parts of its range (Griffiths 1997; Taylor and Piola 2008; Ferguson et al. 2014). The species uses estuaries throughout their life history, which

makes them vulnerable to population decline from habitat destruction (i.e. reduced recruitment via loss of nursery area) and possibly over fishing (Silberschneider and Gray 2008; Ferguson et al. 2014). As such a better biological understanding of their population structure and connectivity is required for improved management and conservation outcomes.

Mulloway biology

In the southern hemisphere mulloway are found in temperate and sub-tropical parts of Australia and South Africa (Taylor et al. 2006). In southern Australia, mulloway are the only recorded sciaenid. Spawning occurs in spring and summer when the water temperature is over 19 °C (Battaglene and Talbot 1994) and is thought to occur just behind the surf zone and sometimes in estuaries (Ferguson and Ward 2003; Parsons et al. 2009). Larvae have a pelagic phase of approximately 30 days (Battaglene and Talbot 1994) and the tides are thought to carry them to nursery grounds in estuaries and other sheltered habitats (Griffiths 1996). In some areas juveniles favour turbid, brackish water of estuaries (Gray and McDonall 1993; Griffiths 1996; Silberschneider and Gray 2008; Ferguson 2010) or when that is not available other sheltered habitat is utilised, such as reef lagoons (Rogers et al. 2014). Predator avoidance and food availability likely plays a role in their choice of nursery (Ferguson et al. 2008). Juveniles are fast growing, with sexual maturity occurring at ~5 years depending on the area (Gray and McDonall 1993; Griffiths 1996). At the onset of sexual maturity growth slows markedly; the oldest recorded individual is 42 years old (Griffiths 1996). Recruitment success has been correlated with river flows and can lead to variation in year class strength (Ferguson et al. 2008); although in some areas mulloway occur where there are no estuarine habitats (Rogers et al. 2014), demonstrating plasticity of life history.

Mulloway form important recreational and commercial fisheries in South Africa and Australia but as mentioned above, their biomass may be in decline (Whitfield and Cowley 2010; Ferguson et al. 2014). In Australia the main commercial fishery is around the Coorong wetlands and ocean beach, South Australia, with a commercial catch of 69.1 tonnes reported for 2013/14 (Earl and Ward 2014).

There are smaller commercial catches of mulloway taken in other parts of Australia (e.g. <300 kg per annum by the demersal gillnet fleet in the Great Australian Bight). The recreational fishery for mulloway is significant in Australia, however it is hard to quantify due to a lack of reporting. However, one national survey was done in 2000/01 and the estimated annual catch rate of mulloway was 323 459 individuals (see Henry and Lyle 2003). A recent survey of mulloway recreational fisher-people on the far west coast of South Australia suggested that over 300 adult mulloway are captured a year but once again quantification is difficult (e.g. some fish are released) (Rogers et al. 2014). Better reporting on the mulloway harvest combined with an understanding of their population structure will help ensure the future sustainability of the species, important as predatory fish are not only a food source but they also help ensure ecosystem health and resilience (Maxwell et al. 2013).

Aims of thesis

The broad objective of my thesis was to assess population structure using different 'tags' singularly and some in combination within a poorly understood predatory demersal sciaenid, namely mulloway (*Argyrosomus japonicus*). Specifically I aimed to:

1a Develop microsatellite markers for mulloway and test their amplification in other members of the family;

1b Use the markers developed in 1a, to investigate genetic population structuring of mulloway

2 Test the feasibility of integrating three different natural tags, microsatellite DNA, otolith shape and otolith chemistry, and their utility in describing fine scale population structure of mulloway

3 Determine the effects of intrinsic (genetic) and extrinsic (temperature and salinity) factors on a widely used natural tag, otolith elemental chemistry

4 Test the utility of an electronic tag for describing movement with particular reference to population connectivity and interaction with marine parks.

Chapters 2a, 2b and 4 have been published in scientific journals, with Chapters 3 and 5 to be submitted shortly. Therefore the style of formatting and references conforms to the instructions for authors from the relevant scientific journals. Each chapter can be read as a standalone paper but each also contributes to a logical progression of ideas that were developed during my graduate research.

Each chapter is preceded by a statement of authorship that provides information on the contribution of the authors and publication status of the chapters at the time of thesis submission. Chapter 5, a general discussion, summarises the main findings, highlights implications and knowledge gaps of the study and suggests limitations and future research directions. The appendix has a copyright permission forms from publishers, advising of their approval to include published manuscripts in my thesis.

Chapter 2a: Statement of authorship

Statement of Authorship

Title of Paper	Development of 15 microsatellite loci from mulloway Argyrosomus japonicus (Pisces: Sciaenidae) using next generation sequencing and an assessment of their cross amplification in other sciaenids
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
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Principal Author

Name of Principal Author (Candidate)	Thomas C. Barnes		
Contribution to the Paper	Contributed to intellectual development of ideas, collected majority of samples, undertook all laboratory work, conducted molecular analyses, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	85		
Signature		Date	19/08/2015

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 in 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.

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Please cut and paste additional co-author panels here as required.

Chapter 2a: Development of 15 microsatellite loci from mulloway, *Argyrosomus japonicus* (Pisces: Sciaenidae) using next generation sequencing and an assessment of their cross amplification in other sciaenids

Collecting DNA in the field

Barnes, T.C., Izzo, C., Bertozzi, T., Saint, K.M., Donnellan, S., Hammer, M.P. & Gillanders, B.M. (2014). Development of 15 microsatellite loci from mulloway, *Argyrosomus japonicus* (Pisces: Sciaenidae) using next generation sequencing and an assessment of their cross amplification in other sciaenids.
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Chapter 2b: Statement of authorship

Statement of Authorship

Title of Paper	Population structure in a wide ranging coastal teleost (mulloway, Argyrosomus japonicus) reflects marine biogeography across southern Australia
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Name of Principal Author (Candidate)	Thomas C. Barnes		
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Overall percentage (%)	85		
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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);

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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter 2b: Population structure in a wide-ranging coastal teleost (*Argyrosomus japonicus*, Sciaenidae) reflects marine biogeography across southern Australia

Returning a sampled fish

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Population structure in a wide-ranging coastal teleost (*Argyrosomus japonicus*, Sciaenidae) reflects marine biogeography across southern Australia

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Abstract. Population structure in marine teleosts is often investigated to aid conservation and fisheries management (e.g. to assess population structure to inform restocking programs). We assessed genetic population structure of the important estuary-associated marine fish, mulloway (*Argyrosomus japonicus*), within Australian waters and between Australia and South Africa. Genetic variation was investigated at 13 polymorphic microsatellite markers. F_{ST} values and Bayesian estimates in STRUCTURE suggested population differentiation of mulloway within Australia and confirm strong differentiation between South Africa and Australia. The 12 Australian sample sets fell into one of four spatially separated genetic clusters. Initially, a significant signal of isolation-by-distance (IBD) was evident among Australian populations. However, further investigation by decomposed-pairwise-regression (DPR) suggested five sample sets were influenced more by genetic-drift, rather than gene-flow and drift equilibrium, as expected in strong IBD cases. Cryptic oceanographic and topographical influences may isolate mulloway populations from south-western Australia. The results demonstrate that DPR is suitable to assess population structure of coastal marine species where barriers to gene flow may be less obvious than in freshwater systems. Information on the relative strengths of gene flow and genetic drift facilitates a more comprehensive understanding of the evolutionary forces that lead to population structure, which in turn informs fisheries and assists conservation management. Large-bodied predatory scale-fish may be under increasing pressure on a global scale, owing to a variety of anthropogenic reasons. In southern Australia, the iconic sciaenid *A. japonicus* (mulloway, jewfish or kob) is no exception. Despite the species supporting important fisheries, much of its ecology is poorly understood. It is possible that a greater understanding of their genetic population structure can help ensure a sustainable future for the only southern Australian sciaenid.

Additional keywords: jewfish, kob, population genetics.

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Introduction

Insight into the population structure of exploited fishes facilitates the identification of appropriate management units (e.g. stock rather than management agency boundaries). For example, sub-populations from different geographic regions may have different population dynamics (i.e. growth rates, size at maturity or natural mortality), and would benefit from different management such as levels of exploitation rate or legal minimum lengths (Begg and Waldman 1999). The intraspecific exchange of genes relies on the connectivity between different areas, and physical barriers to such connectivity can lead to population substructure. Many examples of this are observed in

freshwater environments, such as where mountain ranges and waterfalls limit dispersal (Koizumi *et al.* 2006). These often impermeable barriers can form discrete population units where genetic drift has a much greater influence on population dynamics than gene flow, described as phylogeographic populations (Ovenden 2013) or pattern 1 (Koizumi *et al.* 2006). In the marine environment, phylogeographic fish populations are less common but structuring still occurs as a result of permeable physical barriers to gene flow, such as deep water trenches and currents. Permeable barriers can be breached by fish but in some cases there are insufficient migrants to create panmixia because of a variety of reasons such as thermal thresholds (Junge *et al.* 2011), currents (Ovenden *et al.* 2009), or behavioural factors (Gold and Turner 2002). Thus, permeable barriers can form discrete population units (termed restricted connectivity fish populations or pattern 2, genetic drift slightly more influential than gene flow), or when permeable barriers to migration in the marine environment are coupled with aspects of organismal biology, they may produce an influence comparable to an impermeable barrier (Koizumi *et al.* 2006; Ovenden 2013). The influence of barriers may be a factor in the population dynamics of fish but their influence is not easily assessed or even considered in isolation by distance (IBD), the model that is commonly used to explain structure in widely distributed fish species. Therefore, to better understand the mechanisms of population structuring, the influence of physical (e.g. barriers) and biological (e.g. dependence on estuarine nurseries) factors needs to be assessed.

Estuaries provide important habitat for many fish species, and associations with this habitat type are shown to help drive population structuring (Watts and Johnson 2004). Populations of estuary-dependent fish species are often genetically structured in a manner explained by geographic distance between estuaries and hence the structuring is readily called IBD (O'Donnell *et al.* 2014). However, the effect of estuaries on the genetic population structure of wide-ranging estuary-associated marine species may not just be a result of IBD. Indeed, other ecological roles of estuaries such as juvenile nurseries, spawning sites and the closely linked natal homing may aid in driving spatial population structuring (Lavergne *et al.* 2014). Also, purely marine populations of estuary-associated species have been found to be different genetic populations (Gold and Turner 2002).

Large-bodied fish from the family Sciaenidae, are a suite of commercial and recreational species, which regularly utilise estuaries and have in some instances displayed population structuring despite their marine existence (D'Anatro *et al.* 2011; Haffray *et al.* 2012). Sciaenids are generally declining in abundance on a global scale, which is thought to be a combination of overfishing in some regions and degradation of estuaries owing to anthropogenic influences (e.g. reduced freshwater input owing to abstraction) (Joseph 1972; Griffiths 1997; Gold and Turner 2002, Taylor *et al.* 2014). Furthermore, reducing decline through management measures may be especially challenging for sciaenids because of their often complex life-history.

The subtropical to temperate sciaenid mulloway (*Argyrosomus japonicus)* displays characteristics typical of large-bodied members of this finfish family, e.g. estuary association and complex life-history. In general, mulloway stocks are thought to be in decline in Australia (Ferguson *et al.* 2014) and South Africa (Whitfield and Cowley 2010), in a manner similar to sciaenids worldwide. A new genetic investigation is needed across the Australian range of mulloway owing to several shortcomings of the previous attempts (Ferguson *et al.* 2011). These include lack of adequate spatial sampling of mulloway for all previous studies and the use of lower resolution genetic techniques in most cases. For example, two recent investigations into genetic stock structure of the species focussed mainly on the west coast (Farmer 2008) or the east coast (Archangi 2008) of Australia, despite a remnant but still significant commercial fishery occurring on the southern Australian coastline (69.1 tonnes in 2013–2014, Earl and Ward 2014). Another genetic population structure study that had more geographically representative sampling focussed on allozymes (Black and Dixon 1992), a technique not likely to have the fine-scale resolution of contemporary polymorphic microsatellite markers. However, the allozyme study suggested separate populations from the western, southern and eastern Australian coasts (Black and Dixon 1992). This finding is supported in part by Farmer (2008) who found genetic structuring in mitochondrial DNA among south-western and western coastal populations of mulloway, and also suggested western and eastern Australian mulloway had recently diverged. Unfortunately Farmer (2008) did not include any southern Australian samples to allow detailed comparison with the previous studies. Interestingly, in the only microsatellite work to date, Archangi (2008) found a single panmictic Australian mulloway population (east to south coast Australia); however, the study lacked adequate spatial sampling (e.g. only 15 fish sampled from one location in southern Australia). Internationally, Australian and South African mulloway were found to be highly divergent, which is not surprising given the broad scale spatial separation and oceanic water between the two continents (Archangi 2008; Farmer 2008).

Mulloway also have a complex life-history, hence it is unclear if population structuring is likely. First, the species may be expected to be panmictic in Australia because of (i) residency in near-shore marine environments; (ii) high fecundity; (iii) late first reproduction (~6 years); (iv) long larval duration (30 days) (Battaglene and Talbot 1994); (v) long-lived (~40 years) (Griffiths 1996); (vi) overlapping generations; and (vii) schooling at various size classes. Alternatively, near shore coastal populations may be exposed to cryptic barriers suggesting possible population structuring. In addition, mulloway often use estuarine environments for the juvenile part of their life-history. Tag–recapture studies show only medium spatial-scale migration (up to ~400 km) of sub-adults and adults, which similarly suggests population structuring is possible (Hall 1986, J. A. Lieschke, unpub. data). Based on previous studies and the life-history attributes of mulloway, a comprehensive study investigating population structure across the Australian range of the species is needed.

To investigate the structure of Australian mulloway, we tested the null hypothesis of no genetic population structure (e.g. panmixia) across their southern Australian range. A South African sample was included but it was expected that these fish would form a different genetic population to Australia. In the event of significant population structure in Australia and evidence of IBD, we assessed whether DPR could provide a greater level of insight into population structuring in the marine environment.

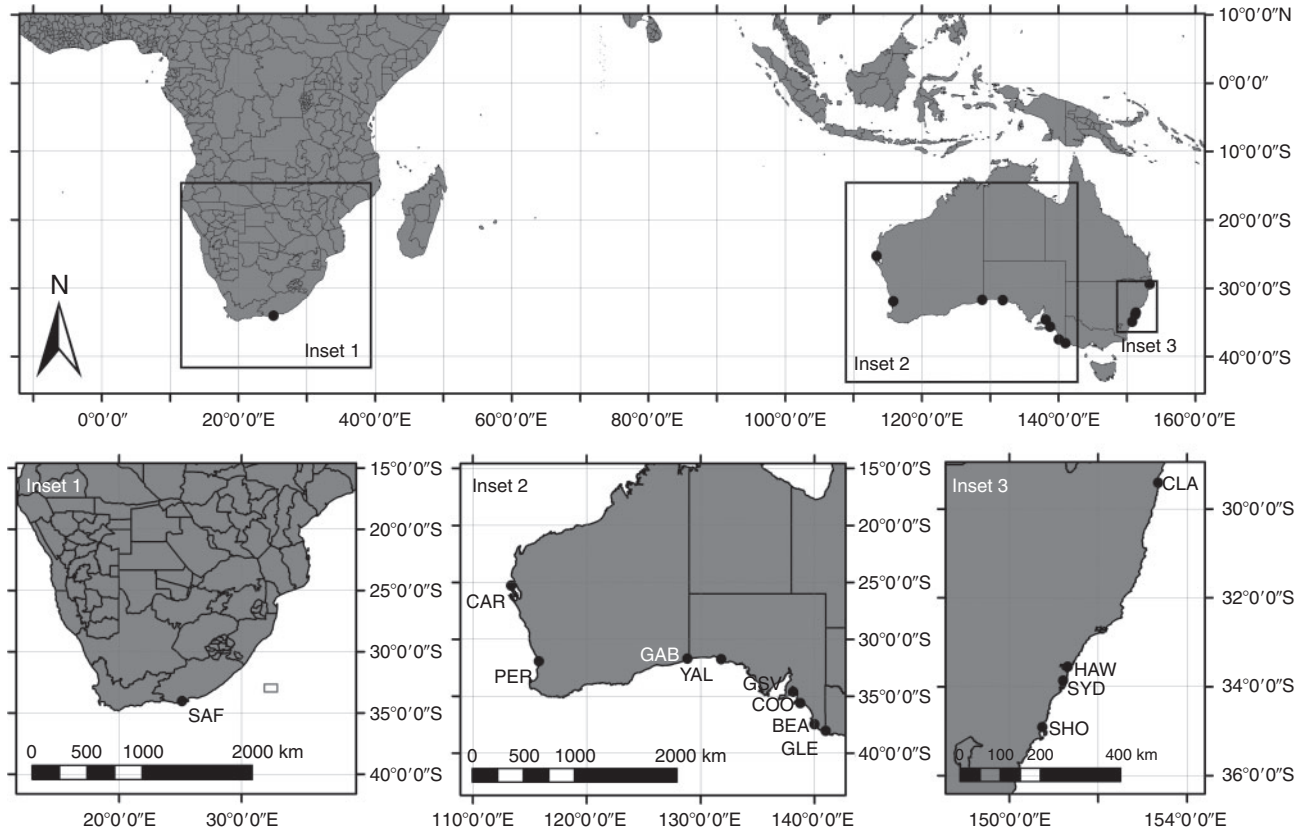

Fig. 1. Sampling locations for mulloway examined for allelic diversity, showing relative location across the southern hemisphere, and locations or sample sets (putative populations) labelled in inset maps on the lower panels. Abbreviations correspond to those listed in Table 1.

Materials and methods

Sample collection and genotyping

Mulloway were sampled from 12 locations throughout their southern Australian range; except for south-east Queensland (Fig. 1). Samples from South Africa (SAF) were also analysed to include an *a priori* divergent group. Fish were sampled from two habitats, estuarine and coastal marine, which are the typical environments frequented by *Argyrosomus japonicus*.

A total of 427 individuals were collected between 2000–2001 and 2012–2013 from the 13 localities (12 Australian and 1 SAF), with sample sizes ranging from 12 (Beachport, BEA) to 97 (Coorong, COO) (Table 1). Tissue, stored in ethanol, was sourced from fish provided by the commercial and recreational fishing sectors. Most fish were mature adults (i.e. total length >80 cm and age of >5 years); however, a small proportion of immature fish were included from some localities (COO; Clarence River, CLA; Gulf St Vincent, GSV; Hawkesbury River, HAW; SAF; and Shoalhaven River, SHO). Juvenile and mature fish were combined for all analyses, as juvenile fish were a small proportion of all samples and fish-length data were unavailable at some locations. We used a minimum sample size of 12 individuals per locality to ensure reasonably robust population analysis. Any duplicate genotypes as a result of recaptures were removed from the dataset.

DNA was extracted using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN) and samples were genotyped for a set of 13 microsatellite markers (Barnes *et al.* 2014; Table S1 of the Supplementary material). Optimised primer concentrations were 10, 20, 40 or 60 (nM) and forward primers were labelled with one of four fluorescent dyes, FAM, NED, PET and VIC, (Table S1). PCR conditions followed the protocol outlined by Barnes *et al.* (2014) and pooled fragments (for details see Table S1) were analysed using capillary electrophoresis on an ABI3730 Genetic Analyzer with the LIZ 500 size standard (Applied Biosystems, Carlsbad, CA, USA). Positive and negative controls and one column inverted and repeated were incorporated in all PCR plates (Hayden *et al.* 2008). Allele scoring was performed using GENEMAPPER 3.7 (Applied Biosystems).

Descriptive statistics, temporal variation and equilibrium

At least 10% of mulloway per sample set were genotyped twice to determine genotyping error rates. Initially, individuals that were sampled from the same localities in multiple years were tested for temporal genetic variation using a hierarchical (locus by locus) analysis of molecular variance (AMOVA) in the program Arlequin v3.11 (Excoffier and Lischer 2010). We partitioned the variance components to reflect among locality (spatial component), among year within localities (temporal component) and among individuals within the same year and locality (within site component) variation. All basic tests used 10 000 permutations for 1000 batches. The analysis showed no variation among years within the same localities (see Results),

Table 1. Sampling details and genetic diversity indices showing locations fish were collected from, sample codes (Sampcode) and years of collection
Also shown are sample sizes (*n*). Codes for genetic diversity indices are: number of alleles (Na), number of effective alleles (Ne), unbiased expected (UHe) and observed (Ho) heterozygosity

Sampling details					Genetic diversity			
Country	Location	Sampcode	Year	*n*	Na	Ne	UHe	Ho
South Africa	Eastern Cape	SAF	2006	8				
			2007	1				
			2009	18				
			2010	15				
				42 total	10.154	5.471	0.732	0.622
Australia	Carnarvon	CAR	2004	14	6.231	4.208	0.685	0.667
Australia	Perth	PER	2000	11				
			2001	4				
				15 total	6.308	4.471	0.706	0.652
Australia	Great Australian Bight	GAB	2010	17	6.077	3.726	0.654	0.620
Australia	Yalata	YAL	2009	8				
			2010	13				
			2011	9				
			2012	2				
				32 total	6.923	3.752	0.653	0.632
Australia	Gulf St Vincent	GSV	2010	2				
			2011	1				
			2012	32				
				35 total	7.615	4.452	0.663	0.662
Australia	Coorong	COO	2009	39				
			2010	41				
			2011	17				
				97 total	9.462	4.118	0.654	0.659
Australia	Beachport	BEA	2011	11				
			2012	1				
				12 total	6.000	3.786	0.668	0.627
Australia	Glenelg River	GLE	2008	24	6.846	3.713	0.649	0.677
Australia	Shoalhaven River	SHO	2011	41	9.385	5.234	0.711	0.694
Australia	Sydney Area (pre-2004)	SYD	2004	20	7.385	4.397	0.697	0.668
Australia	Hawkesbury River	HAW	2011	37	8.231	4.833	0.695	0.665
Australia	Clarence River	CLA	2010	3				
			2011	38				
				41 total	9.000	5.427	0.711	0.682

therefore we pooled samples across years from the localities ($n = 13$) (hereafter termed sample sets) for analysis that produced location-based summary statistics, e.g. $F_{ST.}$ Descriptive tests were performed for each sample set to calculate the number of alleles (N_A), effective alleles (N_E) and unbiased expected and observed heterozygosity (UH_E, H_O) in GenAlEx v6.4 and allelic richness (AR) in FSTAT (Goudet 2001).

We tested for significant deviations from Hardy–Weinberg equilibrium and linkage equilibrium in GENEPOP v4.2 (Rousset 2008). The Monte Carlo Markov Chain (MCMC) parameters were set at the maximum dememorisation number (10 000) and iterations (10 000) for 1000 batches. We corrected for multiple tests by applying sequential Bonferroni corrections (Rice 1989).

Population differentiation and statistical power

Population differentiation was tested using exact G tests in GENEPOP, to assess significant pairwise genic differentiation between each sample set using MCMC specifications described previously and subsequently corrected for multiple tests (as above). Pairwise F_{ST} values were calculated in GENEPOP to test the degree of differentiation between sample sets (Weir and Cockerham 1984).

Several simulations were conducted using POWSIM to assess the statistical power when testing for genetic differentiation (Ryman and Palm 2006). This program simulates sampling from a sample set at a predefined level of expected divergence through random-number computer simulations under a classical Wright–Fisher model, without migration or mutation. Significance estimates were based on 1000 independent simulations and a χ^2-test.

Population differentiation and structure

Bayesian clustering analysis was used to investigate the potential for population structuring using the program STRUCTURE v2.3.2 (Pritchard *et al.* 2000). The admixture model, assuming correlated allele frequencies, was used. The influence of different hyper-parameter priors on our data was evaluated by running MCMC using different prior combinations. The best outcome based on the normal distribution of allele frequencies and likelihood of a large degree of admixture was using mean

allele frequency (0.07) with default standard deviation of 0.05 and lambda of 1.0 (Pritchard *et al.* 2000). After testing, we chose a conservative burn-in of 300 000 iterations and a post-burn-in chain length of 1 000 000 iterations, which we applied to all analyses. 20 MCMC replicates were run for each analysis as recommended by Gilbert *et al.* (2012), for K = 1–13 (K is the number of clusters). The LOCPRIOR model was used as the increased power when investigating weak structuring was deemed appropriate in this case (Hubisz *et al.* 2009). We subsequently used two methods to infer K: (1) the mean estimated logarithm of probability of the data L(K); and (2) the second order of change of the logarithm of the probability of the data (ΔK) as a function of K (Evanno *et al.* 2005) using STRUCTURE HARVESTER (Earl 2012). The average ancestral membership coefficients were then determined for the true K based on all of its replicates using CLUMPP software v1.1.2 (Jakobsson and Rosenberg 2007) and visualised using DISTRUCT (Rosenberg 2004). The most divergent genetic clusters were removed sequentially and the remaining sample sets reanalysed until no further structuring was found.

The Mantel test in GenAlEx v6.4 was used to determine if genetic variation could be explained by geographic distance. To facilitate the test, genetic variation (Pairwise F_{ST}) was calculated using GENEPOP version 4.2, then the data were transformed ($F_{ST}/(1 - F_{ST})$) (Nei 1977). Pairwise geographic distance was calculated using the measure function in Google Earth (Mountain View, CA, USA) based on the shortest route mulloway would likely take. The Mantel test comprised 999 permutations and we plotted all pairwise comparisons of genetic variation against geographic distance.

To determine if there were non-conforming (or outlier) sample sets to the isolation by distance (IBD) model we implemented a decomposed pairwise regression (DPR) analysis (Koizumi *et al.* 2006). Briefly, interpretation of IBD results may be hampered by 'outlier' sample sets, as regression methods assume the same degree of isolation and that other characteristics (e.g. sample set size) are similar for all sample sets. DPR consists of three phases: (1) systematic bias of regression residuals; (2) Akaike's information criteria (AIC); and (3) regression of each sample set against all non-outlier sample sets. These phases modify regression to extract unique elements of local sample sets whilst assessing the contributions of genetic drift *v.* gene flow. Based on the relative effects of genetic drift and gene flow, populations can be classified as being influenced by one of four categories: (i) pattern 1, drift ≫ flow; (ii) pattern 2, drift > flow; (iii) pattern 3, drift = flow; and (iv) pattern 4, drift < flow (for further methodological details see Koizumi *et al.* 2006).

Results

Descriptive statistics, temporal variation and equilibrium

A total of 427 mulloway were genotyped successfully at 13 microsatellite markers. The genotype error rate was less than 0.01% indicating acceptable accuracy. Less than 1% of alleles were not scorable or were missing altogether (76 out of 11 102 or 0.69%). The mean number of alleles (Na) and effective alleles (Ne) measured across loci and sample sets was 7.663 ± 0.303 s.e. (range from 6.000 (BEA, Beachport) to 10.154 (SAF, South Africa)) and 4.430 ± 0.198 s.e. (range from 3.713 (GLE, Glenelg River) to 5.471 (SAF)) respectively (Table 1); suggesting our allele set had moderate differences in frequencies. Observed and unbiased expected heterozygosities (Ho and UHe) had similar ranges (0.620–0.694 and 0.648–0.732 respectively) (Table 1).

AMOVA showed that most of the genetic variation (86.7%, variance component (VC) = 3.985, $F_{CT} = 0.110$, $P < 0.001$) was within individuals collected in the same year from a locality, with variation among localities (sample sets) driving the next greatest genetic variation (11.0%, VC = 0.493, $F_{ST} = 0.113$, $P < 0.001$). The genetic variation among years within the same locality (temporal genetic variation) was low (0.38%, VC = 0.017, $F_{SC} = 0.004$, non-significant). Therefore all sampling years within localities were pooled to provide 13 sample sets for subsequent analyses.

Four of the 13 sample sets (SAF, CAR (Carnarvon), HAW (Hawkesbury River) and SYD (Sydney)) showed non-conformance to Hardy–Weinberg equilibrium at ~1 locus after Bonferroni corrections. Deviation could be explained by heterozygote deficiency in all cases, with the likely causes being Wahlund effect in SAF and SYD and inbreeding possible in CAR and HAW; further investigation into potential causes was beyond the scope of the current study. No locus pairs deviated significantly from linkage disequilibrium in any sample sets.

Population differentiation and structure

We found that 69 out of 78 pairwise comparisons within all sample sets showed significant genetic differentiation (63 after Bonferroni corrections). Pairwise F_{ST} values ranged from −0.007 to 0.278 (Table S2 of the Supplementary material). Power simulations indicated that given the number of loci, their polymorphism, and the sample sizes available, the probability of detecting F_{ST} values as low as 0.003 was >0.99 and for $F_{ST} = 0.001$, it was still reasonably high at 0.54. Given that the two smallest F_{ST} values between two significantly diverged sample sets were 0.002 and 0.001, the significant F_{ST} values we observed are likely realistic, yet the latter comparison (SHO–CLA) needs to be treated with some caution.

The program STRUCTURE revealed two most likely genetic clusters when all 13 sample sets were included in the analysis (Fig. S1 of the Supplementary material). Genetic clusters showed spatial structuring, with one cluster (cluster 1) comprising all individuals from SAF, and the other (cluster 2) comprising most individuals from Australian locations, with the exception of the two western most Australian populations (CAR and PER (Perth)); individuals in these locations had mixed genotypes, with ~75% and 50% membership, respectively, to cluster 1 (Fig. 2*a*). One individual from YAL (Yalata) also showed evidence of a mixed membership, with ~35% membership to cluster 1 (Fig. 2*a*).

After removing the most divergent sample set (SAF), STRUCTURE again revealed two genetic clusters (Fig. S1). The genetic clusters showed spatial structuring with the two West Australian sample sets (CAR and PER) comprising one cluster, whereas the other Australian sample sets predominantly belonged to the other cluster (cluster 2). However, the two most western south coast sample sets (GAB (Great Australian Bight) and YAL) showed a mix of genotypes, with the second cluster more prominent (70–75% membership) (Fig. 2*b*).

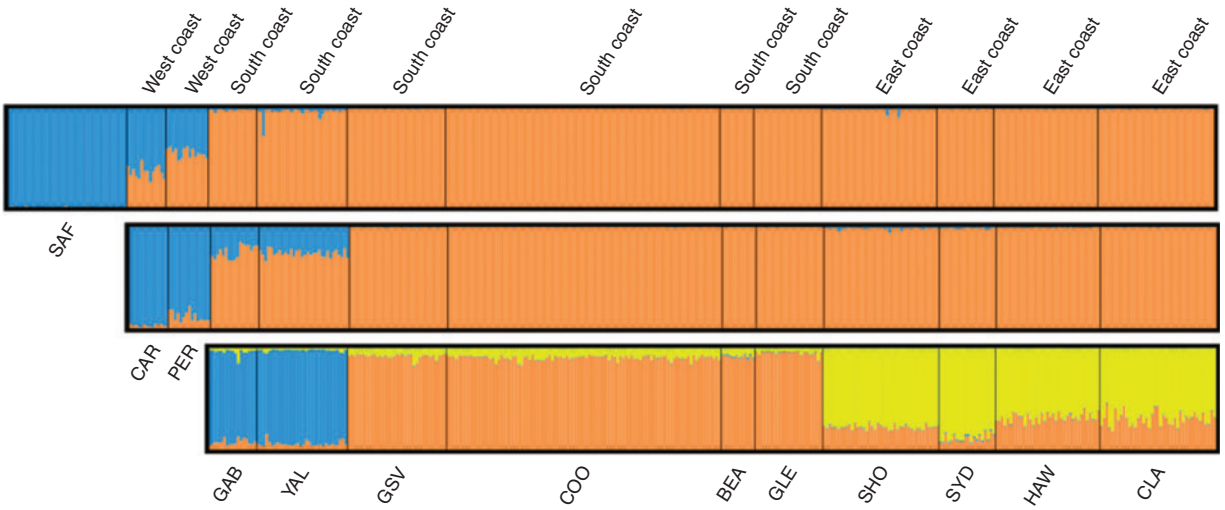

Fig. 2. STRUCTURE Bayesian assignment for mulloway: (top) 13 sample sets; (middle) 12 Australian sample sets; and (bottom) the 10 central to eastern Australian sample sets. Sample sets are ordered from west to east. Each vertical line represents an individual and each shade represents a genetic cluster or K. For more information on the sample codes see Table 1.

After removing the next most divergent sample sets (CAR and PER), STRUCTURE revealed three most likely genetic clusters (Fig. S1). The two most western south coast sample sets (GAB and YAL) belonged to cluster 1, with very small proportions of the other genotypes evident. Both the eastern south coast sample sets and the east coast sample sets were a mixture of genotypes (Fig. 2*c*); however, in different proportions (e.g. 90% and 30% of cluster 2 respectively). The eastern south coast sample sets belonged to cluster 2 and the east coast sample sets to cluster 3. Interestingly one east coast sample set (SYD) showed stronger membership (~80%) to the third cluster than other sample sets in the proximity (~60–70%). The most divergent sample sets were again removed, but no further structuring was found.

Isolation by distance and decomposed pairwise regression

The Mantel test showed a significant signal of genetic variation being explained by geographic distance, with a reasonable fit of the model ($P = 0.003$, $R^2 = 0.588$, Fig. 3). During phase 1 of decomposed pairwise regression (DPR), residuals of the regression were biased with high genetic divergence for the distance (e.g. population CAR, Fig. 4); however, one sample set exhibited only moderate genetic divergence for the distance (SYD). All of the five putative outliers from DPR phase 1 were recognised by Bayesian clustering in STRUCTURE as being divergent sample sets and potentially affected by a cryptic migration barrier. All combinations of models with and without putative outliers were tested based on Akaike's information criteria (AIC_C) and the best model included five putative outliers (CAR, PER, GAB, YAL and SYD). These five outliers became the 'true outliers' as this model did not differ by more than two from the highest possible AIC_C and had the highest R^2 of all models that were $\Delta AIC_C < 2$ (Table 2). In the final phase of DPR (phase 3) each of the five true outlier sample sets were regressed with the seven non-outliers separately and categorised into one of the relevant patterns (Fig. 5, Table 3). All true

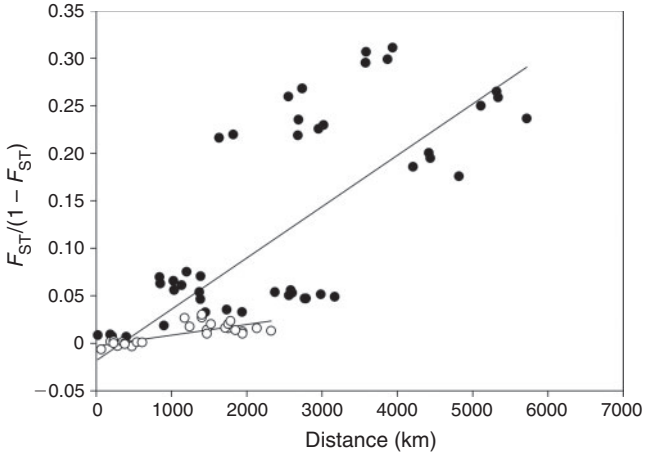

Fig. 3. Relationship between genetic ($F_{ST}/(1 - F_{ST})$) and geographic distance (km) for all 12 Australian sample sets (open and closed circles, upper line; $y = 5.4 \times 10^{-5}\chi - 0.019$, $R^2 = 0.591$) and excluding five outlier sample sets (only open circles; $y = 1.1 \times 10^{-5}\chi - 0.003$, $R^2 = 0.611$, lower line).

outliers except SYD exhibited pattern 1 (genetic drift ≫ gene flow (i.e. drift is a much greater influence)), whereas SYD exhibited pattern 2 (genetic drift > gene flow (i.e. drift is moderately more influential)). All the true outliers were significantly divergent from neighbouring and more distant sample sets, except in the case of GAB and YAL (Fig. 5). The neighbouring sample sets GAB and YAL appear to be the same genetic cluster. The seven non-outlier sample sets followed pattern 3 (genetic drift = gene flow (i.e. in equilibrium)), which was expected as all of these sample sets had similar regression lines (Fig. 5). There was a significant correlation ($P < 0.050$) between genetic and geographic distance in most sample sets; except in one true outlier sample set (GAB) and three

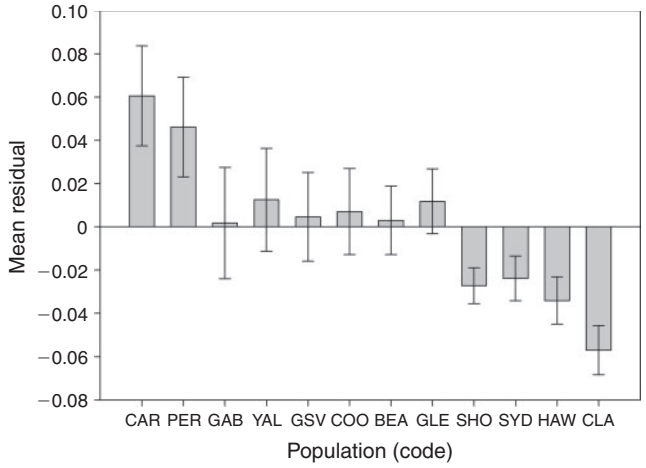

Fig. 4. The means and standard error of residuals from the least-squares regression (calculated from Fig. 3) with all Australian sample sets included (CAR was removed after this analysis).

Table 2. Fit of alternative isolation by distance models with and without putative outlier sample sets (see Table 1 for codes)

n refers to the number of sample sets, AIC_C refers to Akaike's information criteria corrected for small sample sizes, ΔAIC_C refers to the difference in AIC_C between alternative models (post ranking from highest to lowest)

Population excluded	*n*	R^2	*P*	AIC_C	ΔAIC_C
CAR, PER, YAL and GAB	8	0.452	<0.001	−61.480	0
CAR, PER, YAL, GAB and SYD	7	0.595	<0.001	−60.126	−1.354
CAR, PER	10	0.259	<0.001	−59.941	−1.539
CAR, PER and YAL	9	0.342	<0.001	−58.167	−3.313
CAR	11	0.439	<0.001	−43.590	−17.890
None	12	0.588	<0.001	−42.426	−19.054

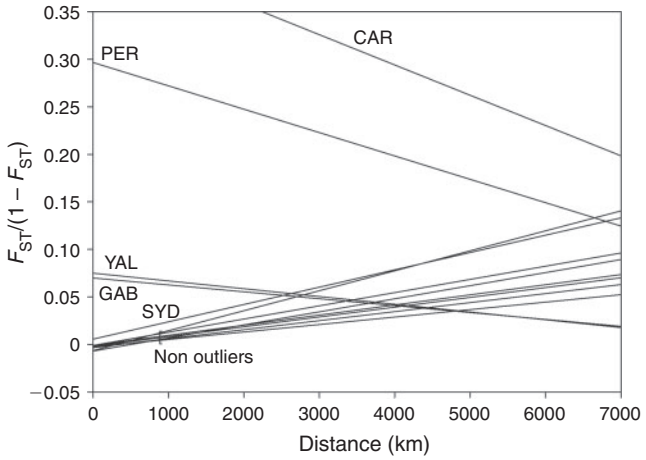

Fig. 5. Pairwise regression for genetic *v.* geographic distance for true outliers (sample sets CAR, PER, GAB, YAL and SYD) regressed individually against the non-outliers (sample sets GSV, COO, BEA, GLE, SHO, HAW and CLA), with the non-outliers regressed against the other non-outliers; non-significant regressions included (sample sets GAB, SHO, HAW and CLA).

Table 3. The intercept and slope (with standard error) of the third and final stage of the decomposed-pairwise-regression (DPR) for non-outlier and outlier sample sets

Also shown are the fit (R^2), sample size (*n*) and category where pattern 1 refers to phylogeographic populations (drift >> flow), pattern 2 to restricted connectivity fish populations (drift > flow) and pattern 3 to signal of isolation by distance (IBD) (drift = flow). See Table 1 for location codes. *, $P < 0.050$

	Intercept	Slope	R^2	*n*	Category
Non-outlier sample sets					
GSV	−0.003 (0.003)	7.856×10^{-6} (<0.001)*	0.801	7	Pattern 3
COO	−0.002 (0.003)	1.032×10^{-6} (<0.001)*	0.834	7	Pattern 3
BEA	−0.007 (0.003)	1.374×10^{-5} (<0.001)*	0.848	7	Pattern 3
GLE	−0.006 (0.005)	2.100×10^{-5} (<0.001)*	0.859	7	Pattern 3
SHO	−0.001 (0.007)	1.389×10^{-5} (<0.001)	0.583	7	Pattern 3
HAW	0.001 (0.007)	1.003×10^{-5} (<0.001)	0.511	7	Pattern 3
CLA	−0.003 (0.005)	9.490×10^{-6} (<0.001)	0.691	7	Pattern 3
Outlier sample sets					
CAR	0.421 (0.021)*	-3.182×10^{-5} (<0.001)*	0.907	8	Pattern 1
PER	0.297 (0.013)*	-2.458×10^{-5} (<0.001)*	0.900	8	Pattern 1
GAB	0.070 (0.006)*	-7.266×10^{-6} (<0.001)	0.545	8	Pattern 1
YAL	0.075 (0.005)*	-8.167×10^{-6} (<0.001)*	0.679	8	Pattern 1
SYD	0.006 (0.005)	1.822×10^{-5} (<0.001)*	0.776	8	Pattern 2

non-outliers (SHO, HAW and CLA) (Fig. 5). The new Mantel test showed a more significant signal of genetic variation being explained by geographic distance for the non-outliers with an improved but still only reasonable fit of the model ($P < 0.001$, $R^2 = 0.611$, Fig. 3).

Discussion

Data from the 13 microsatellite markers provided insight into structuring of mulloway on a broad scale between Australia and South Africa and also throughout Australia. Mulloway from South Africa were clearly differentiated from those in Australia, confirming earlier studies (Archangi 2008; Farmer 2008). Within the Australian range, the most divergent sample sets were from the west coast and western-central part of the south coast, whereas mulloway around the south-eastern and eastern coast had a common ancestry. A significant signal of isolation by distance (IBD) was found among the 12 Australian sample sets; however, outliers to the IBD model were found by decomposed pairwise regression (DPR). The DPR analysis provided a useful tool for analysis of genetic population structure in the marine environment by identifying those sample sets more influenced by genetic drift rather than genetic drift and gene flow equilibrium (expected under IBD). Hence DPR could indicate barriers to gene flow in coastal marine environments, which has not been extensively tested before. Our results, demonstrating significant population structuring of mulloway, suggest that fisheries managers should consider these stock boundaries in management plans.

The population structure of mulloway and comparison to other sciaenids

Mulloway populations showed significant spatial structuring. The most divergent sample set was SAF (South Africa), which

was expected, given the enormous spatial separation between the South African and Australian continents (8315 km). Similar divergence between South Africa and Australia was also found by two recent genetic studies (Archangi 2008; Farmer 2008). Our study is the first to demonstrate that there are four mulloway populations in southern Australia. Broadly these populations are the west (CAR and PER), the western-central-south (GAB and YAL), the south-east (GSV, COO, BEA and GLE) and east (SHO, SYD, HAW and CLA) coasts. Weaker structure within some of these populations is also evident (west and east coasts). Some aspects of the structuring demonstrated in the present study are supported by some of the previous Australian genetic mulloway studies (Black and Dixon 1992; Farmer 2008). It is likely that the lack of adequate sampling (small number of replicates from a small number of regions) led to the panmictic result of the other microsatellite DNA study by Archangi (2008). For example, the current study sampled mulloway from four regions (GSV–GLE) in the important south-east Australian bioregion, whereas Archangi (2008) sampled one (Port Lincoln). In South Africa, population structuring has been suggested as likely (Klopper 2011), although there is not yet conclusive genetic evidence. Structuring of mulloway populations has also been found using otolith based techniques (e.g. Ferguson *et al.* 2011). Importantly, otolith chemistry results are similar to the findings of the current study and may have a genetic component (Barnes and Gillanders 2013).

Genetic population studies of other similar large-bodied sciaenids have found structuring. For example, meagre (*Argyrosomus regius*), red drum (*Sciaenops ocellatus*) and white croaker (*Genyonemus lineatus*) show population structuring but with differing drivers, such as physical oceanographic forces, limited adult dispersal leading to IBD and adaptions to different habitats (Gold and Turner 2002; D'Anatro *et al.* 2011; Haffray *et al.* 2012). There is a possibility that fishing mortality may enhance population structuring in sciaenids, by selective pressures imposed by fisheries (D'Anatro *et al.* 2011).

Biogeographic factors contributing to genetic structure of mulloway

Mulloway are distributed in Australian coastal waters spanning over 6000 km and as such the population structure reflects multiple biogeographic forces. IBD is often used to explain structuring (e.g. Gold and Turner 2002) and it plays a role in mulloway population structuring as previous tagging studies (mark–recapture and acoustic) across the species range suggest limited adult movement (Hall 1986; Taylor *et al.* 2006; Farmer 2008; J. A. Lieschke, unpub. data). However, the DPR suggests gene flow and genetic drift were not always in equilibrium, which was expected under IBD. For example, mulloway from the western section of Australia did not conform to IBD, suggesting that gene flow with the east populations was blocked by migration barriers.

There are two impermeable physical barriers that may block gene flow and hence cause some populations to be more divergent (characteristics of phylogeographic or pattern 1) than expected under IBD. One barrier is on the south-western coast in the form of unsuitable habitat. The high energy coastline between the western-central-south coast (where GAB and YAL are located) and western coast (where PER and CAR are located) forms a barrier as there is a lack of suitable sheltered habitat (Farmer 2008). This barrier is supported by the lack of mulloway found between the western-central-south coast and southern part of the western Australian coastline (where PER is located) (Farmer 2008). Although mulloway occur at Oyster Harbour, Albany, on the south coast of Western Australia (WA), no DNA samples were available. It has been suggested previously that these fish represent an inbred population, which are estuary-dependent (Farmer 2008). The second barrier to gene flow is on the south-central coastline of Australia, in the form of physical oceanographic fronts derived from reasonably warm and saline water masses from the gulfs in central South Australia (SA), which do not mix readily with the less saline, generally cooler, Southern Ocean water (O'Hara and Poore 2000; Petrusevics *et al.* 2011). The fronts and upwelling in this area have been shown to create a barrier to gene flow in other large predatory marine animals (e.g. Bilgmann *et al.* 2007).

There are also two permeable barriers that affect mulloway gene flow, which cause some weak population structuring compared with the phylogeographic structuring discussed above. The first permeable barrier is very likely owing to the influence of the Leeuwin current running north to south and the topography of Shark Bay. The Leeuwin current and the topography of the north facing and extensive Shark Bay (near where Carnarvon is situated) means that gene flow from PER to CAR (south to north) is unlikely (Caputi *et al.* 1996; Farmer 2008). Therefore, one-way gene flow may account for slight genetic differences between the two western sample sets. The second permeable barrier that sometimes separates the south-eastern and eastern populations is Bass Strait. This water body is shallow and narrow at the junction of two oceans (Southern and Pacific), and as such has special characteristics such as strong currents and tidal movements (Ward and Elliott 2001). Bass Strait is historically a marine migration barrier owing to the impermeable effects of the land bridge (Bassian Isthmus) between mainland Australia and Tasmania during periods of low sea level, such as at the last glacial maximum (Ward and Elliott 2001; Waters 2008). In modern times of increased sea level, gene flow among mulloway across Bass Strait is likely to be spasmodic for several reasons. First, their presence around the south-east corner of Australia (e.g. between Wilson Promontory and Sydney) is patchy at best. Second, larval advection between the south-east and eastern seaboard may also be inconsistent because of the timing and direction of Bass Strait currents being driven by prevailing winds (O'Hara and Poore 2000; Ward and Elliott 2001). Third, at times there is little flow-through of water via Bass Strait (Ward and Elliott 2001). Consequently, there is not enough gene flow for panmixia and thus the weak structuring among the south-east and eastern populations fits reasonably into the IBD model (pattern 3, genetic drift and gene flow equilibrium). Furthermore, similar patterns have been observed in other estuary-associated fish species across Bass Strait (see Shaddick *et al.* 2011).

Interestingly, SYD was the only eastern Australian sample set found by DPR to be an outlier to the IBD model and was deemed to exhibit differentiation consistent with moderate restricted connectivity or pattern 2 (genetic drift > gene flow). This restricted connectivity and hence partial barriers to gene flow

appears unlikely, given SYD is located in close proximity to other east coast sample sets. Rather, the differentiation of SYD could be driven by the temporal difference of sampling of east coast populations (~7 years). Therefore, it is possible that there may be some medium term (decadal) temporal structure of east coast mulloway, which could not be determined with the sample sets in the present study. Stocking of hatchery-reared mulloway has occurred along parts of the New South Wales (NSW) coast during this temporal period (Taylor and Piola 2008), but the creation of a modified east coast gene pool due to a hatchery bottleneck is extremely unlikely, given the large overlap of generations of mulloway and the fact that broodstock were sourced from the NSW coast.

Assessment of DPR applied to a marine fish

DPR has primarily been used in freshwater systems, but was informative in this marine fish application. Importantly DPR recognised the most differentiated populations of mulloway and suggested that four out of five of these outliers to IBD were affected by formidable barrier(s) to gene flow, and as such they are phylogeographic or pattern 1 (genetic drift $\gg$ gene flow) populations. Thus, DPR provided additional information on the dynamics of mulloway population structure to that supplied by IBD. Population analysis using STRUCTURE supported the findings of DPR providing a useful cross-check and hence solidifies the results of DPR. DPR was also applied to a marine fish, Pacific cod (*Gadus macrocephalus*) in at least one previous example. Pacific cod had outlier populations to the IBD model, which were due to impermeable barriers to gene flow (Cunningham *et al.* 2009).

Possible implications for management of mulloway

The population structure described in the present study suggests that current management of mulloway conducted on the scale of state boundaries could be enhanced by accommodating differences among individual stocks. Currently, almost all management regulations for recreational and commercial mulloway exploitation apply to each state; except for Commonwealth Commercial Shark Fishery, which reports low mulloway catches ($<$300 kg year^{-1}; G. J. Ferguson pers. comm.). Considering regulations are generally at the state level, our results indicate that management agencies in WA and SA could benefit from considering the multiple stocks in their jurisdictions. For example, higher growth rates in western SA compared with the south-east of the state suggest differences in size or age at maturity (Ferguson 2010), although the legal minimum size of 750 mm total length applies across all SA marine waters. Additionally, other life-history traits, such as dependency on juvenile habitats in estuaries or alternative near-shore reef habitats found in south-eastern and western SA, respectively, may result in regional differences in recruitment but there is a single statewide bag limit for the recreational fishery, which is substantial (e.g. similar in size to the commercial fishery, Henry and Lyle 2003). Further, those states sharing the eastern Australian population (SA, Victoria and NSW) could also benefit by collaborating on management objectives.

Similarly, the restocking of mulloway on the west and east coasts of Australia could help to maintain the genetic structure in those regions, by sourcing brood stock from the populations, which are found in the proposed release areas. Potential longer term temporal structuring (some evidence in NSW but also possible elsewhere) suggests some periodic genetic testing is important before stocking operations. The spatial structuring observed by the present study is particularly important in terms of restocking, given that some previous works suggested a panmictic Australian population (Archangi 2008). The potential benefits for management of mulloway highlights the importance of applying resources (i.e. ensuring adequate spatial sampling) to improve our understanding of exploited far-ranging teleosts by genetic population structure investigations.

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Supplementary material

Population structure in a wide-ranging coastal teleost (*Argyrosomus japonicus*, Sciaenidae) reflects marine biogeography across southern Australia

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Table S1. Showing locus, primer sequence and other information for microsatellite data

Locus	Primer sequence	Motif	Initial product size (bp)	Size range (bp)	Pool	Colour	Concentration (nM)
Arja14	F–GGTGGGGCCAGTGATCTTC R–TCAACACCTCCTATCATCACC	(GAT)^18	171	156–190	1	VIC	60
Arja18	F–GAGGTGGTCACAGTCCCTC R–ACCTTCTCCCTGCGAACTC	(AGC)^8	174	182–230	1	FAM	10
Arja19	F–CACGAGTGGAGTCTGGTGG R–TCGTTGCCGTCGTGATTTC	(GAT)^9	176	179–200	1	PET	10
Arja21	F–TCAACATCCCGAGTGAATGTG R–GACTGTCAATGAAATAAAGGGAGC	(AAT)^15	179	180–198	1	NED	10
Arja22	F–CGGTTGGTAAATGCTTGCAG R–AAGCTCACCTGAACCCCAC	(AGAT)^14	207	186–238	2	FAM	60
Arja26	F–CAGACACTAGATGGCAGTATTGG R–ACCTCCAGGAAGCAGTCATC	(AGAT)^18	218	179–238	2	NED	40
Arja28	F–AGCCTCCACAGTGAGCTTC R–TCACTGATATCTTGAATTCTTCGG	(AGAT)^22	223	185–253	2	PET	20
Arja33	F–GGGACTGTTCCCTGACCTTC R–AACATAGGACCTTGCCTGC	(ATCT)^17	243	240–296	1	VIC	10
Arja34	F–ATTACCCTGCCTGTCCAGC R–CTTGAAACAGCCGAAGGGG	(AGAGG)^12	246	240–270	1	NED	10
Arja37	F–TTTGTAAACAGCATCGTTTGTG R–AACATATCGGTGCATATCGTTC	(CTT)^13	254	253–265	2	VIC	
Arja39	F–GCCACTGTGCATGTGGAG R–AATTCAGTTCAACAGCCTTGG	(ATT)^9	257	265–275	1	FAM	10
Arja41	F–CCCTTTTAACGCGTGGGAC R–CTCAGGTAATTGTAGGTTGAGGC	(CTTT)^14	261	250–280	1	PET	20
UBA42	F–ACGACGTTGTAAAATGCCAGCAGACAGCATTATC R–CATTAAGTTCCCATTAGCTCGCAGGTCTTGAGATTG	(TGC)^21	–	171–217	2	VIC	40

Table S2. Pairwise F_{ST} values with exact G tests (significant pairwise comparisons of exact G test indicated by asterisk) (above diagonal) and pairwise geographic distance (km) (below diagonal)

Location codes are in Table 1. *, $P < 0.05$; **, $P < 0.001$

	CAR	PER	GAB	YAL	GSV	COO	BEA	GLE	SHO	SYD	HAW	CLA	SAF
CAR		0.018*	0.206**	0.212**	0.228**	0.235**	0.230**	0.237**	0.200**	0.210**	0.206**	0.191**	0.224**
PER	900	–	0.178**	0.180**	0.180**	0.191**	0.184**	0.187**	0.157**	0.167**	0.163**	0.150**	0.221**
GAB	2551	1633	–	0.009	0.062*	0.053**	0.051*	0.066*	0.048**	0.045*	0.0445**	0.047*	0.262**
YAL	2735	1818	185	–	0.065**	0.059**	0.058*	0.070**	0.051**	0.053*	0.050**	0.049**	0.267**
GSV	3578	2678	1025	840	–	0.002	–0.003	0.001	0.016**	0.032*	0.012*	0.013*	0.264**
COO	3586	2686	1035	850	190	–	–0.003	0.001	0.020**	0.034**	0.016**	0.016*	0.278**
BEA	3870	2952	1369	1134	474	284	–	–0.007	0.017*	0.031*	0.014*	0.013*	0.255**
GLE	3936	3018	1385	1200	540	350	66	–	0.026*	0.044*	0.026*	0.023*	0.269**
SHO	5109	4209	2558	2373	1713	1523	1239	1173	–	0.007*	0.002*	0.001*	0.240**
SYD	5319	4419	2768	2583	1936	1733	1449	1383	210	–	0.008*	0.007	0.242**
HAW	5339	4439	2788	2603	1943	1753	1469	1403	230	20	–	–0.001	0.248**
CLA	5718	4818	3167	2982	2322	2132	1848	1782	609	399	379	–	0.241**

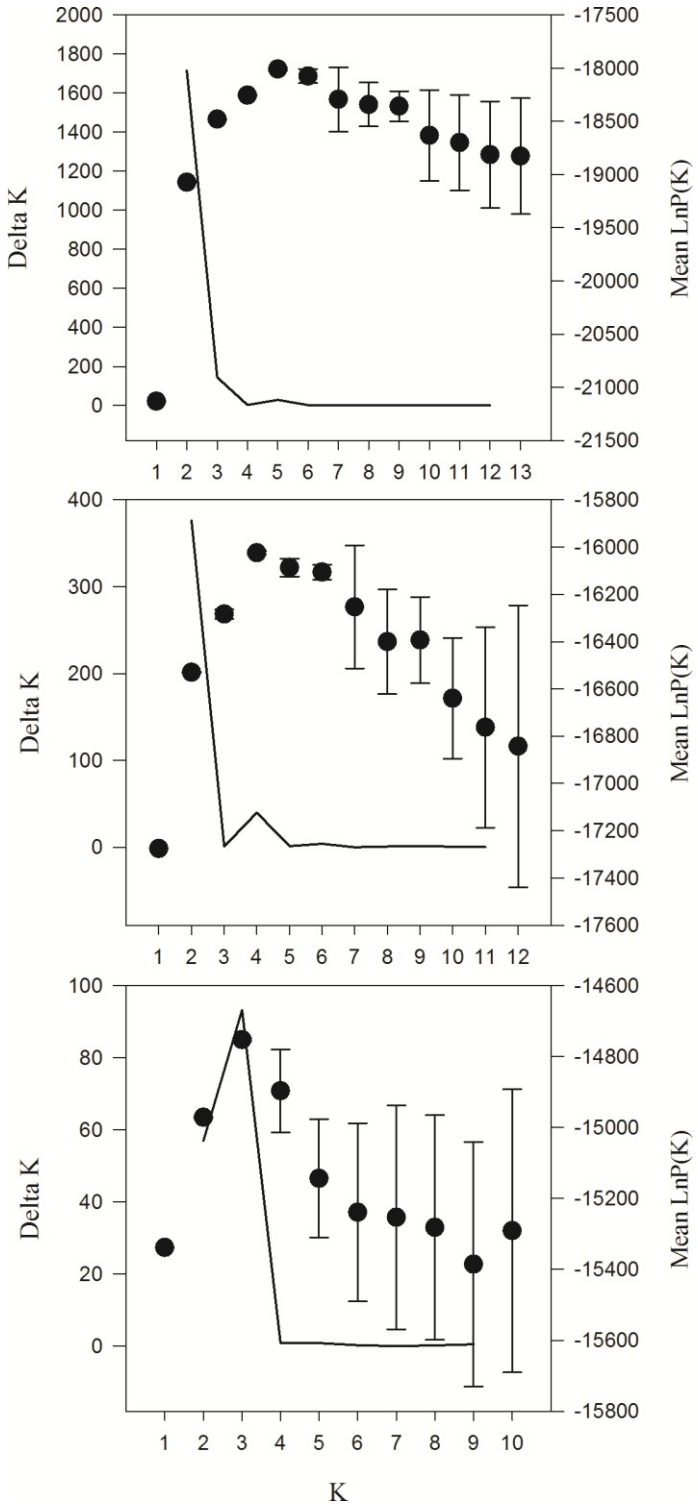

Fig. S1. ΔK (line-plot) and mean ln probability (P) (scatterplot) from the STRUCTURE runs of the: upper graph) 13 sample sets, middle graph) 12 Australian sample sets (i.e. all except for the previously most divergent (SAF) and lower graph) the 10 central to eastern Australian sample sets (i.e. all except for the previously most divergent (CAR and PER)).

Chapter 3: Statement of authorship

Statement of Authorship

Title of Paper	Integrating molecular, geochemical and morphological methods for a robust investigation into population structure
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Publication Style
Publication Details	This paper has been written up and formatted in line with the requirements of the target journal "Canadian Journal of Fisheries and Aquatic Sciences". Barnes T C; Izzo C; Junge C; Gillanders B M (In prep) Integrating molecular, geochemical and morphological methods for a robust investigation into population structure, Canadian Journal of Fisheries and Aquatic Sciences.

Principal Author

Name of Principal Author (Candidate)	Thomas C. Barnes		
Contribution to the Paper	Contributed to intellectual development of ideas, collected majority of samples, undertook most laboratory work, conducted analyses, wrote manuscript.		
Overall percentage (%)	85		
Signature		Date	9/09/2015

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 in 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	Contributed to intellectual development of ideas, undertook some laboratory work and analysis and helped write the manuscript.		
Signature		Date	9/09/2015

Name of Co-Author	Claudia Junge		
Contribution to the Paper	Contributed to intellectual development of ideas, undertook some analysis and helped write the manuscript.		
Signature		Date	9/09/2015

Name of Co-Author	Bronwyn M Gillanders		
Contribution to the Paper	Contributed to intellectual development of ideas, design, implementation and also helped write the paper via commenting and providing direction on multiple drafts and helped organise funding.		
Signature		Date	9/09/2015

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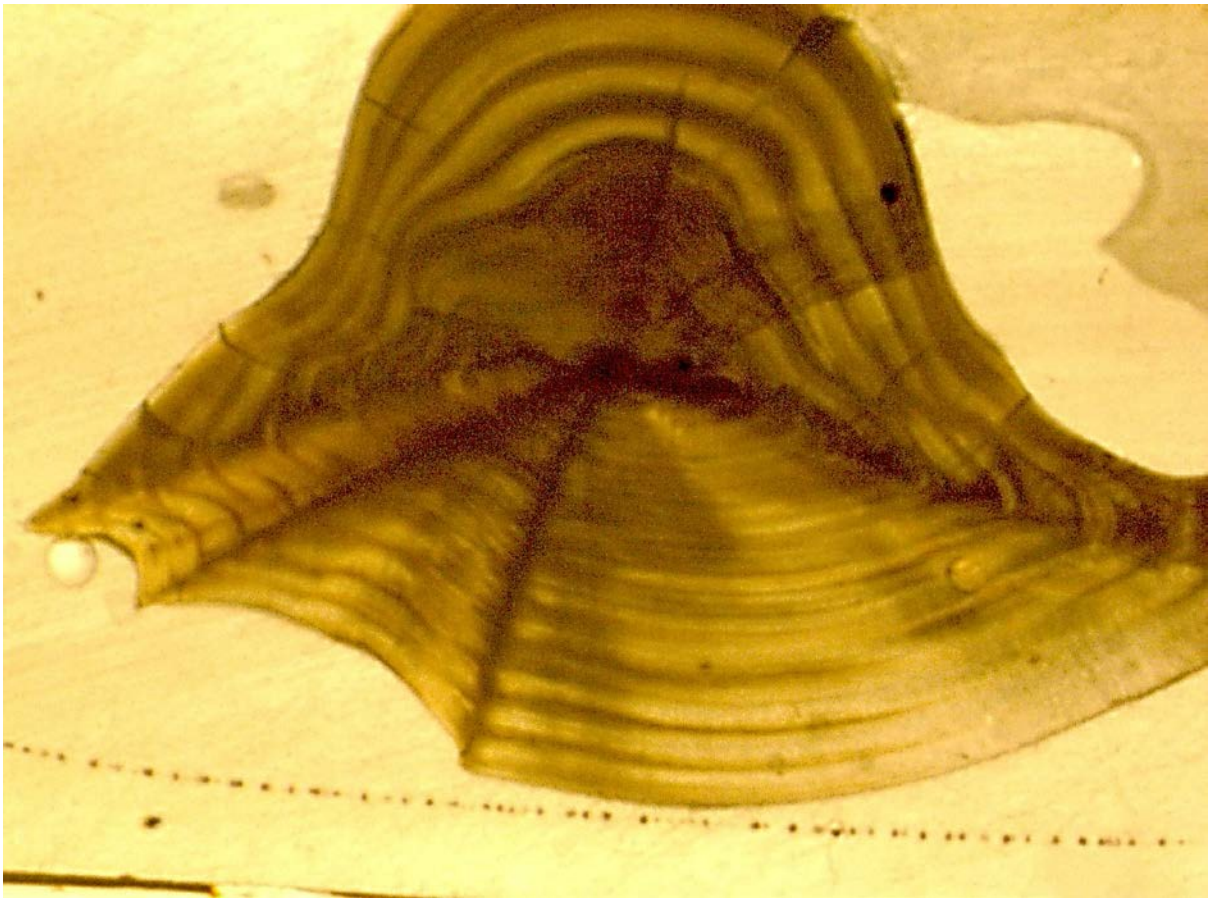
Chapter 3: Integrating molecular, geochemical and morphological methods for a robust investigation into population structure



Cross section of a mulloway otolith

Integrating molecular, geochemical and morphological methods for a robust investigation into population structure

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Key words

Otolith, stock, differentiation, genetics, *Argyrosomus japonicus,* mulloway, kob, jewfish

Abstract

An understanding of population structure is required to properly manage and conserve biota. Various approaches are used to identify population structure, each uniquely informative. To overcome the shortcomings of any one approach, the application of multiple approaches has been recommended. In the present study we integrated molecular (microsatellites), geochemical (otolith chemistry) and phenotypical data (otolith shape) from the same individuals to investigate fine scale population structure of the sciaenid *Argyrosomus japonicus*. The three different data sets were analysed individually and integrated into data sets encompassing all combinations of approaches prior to statistical analysis. We found that a combination of all three methods had an equally good allocation success to just using microsatellites (99%), whilst just otolith chemistry and shape were weaker at 69 and 82% classification success, respectively. Combining the two non-molecular approaches improved the allocation success (86%) and both were improved substantially when combined with the molecular data (99% all tests). However, the otolith approaches also provide some evidence of more complex structuring. The results of this study provide new insight into the population structure of an important sciaenid fish but also allow the comparison of different approaches. Integrating population structure approaches is more robust than using just one as it provides a weight of evidence through assessment at a variety of timescales and extrinsic and intrinsic influences.

Introduction

The definition and delineation of population structure provides a spatial context for fisheries and more broadly wild animal science and management. Although many definitions of stock or population (population herein) exist, in fish ecology and biology a population is defined as a self-reproducing unit which possesses similar life histories within a greater species distribution (Pawson and Jennings 1996; Begg and Waldman 1999; Secor 1999). Identifying these population units and therefore enhancing our understanding of fish population dynamics assists in promoting sustainable harvesting and conservation of species (Begg et al. 1999; Ovenden et al. 2009). For example differences in natural mortality, year class strength, growth rates, life history and size at maturity among population units may mean that discrete sub-populations respond differently to negative pressures such as habitat destruction, pollution and over exploitation (Pawson and Jennings 1996). Despite the benefits of population identification to fisheries and conservation efforts, defining conspecific populations remains challenging (Begg and Waldman 1999; Welch et al. 2015).

There are numerous approaches to determining population structure in fish. Molecular genetics, which identifies populations via shared allele frequencies or genomic sequences, with such information spanning many generations, is widely used (Waples and Gaggiotti 2006). Another approach, the geochemical signatures of hard parts such as otoliths can also be used to identify populations but at an ecological timescale (Campana et al. 2000) although several studies also suggest a genetic influence on otolith chemistry (Clarke et al. 2011; Barnes and Gillanders 2013, Chapter 5). Phenotypic markers such as the shape of otoliths can also be used to identify populations, via combined hereditary and ecological information, informative at the lifetime scale (Campana and Casselman 1993). Other widely used population identification approaches include the use of parasites and mark and recapture (Begg and Waldman 1999, Catalano et al. 2014). However, there are advantages and disadvantages to the varying population identification approaches (Smith and Campana 2010), with suggestions that multiple complimentary approaches will provide a more robust understanding of population structure (Begg and Waldman 1999; Welch et al. 2015).

The use of multiple approaches to assess population structure creates a “body of evidence” and thus, more accurate ecological outcomes due to the approaches being informative at different spatial and temporal scales. For example, recent divergence may not be shown by molecular genetics due to the long time scale (Begg and Waldman 1999). However, it may be confirmed by otolith chemistry, as otolith approaches are informative over a recent time period (Ferguson et al. 2011). Furthermore, as weak genetic population structuring is often the case in the highly connected marine environment, it is more likely structuring will be recognised under the greater scrutiny of multiple approaches (Begg and Waldman 1999).

The use of a holistic approach creates new challenges in data analysis and interpretation of results (Tanner et al. 2014; Welch et al. 2015). Integration of approaches has been done at the inter-study level (e.g. Roques et al. 2002; Marcogliese et al. 2003) where initial frameworks have been built on. However, this style of integration does not lend itself to outcomes readily synthesised and implemented by fishery managers. At the intra-study level researchers have historically compared the results of data sets of different approaches after running statistical tests separately (e.g. Abaunza et al. 2008), but synthesising results of multiple tests can be complex and therefore potentially still challenging for end users such as managers. To reduce the complexity of synthesising the results of separate statistics (i.e. a separate test for each approach) results of two independent approaches have been plotted in two dimensional space (Perrier et al. 2011). Whilst an ‘integrated stock definition’ has also been used to integrate results (see Welch et al. 2015), this creates a potentially arduous extra step in analysis. Alternatively, at least one study has integrated two heterogenous data sets before statistical testing (Tanner et al. 2014). We propose that this pre statistics integration as possibly the best practice as it produces a streamlined result such as a single P-value. However, to our knowledge pre-test data integration has not been attempted before on more than two heterogenous data sets from independent approaches, as such empirical testing is required to determine the feasibility of integrating three or more data sets. Supplying simplified, but informative results, is important in gaining end users support of the uptake of a holistic approach.

Mulloway (*Argyrosomus japonicus*) are a popular large bodied sciaenid, which possess a life history characterised as being long-lived and late to mature (Griffiths 1996), with the exact timing of maturity varying among regions (Ferguson 2010). These fish usually have spatially and temporally predictable habits (e.g. entering estuaries at certain periods during their life history), which increases their vulnerability to the activities of humans (Silberschneider and Gray 2008; Ferguson et al. 2014). Current management in Australia is at the state level, however, a molecular study suggests genetic management units do not align with these boundaries (Barnes et al. 2015, Chapter 2).

Despite marine life stages and a pelagic larval stage, broad scale genetic population structuring of mulloway has been found in the southern hemisphere (Barnes et al. 2015, Chapter 2). In South Africa, the species has long been described as a single stock, largely due to the results of a mark recapture study (Griffiths 1996). However, recent genetic testing indicates weak structuring in South Africa (Mirimin et al. 2015). Population genetics suggests that South African and Australian populations differ and that four populations occur across the species' Australian range (Barnes et al. 2015, Chapter 2). Two of the Australian populations are found within the waters of South Australia. Within South Australia, otolith based approaches also support the presence of multiple populations at a finer scale (Ferguson et al. 2011).

As genetic testing has suggested broad scale structuring, we set out to test whether incorporating three different population identification approaches could identify potential populations at the fine scale in a highly vagile environment. Specifically, we apply three approaches singularly and in combination to assess population structure in mulloway: (i) genetics (microsatellites), (ii) geochemistry (otolith chemistry) and (iii) phenotypes (otolith shape) to the same samples, and subsequently integrate all data to enable a single statistical test of population structure. In addition to testing the appropriateness of our approach, the results of this study provide a robust investigation into fine scale population structuring of the South Australian mulloway "meta-

population". Importantly, this investigation could suggest more appropriate management units to those currently implemented by management agencies.

Methods

Fish collection

Tissue samples and otoliths of mulloway were collected from anglers or via the commercial fishing sector at the point of landing and markets in South Australia. Samples came from seven broad geographical regions and were collected between 2009 and 2013 (Table 1, Figure 1).

Table 1. Sample set names with corresponding sample code and the number of mulloway sampled from each South Australian regional location for each approach.

Sample set location	Sample code	Genotype	Otolith shape	Otolith chemistry
Great Australian Bight	GAB	17	17	17
Yalata	YAL	13	13	12
Spencer Gulf	SPE	6	6	6
Gulf St Vincent	GSV	7	8	7
Coorong ocean	COO	40	39	40
Coorong estuary	COOE	21	22	22
Beachport	BEA	10	9	10
Total	114	114	114	113

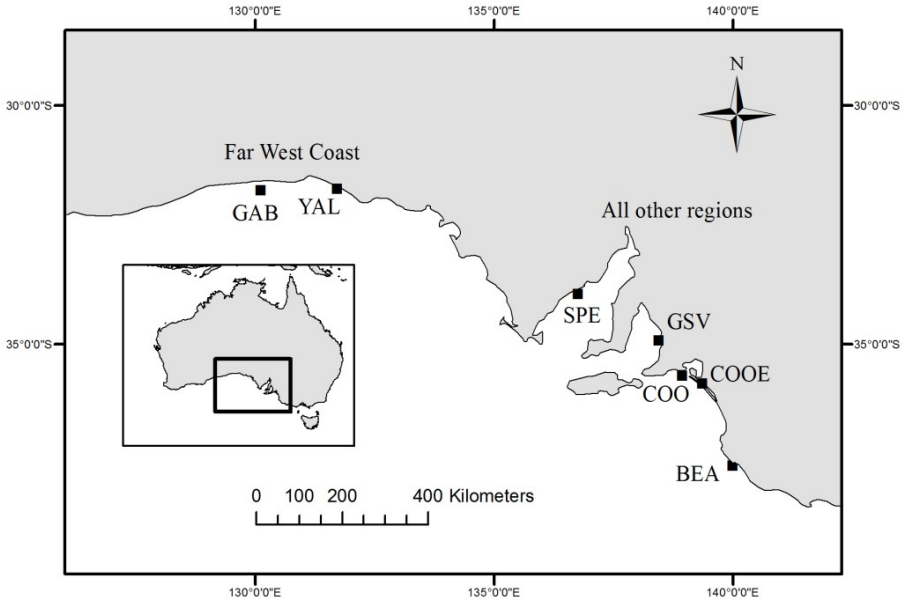

Fig. 1. Map showing regional locations and broader regions where mulloway (DNA and otoliths) were collected.

Genotyping and population genetics

DNA was extracted from tissue samples and genotyped for 13 microsatellite loci (see Barnes et al. 2014; Chapter 2). GenAlEx v6.5 (Peakall and Smouse 2006) was used to convert genotype data to a suitable format for subsequent analyses, and to calculate descriptive statistics of microsatellite diversity, i.e. unbiased expected and observed heterozygosity, and allele frequencies. Allelic richness was estimated using FSTAT v2.9.3.2 (Goudet 2001), assuming a sample size of 6 individuals, which was the minimum number from a single region (i.e. Spencer Gulf). The program GENEPOP v4.0 (Rousset 2008) was used to test for significant deviations from Hardy–Weinberg and linkage equilibrium with Markov Chain (MC) parameters set at dememorization number and maximum number of iterations per batch (10 000) for 1 000 batches. Corrections for multiple tests were applied using sequential Bonferroni corrections (Rice 1989), and the Bernoulli method (Moran 2003).

To examine population structure several simulations were conducted using the computer program POWSIM to assess the statistical power when testing for genetic differentiation (Ryman and Palm 2006). The program mimics sampling from populations at a predefined level of expected divergence through random simulations under a classical Wright-Fisher model without migration or mutation. Significance estimates were based on 1 000 independent simulations. In GENEPOP, population differentiation tests were performed using exact G tests, for all population pairs at every locus and over all loci (Markov Chain Monte Carlo (MCMC) settings and multiple test correction procedures as described above), and pairwise F_{ST} values were calculated to estimate the degree of differentiation (Weir and Cockerham 1984).

The program STRUCTURE v2.3 (Pritchard et al. 2000) was used to infer population structuring, based on MCMC simulations to assign individuals to genetic clusters (K) inferred from multi-locus genotypes. MCMC simulations were performed using the admixture model, assuming correlated allele frequencies (Pritchard et al. 2000; Falush et al. 2003), with a burn-in of 500 000 and 1 million MCMC iterations, and the 'locprior' option to detect weak structuring (Hubisz et al. 2009). Twenty

replicate runs for each K value representing the number of sampled sites (n = 7) were performed. The optimum number of clusters for the data was found by comparing the log likelihood of the data given the number of clusters [ln P(X|K)] (Pritchard et al. 2000), by estimating ΔK (Evanno et al. 2005), and by evaluating individual membership coefficients (Q) for different values of K (Pritchard et al. 2000). The number of distinct local populations was visualized in DISTRUCT v1.1 (Rosenberg 2004).

Otolith shape

High contrast images of whole otoliths were captured using Image Pro-Plus (v7.0) and a Leica DC-300 digital camera mounted to a Leica stereo microscope. Images were taken using reflected light and a black background to ensure a clear outline of the otolith. Right otoliths were oriented so that the sulcus was face down (distal orientation) and the rostrum horizontally aligned.

To determine if otolith shape varied among regions, images were imported into the Shape (v1.3) software program for elliptical Fourier analysis of contours based on outlines of otoliths (Iwata and Ukai 2002). The Fourier power spectrum was used to determine the number of Fourier descriptors required to accurately describe the shape of mulloway otoliths.

Otolith chemistry – outermost edge

One whole otolith per fish was embedded in indium-spiked resin before transversely sectioning through the primordium, using a low speed saw. The thin section was polished on each side and mounted onto a microscope slide with indium-spiked epoxy. Slides were then cleaned in ultrapure water and air-dried for 24-h under a laminar flow hood.

Otolith element analysis was performed using a laser ablation inductively coupled plasma mass spectrometer (LA ICP-MS) system housed at Adelaide Microscopy, University of Adelaide. The LA ICP-MS system consisted of a New Wave UP-213 high-performance ultraviolet laser connected to an Agilent 7500cs ICP-MS. The elemental isotopes chosen for analysis were: sodium (^{23}Na), magnesium (^{24}Mg), manganese (^{55}Mn), strontium (^{88}Sr), indium (^{115}In), barium (^{138}Ba), and calcium (^{43}Ca); the latter was used as an internal standard. All trace element concentrations were ratioed to ^{43}Ca. A

reference standard (National Institute of Standards and Technology: NIST 612) was analysed after every eight to ten otolith ablations to correct for machine drift. A certified reference material (microanalytical carbonate standard, MAC-3) was analysed at the beginning and end of each session and used to calculate precision. Elemental concentrations (in ppm) were determined using GLITTER v5.3 (www.glitter-gemoc.com). All data processing was performed using Excel (Microsoft).

All element:Ca ratios were above detection limits for all samples except Mn:Ca. Precision based on coefficient of variation of repeated measures of the standard samples (NIST612) and a certified reference material (microanalytical carbonate standard, MAC-3) was less than 6% and 10% for all isotopes, respectively (except ^{23}Na for the MAC-3, which was 11.8%).

Statistical analysis

Microsatellite data are codominant therefore they cannot easily be combined with the otolith shape and chemistry data. We therefore used the assignment proportions to the two genetic clusters reported by STRUCTURE (far west coast and other regions) as the genetic scores in subsequent analyses. Therefore, assignment proportions of genetic clusters, Fourier descriptors of otolith shape and trace elemental concentrations from the edge of otoliths were analysed using permutational multivariate analysis of variance (MANOVA). This analysis was initially performed separately on the three datasets. Euclidean distances between each pair of samples were calculated to obtain a distance matrix. A one-factor MANOVA involving permutation of the raw data was used to determine whether differences occurred among the seven sample sets. A Canonical Analysis of Principal (CAP) coordinates was used to ordinate the data and determine the success of classifying individuals back to their sample set and therefore regional capture location. Such an analysis finds the axis (or axes) that are best at distinguishing among *a priori* groups based on the principal coordinate space (Anderson and Willis 2003). Fish were classified to their sample set, and more broadly to the two genetic populations evident from the structure analyses. The "leave-one-out"

approach was used to calculate misclassification errors. All statistical tests were performed on the 7 sample sets and on the two genetic clusters obtained from the microsatellites.

Results

Population genetics and structure

The average number of alleles per locus within a sampling site varied between 4.5 and 8.2 (Table 2), and allelic richness ranged from 4.3 to 4.8 (Table 2). Observed and expected heterozygosities ranged from 0.61 to 0.71, and 0.64 to 0.67, respectively (Table 2). One sample set showed non-conformance to Hardy Weinberg equilibrium at one locus (GAB – Arja39). All locus pairs were in linkage equilibrium except for one in one sample set (Arja41 & Arja42 – GAB). After removal of either Arja41 or Arja42, the results did not vary; therefore these locus pairs were included in all subsequent analyses.

Table 2. Genetic diversity indices for regional sample sets of mulloway with number of different alleles (Na), allelic richness (Ar), unbiased expected heterozygosity (Uhe), observed heterozygosity (Ho) (see table 1 for an explanation of sample codes).

		Genetic diversity			
Sample code	**n**	**Na**	**Ar**	**Uhe**	**Ho**
GAB	17	6.1	4.3	0.66	0.62
YAL	13	5.2	4.3	0.67	0.63
SPE	6	4.5	4.5	0.70	0.71
GSV	7	5.2	4.8	0.64	0.63
COO	40	8.2	4.6	0.67	0.67
COOE	21	6.6	4.3	0.64	0.63
BEA	10	5.5	4.5	0.65	0.61

Power simulations indicated that given the number of loci, their polymorphism, and the sample sizes used in the study, the probability of detecting F_{ST} values as low as 0.01 was 0.98 and for F_{ST} of 0.005 was 0.72. Given that the smallest F_{ST} value between two significantly diverged populations was 0.034, the significant F_{ST} values observed in this study are most probably realistic indications of population differentiation.

Eleven out of 21 pairwise comparisons showed significant differentiation in F_{ST}. This is highly significant given the Bernoulli method (i.e. the probability of getting this result with α=0.05 by chance alone, is very small). Ten of those significant pairwise comparisons involved the sample sets

GAB or YAL from the region called the far west coast (FWC). The global F_{ST} (jackknifed over loci) was 0.034 (95% confidence interval (CI): 0.022-0.048), and pairwise F_{ST} values varied between -0.018 and 1.75 (Supplementary Table 1).

The number of inferred distinct local populations using STRUCTURE was two, one which included sample sets GAB and YAL from the FWC and one which included all other sample sets (termed all other regions for the present study) (Figure 2). The likelihood of the data was highest for K=2 and the measure of ΔK confirmed this (data not shown). The removal of the most differentiated sample sets GAB and YAL (FWC) and subsequent re-analysis of the data did not result in further population structuring.

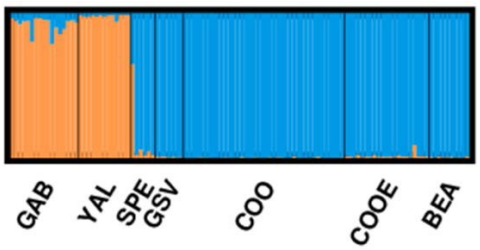

Fig. 2. Individual assignment of mulloway based on the Bayesian clustering method in STRUCTURE. Each bar represents the probability of membership for each individual to one of the two groups (*K*=2). Labels at the bottom are collection locations (see Table 1 for details).

The CAP analysis found that 60% of fish were correctly allocated to their sample sets (collection region) when all seven sample sets were included in the analysis (Table 3). In this analysis GAB and YAL samples only misclassified between each other (7 individuals - data not shown), one SPE fish was erroneously allocated to GAB and the other misallocations were among all other sample sets. Overall allocation success increased to 99% when only two broad regions (FWC and all other regions) were considered with the FWC showing an allocation rate of 100% (Table 3) and the same SPE fish again erroneously allocated to GAB.

Table 3. Classification (%) of mulloway to regional sample sets based on microsatellite (MS), otolith chemistry (OC) and otolith shape (OS) data individually and in combination. Correct classification was estimated using Canonical Analysis of Principal (CAP) coordinates and a leave one out allocation approach. See Table 1 for sample set codes.

	Correct classification						
Sample set	**MS**	**OS**	**OC**	**OC & OS**	**MS & OS**	**MS & OC**	**MS, OC & OS**
GAB	65	65	41	82	76	71	88
YAL	92	31	33	42	62	67	75
SPE	0	33	50	33	33	17	33
GSV	57	38	14	29	43	29	29
COO	80	44	37	51	59	38	59
COOE	33	77	32	86	90	38	86
BEA	20	44	50	56	56	70	56
Overall	60	51	37	60	65	47	67
FWC	100	83	69	90	100	100	100
All other	99	82	72	86	99	99	99
Overall	99	82	68	87	99	99	99

Otolith shape

Fourier descriptors of otolith shape varied significantly among the seven regions ($F_{6,107} = 6.496$, $P<0.001$; Figure 3a). Post-hoc tests found that there were significant differences among all pairwise comparisons, with the exception of comparisons between GAB and YAL, YAL and SPE and between SPE and COO sample sets (Figure 3a). The CAP analysis found the overall accuracy of classifying mulloway to sample sets was only 51%. Classification success ranged from 31 (YAL) to 77% (COOE). There was no clear pattern regarding the misallocation of individuals (Table 3). Allocation success increased to 82% when only the two broad regions were considered, with the FWC fish correctly classified 83% of the time and all other regions correctly classified 82% of the time (Table 3).

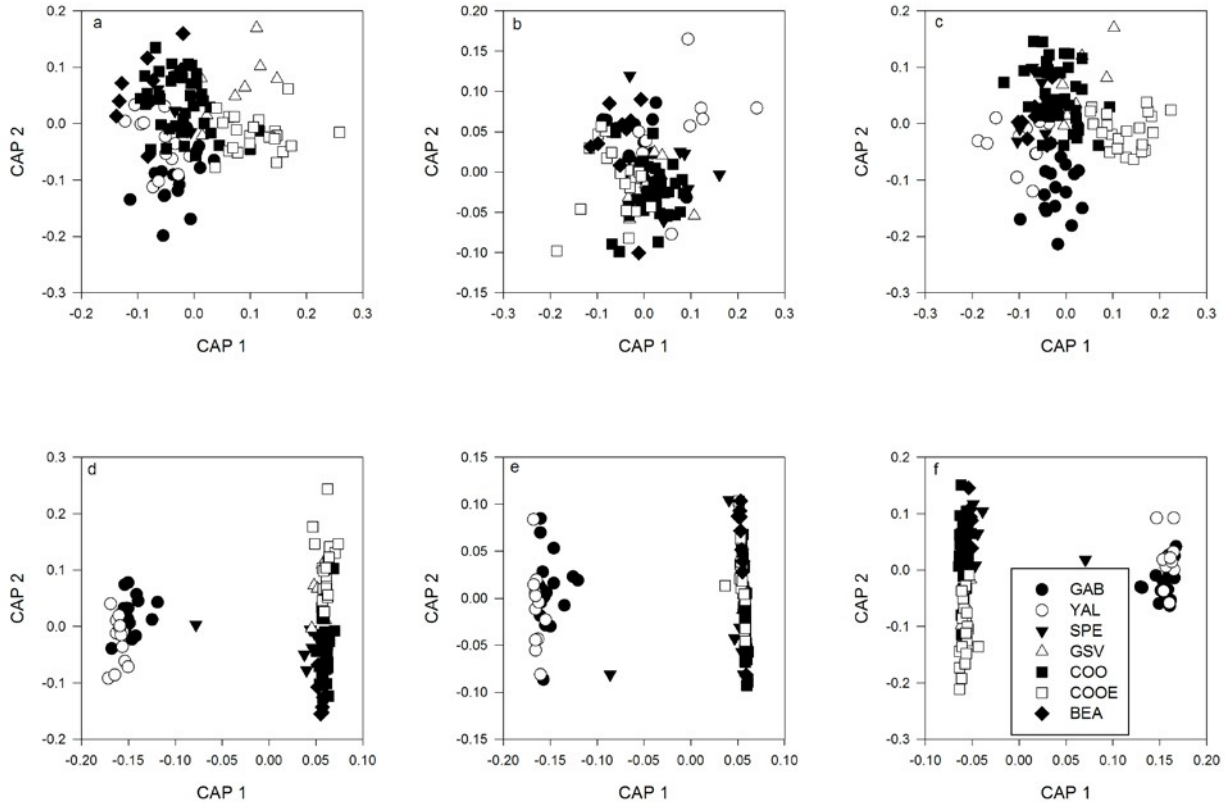

Fig. 3. Canonical Analysis of Principal (CAP) coordinates plots summarising variation in: otolith a) shape, b) chemistry and c) shape and chemistry combined and genotypes with d) shape, e) chemistry and f) shape and chemistry from different regional sample sets of mulloway.

Otolith chemistry

The multielement signature comprising Na:Ca, Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca differed significantly among sample sets ($F_{6,107} = 4.673$, $P<0.001$; Figure 3b). The sample sets separated broadly into two groups based on post-hoc tests, namely a group comprising GAB, YAL, SPE and BEA fish and a group comprising COO and COOE fish along with GSV. Although significant differences among regional sample sets were found, the overall accuracy of classifying fish back to their sample sets based on the multielement signature was less than that of otolith shape (37% cf. 51%). Overall classification success ranged from 14 (GSV) to 50% (SPE and BEA) (Table 3). Individual fish allocation success increased to 68% when only the two broad regions were considered, with the FWC allocation success 69% and all other regions allocation success 72% (Table 3).

Comparison of approaches

When considered singularly (as above), the results of the three independent approaches broadly suggest 2 main populations of mulloway in South Australian waters, although there was a lack of consistency between approaches. The combination of otolith shape and chemistry suggest the sample sets can be separated into two main groups (similar to just otolith chemistry), but with potential for a smaller subgroup. For example, pairwise post hoc analysis again suggests grouping of GAB, YAL, SPE and BEA fish in one group and GSV, COO and COOE fish in another, which is supported by the CAP coordinates plot (Figure 3c). Also, pairwise post hoc showed there was evidence of a sub-group (SPE and GSV).

The combination of the two otolith-based approaches generally increased individual fish allocation to sample sets success compared to either approach used individually. Individual fish allocation success improved when 7 sample sets were considered and when the two broad regions were considered (individual fish allocation success was 51, 37, 60% for all 7 sample sets and 82, 68 and 87% for otolith shape, otolith chemistry and the combination, respectively) (Table 3, Figure 3c).

Visualisation from CAP coordinates plots clearly shows separation of the two broad regions (FWC and all other regions) for the otolith shape and microsatellites (Figure 3d) and the otolith chemistry and microsatellites (Figure 3e). The addition of microsatellites improved allocation of fish to sample sets for both otolith approaches (Table 3, Figures 3d and 3e). Alternatively, the addition of the otolith approaches to microsatellites only improved the allocation at 3 sample sets namely GAB, SPE and COOE (Table 3, Figures 3d and 3e). When only considering the two broad regions, the addition of microsatellites to either otolith approach improved the individual fish allocation rate to near perfect (99%) for either combination (Table 3).

When considering all seven sample sets the three way combination of microsatellites, otolith shape and otolith chemistry supported the separation of two main groups with a sub-group of sample sets in a similar manner as suggested by both pairwise post hoc and CAP analysis involving otolith chemistry (Figure 3f). For example the CAP coordinates plot clearly separates FWC but similarly suggests the sub grouping within the all other regions (e.g. SPE and BEA and GSV, COO, COOE) (Figure 3f). The individual fish allocation success of sample sets was only improved in one case (GAB) when comparing the three way combination to all other combinations or single approaches (Table 3, Figure 3). However, like all approaches involving microsatellites the individual fish allocation to the two broad regions was near perfect (Table 3).

Discussion

Our results demonstrate that it is feasible to combine three data sets from the same individuals to increase the discriminatory power when looking at population structure and to produce integrated, readily interpreted outcomes for fisheries managers. Our integrated approach suggested there were two populations among the sample sets investigated. However among the individual approaches, there was some disparity as to which sample sets formed the two populations, but no consistent evidence of finer scale structuring was found. Integrated datasets from multiple approaches for identifying population structure have the potential to facilitate a better understanding of animal ecology and could lead to better management outcomes.

Microsatellite data suggested a major genetic spilt was evident in South Australia (Chapter 2, Barnes et al. 2015). However the present study includes an extra sample set (SPE – Spencer Gulf), which is possibly located in the vicinity of the population boundary. These data suggest Spencer Gulf may be a region of overlap of the two South Australian gene pools, with one fish having a large proportion of the western genotype (i.e. the predominant genotype from the FWC), whilst the other fish in the sample set have the all other regions genotype. Unfortunately, Spencer Gulf fish can be hard to obtain, and as such we only acquired six fish for the present study. Furthermore, this region has previously had sea cage aquaculture of mulloway from which there have been escapees (Ferguson et al. 2011). In addition, the brood stock for aquaculture in Spencer Gulf was acquired from regions other than Spencer Gulf such as the Coorong (COO), creating uncertainty about the true genetic identity of mulloway from this region (W Hutchinson, SARDI Aquatic Sciences, pers. com.). Nonetheless the findings of the present study support the theory of a phylogeographic boundary created by the large South Australian gulfs (Barnes et al. 2015, Chapter 2).

Otolith shape and chemistry have been useful as a natural tag for many fish species, including mulloway (Ferguson et al. 2011), but the results can be complex as the response of fish to intrinsic and extrinsic factors can be species specific and require background understanding via controlled

testing (Campana 1999; Thresher 1999; Barnes and Gillanders 2013, Chapter 4). Our results from the otolith approaches used singularly and in combination support the presence of two broad populations, as evident from the genetics, but also suggest finer structuring. Interestingly other studies have found disparity between genetics and otolith approaches (e.g. Milton and Chenery 2001, Bradbury et al. 2008, Collins et al. 2013). A review of the literature suggests that the otolith approaches provide the best allocation success of individual fish to putative population compared to molecular approaches. For example, at least three studies found otolith chemistry provided the best fish allocation (Thorrold et al. 2001; Smith and Campana 2010, Tanner et al. 2014) and another found otolith shape to give the best allocation success (Wennevik et al. 2008). There are a number of possible explanations for this inconsistency; such as our success with microsatellites and the contradictory findings of other studies. First, the present study utilised 13 microsatellite loci which is over double for some of the comparable studies (e.g. Smith and Campana 2010), hence our genotypes potentially had better differentiation ability. Second, some studies did not use the same individuals for the different approaches (see Bradbury et al. 2008), creating some uncertainty of the true genetic identity of fish used for otolith chemistry. Third, in some coastal regions there are heterogeneous environments, which will affect otoliths differently over short spatio-temporal scales. This means differences in otolith chemistry driven by heterogeneous environments could represent a separate population or just different dispersal of groups within a population.

In the present study all approaches and statistical outcomes suggest a single FWC population but this is less clear for the “all other regions”. This is because the “all other regions” encompass a broad array of environments. Environmental variation is the greatest influence on otolith shape and chemistry but there is also some influence of intrinsic factors including genetics (Vignon and Morat 2010; Clarke et al. 2011; Barnes and Gillanders 2013, Chapter 4) and growth rate (Campana and Casselman 1993; Vignon 2012). The type of environmental variables that influence otoliths include salinity, water chemistry and temperature (Campana et al. 2000; Elsdon and Gillanders 2002; Barnes and Gillanders 2013, Chapter 4) and these variables differ in open ocean, coastal and estuarine

waters which encompass the range of environments that mulloway were collected from. This is particularly the case across the “all other regions” due to estuaries and river mouths facilitating the mixing of river and marine waters (Ferguson et al. 2008), whilst upwelling also occurs in the area (Kämpf et al. 2004). As well as being influenced by different environments otolith analyses and genetics also reflect different ecological and evolutionary time scales (Campana and Casselman 1993; Begg and Waldman 1999; Welch et al. 2015). With microsatellites, otolith shape and otolith chemistry are informative at timescales of: 100s of generations, single generations and recent life history respectively. Thus different influences of intrinsic and extrinsic forces over different timescales may explain why there were different outcomes among approaches but importantly this adds to the robustness of such investigations (i.e. a variety of temporal scales are incorporated into the study) (Welch et al. 2015).

Combining all three approaches provided strong support for two South Australian mulloway populations. However, when all 7 sample sets were considered for statistical testing there was slightly different grouping in terms of the two broad populations. The inconsistency was the inclusion of SPE and BEA into the FWC population; however, this was readily explained by the mixed population at SPE and marine conditions at BEA among estuary associated sample sets in the rest of the region. The slightly different grouping of the two broad populations between approaches demonstrated an advantage of genetics, where the genotype is stable across cohorts (Smith and Campana 2010); whilst the otolith chemistry captures different intra-population dispersal (Perrier et al. 2011). In our example, BEA is clearly a member of the all other regions genetic cluster, but the fish we sampled may have resided for a period of time in marine waters sufficient to allow their otoliths to be significantly affected. As such, this recognition of different dispersal rather than population structure highlighted the advantage of using more than one approach as it provided the opportunity to focus on the best approach, which varies between species and regions (Smith and Campana 2010). The best approach can be determined by reviewing inconsistencies between

approaches and determining the intrinsic and extrinsic factors which could have contributed to the inconsistency.

There was only one stage to integrating three data sets to allow statistical testing on one master data set. The microsatellite scores were co-dominant, as such they needed to be transformed to allow integration. We found the genetic cluster assignment proportion to be a suitable vehicle to allow integration as it converts genotypes into quantitative data and has been used successfully in a previous study (Tanner et al. 2014). Another study has utilised an index to collate results of multiple approaches post testing of each approach individually (Welch et al. 2015); whilst this index is informative, we believe integration of data pre statistical test to be a more simplified approach.

It is feasible to integrate more than two approaches to population structure in a manner that produces readily interpreted outcomes. By utilising multiple approaches we have presented a “body of evidence” drawing on the various information provided by the approaches. This information included intrinsic (e.g. evolutionary) and extrinsic (e.g. environmental) aspects, with these operating at different timescales (generational, life time and short term). For mulloway in South Australia the “body of evidence” supports two long-term populations which are currently managed as a single unit.

It was evident through our fish allocation success that the microsatellites provided the best biological information of the three approaches. Whilst the literature suggests this is normally unusual (i.e. otolith approaches historically provided the best allocation success (e.g. Tanner et al. 2014) it is likely that more variable modern genetic approaches will give a more accurate indication of vagile marine fish population structure than has previously been the case. This is facilitated through modern sequencing techniques providing many more markers than what was traditionally economically viable and by utilising markers under selection as a source of information on recent divergence (see Catalano et al. 2014). Hence the choice of approaches to be incorporated in a study depends on the requirements of the biological question. For example if recent divergence or cohort

dispersal is a possibility then an otolith approach combined with modern molecular approaches is likely to be very informative. Alternatively, in the case that a target fish species range is over large areas of homogenous oceanic environments, otolith chemistry is not likely to be very informative and more appropriate approach(es) should be considered (e.g. genetics coupled with parasites or mark recapture). The most appropriate approaches for different fish population studies have a greater chance of being utilised if multiple approaches are employed.

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Supplementary material

Supplementary Table 1. Pairwise F_{ST} values and significant genic differentiation (*) for sample sets (defined in table 2) of mulloway.

Sample set	GAB	YAL	SPE	GSV	COO	COOE
YAL	-0.005					
SPE	0.066*	0.075*				
GSV	0.065*	0.068*	0.049			
COO	0.057*	0.066*	0.018	-0.002		
COOE	0.051*	0.056*	0.034*	-0.001	-0.003	
BEA	0.061*	0.064*	0.026	-0.018	-0.001	0.003

*P<0.01

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Chapter 4: Statement of authorship

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Thomas C. Barnes		
Contribution to the Paper	Contributed to intellectual development of ideas, undertook all laboratory work, conducted analyses and wrote manuscript.		
Overall percentage (%)	90		
Signature		Date	9/09/2015

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 in 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	Bronwyn M Gillanders		
Contribution to the Paper	Contributed to intellectual development of ideas, design, implementation and also helped write the paper via commenting and providing direction on multiple drafts and helped organise funding.		
Signature		Date	9/09/2015

Chapter 4: Combined effects of extrinsic and intrinsic factors on otolith chemistry: implications for environmental reconstructions

Landing a large mulloway for sampling and release

NRC Research Press

ARTICLE

Combined effects of extrinsic and intrinsic factors on otolith chemistry: implications for environmental reconstructions

Thomas C. Barnes and Bronwyn M. Gillanders

Abstract: Otolith chemistry is widely used to understand patterns of fish movement and habitat use, with significant progress made in understanding the influence of environmental factors on otolith elemental uptake. However, few studies consider the interactive effect that environmental and genetic influences have on otolith chemistry. This study assessed the influence of salinity, temperature, and genetics on the incorporation of three key elements (strontium (Sr), barium (Ba), and magnesium (Mg)) into the otoliths of two discrete stocks of mulloway (*Argyrosomus japonicus*) fingerlings reared in captivity. Elemental analysis via laser ablation inductively coupled – plasma mass spectrometry found that stock (genetics) had a significant interactive effect on otolith Sr:Ca (salinity × temperature × stock) and Ba:Ca (salinity × stock), but did not affect Mg:Ca incorporation. Mg:Ca showed a positive relationship with temperature for both stocks. The incorporation of some elements into the otoliths of fish is the result of complex interactions between extrinsic and intrinsic factors. These findings highlight the necessity to also consider stock along with environmental variables when using trace elemental signatures to reconstruct the environmental histories of fish.

Résumé : L'utilisation de la chimie des otolites pour comprendre les déplacements des poissons et leur utilisation de l'habitat est fort répandue, et des avancées considérables ont été faites dans la compréhension de l'influence de facteurs environnementaux sur l'incorporation d'éléments dans les otolites. Cela dit, peu d'études ont abordé l'effet interactif des influences environnementales et génétiques sur la chimie des otolites. La présente étude a évalué l'influence de la salinité, de la température et de la génétique sur l'incorporation de trois éléments clés (strontium (Sr), baryum (Ba) et magnésium (Mg)) dans les otolites de deux stocks distincts d'alevins d'un an de mulloway (*Argyrosomus japonicus*) élevés en captivité. L'analyse élémentaire par plasma à couplage inductif spectrométrie de masse jumelée à l'ablation au laser a permis de démontrer que le stock (la génétique) avait un effet interactif significatif sur les rapports Sr:Ca (salinité × température × stock) et Ba:Ca (salinité × stock) des otolites, mais n'avait pas d'incidence sur l'incorporation de Mg:Ca. Le Mg:Ca présentait une relation positive avec la température pour les deux stocks. L'incorporation de certains éléments dans les otolites de poisson est le résultat d'interactions complexes entre des facteurs extrinsèques et intrinsèques. Ces constatations soulignent la nécessité de tenir compte du stock en plus des variables environnementales dans l'utilisation des signatures d'éléments en traces pour reconstruire les antécédents environnementaux des poissons. [Traduit par la Rédaction]

Introduction

Understanding patterns of movement and stock structure is vital for the development of spatially appropriate management and conservation action (Campana et al. 1999; Thorrold et al. 2001). It is well recognised that tag and recapture methods for assessing species movement and population structure only provide information on tagged fish, require large numbers of fish to be tagged to get meaningful returns, and can be expensive. Otolith chemistry provides an alternate approach, since all fish contain a natural tag. Furthermore, unlike conventional tag and recapture technologies, otolith chemistry is applicable to all life history stages (Gillanders 2005).

Otoliths are calcium carbonate structures that act as chronometers of environmental change by incorporating information from the surrounding environment into their matrix (Elsdon et al. 2008). This incorporation is permanent (Campana 1999), and has been widely used to reconstruct the environmental histories of fish and to delineate discrete stocks of fish (Campana et al. 2000; Morris et al. 2003; Ferguson et al. 2011). However, a growing body of literature indicates that otolith chemistry is influenced by a range of intrinsic (i.e., physiological and genetic) (e.g., Clarke et al. 2011) and extrinsic (i.e., environmental: salinity and temperature) (e.g., Elsdon and Gillanders 2002) factors. These factors have been shown to interact (Elsdon and Gillanders 2002) or act independently (Martin et al. 2004) on various elements that are incorporated into otoliths in a species-specific manner (Diouf et al. 2006; Martin and Wuenschel 2006; Dorval et al. 2007). Species-specific responses make generalized predictions of environmental effects on otolith chemistry difficult, potentially impeding the ability to reconstruct the habitat use of fish. A greater understanding of how both intrinsic and extrinsic factors affect otolith trace element incorporation is needed.

Inter- and intra-specific genetic differences may affect otolith chemistry but have not been extensively tested (Thresher 1999). Inter- or species-specific differences in otolith chemistry have been demonstrated through tank rearing experiments (Elsdon and Gillanders 2003) and similarly in the wild (Gillanders and Kingsford 2003; Hamer and Jenkins 2007). However, it is possible that within species (intra) genetic differences (stock or population differences) may also cause differences in otolith chemistry. One previous study has reported intraspecific effects (genetics) on otolith chemistry of a teleost (*Menidia menidia*) (Clarke et al. 2011). Similarly extrinsic factors also require further investigation into their influence on otolith chemistry, since variation among species is reported (Elsdon and Gillanders 2003).

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This study sought to determine whether otolith chemistry varied between two genetic stocks of mulloway (i.e., kob, *Argyrosomus japonicus*), a commercially important species, and whether any variation was influenced by extrinsic factors, namely temperature and salinity. Specifically, we aimed to determine the relative and interactive effects of stock (genetic component) and salinity and temperature (environmental component) on otolith elemental chemistry in a controlled laboratory experiment.

Materials and methods

Experimental design

A controlled laboratory experiment was conducted to test the effects of salinity and temperature on elemental chemistry of otoliths of *Argyrosomus japonicus* from two different genetic stocks of hatchery fish. The salinity and temperature ranges were chosen to represent natural conditions experienced by the species (e.g., brackish to hypersaline). Limitations with obtaining juvenile fish at the same time meant that experimental rearing of each genetic stock of *A. japonicus* was conducted separately. The first experiment focused on a New South Wales (NSW) stock and used seven nominal salinity levels (10‰, 20‰, 30‰, 35‰, 40‰, 45‰, and 50‰) at a single temperature (20 °C). A further experiment used four of these salinities (10‰, 30‰, 40‰, and 50‰), each replicated at three different temperatures (16, 20, and 24 °C) to investigate the interactive effect of temperature and salinity. The Western Australian (WA) stock was exposed to four salinity (10‰, 30‰, 40‰, and 50‰) and two temperature (20 and 24 °C) treatments; treatment levels were reduced owing to decreased numbers of fish being available.

Fish rearing

Both stocks of *A. japonicus* were sourced from hatcheries. The NSW stock came from the New South Wales Department of Primary Industries hatchery at Port Stephens, and fish were ~0.8 g at the start of the experiment. The WA stock was sourced from Challenger TAFE in Perth, WA, as larvae and reared at the South Australian Research and Development Institute (SARDI) Aquatic Sciences hatchery at West Beach until the fish attained an approximate mass of 0.8 g.

Fish were initially housed in 250 L polypropylene tanks and held for at least 1 week to acclimate or in the case of larvae to metamorphose and grow to the desired size. During acclimation, temperature was maintained at a nominal 21.5 °C to encourage growth. Fresh ultraviolet-filtered seawater was sourced from SARDI Aquatic Sciences (40‰) and supplied to the holding tanks in half-volume water changes twice weekly. Fish were fed daily on commercially available pellets (Grobest Pty Ltd.; barramundi feed: floating, 0.75 and 2 mm diameter), except during the larval development phase, where the diet initially comprised rotifers and *Artemia* spp. until fish were pellet weaned (consistent with standard hatchery practice) (e.g., Battaglene and Talbot 1994).

On completion of the acclimation or development period, all fish were fasted for 24 h and then immersed in an alizarin complexone ($C_{19}H_{15}NO_8$) bath at a concentration of 35 mg·L^{-1} for 24 h to mark the otoliths (de Vries et al. 2005). The alizarin mark distinguished the experimental growth from the hatchery growth.

Fish were randomly assigned to experimental tanks at a nominal density of either 10 fish per tank (NSW) or 7 fish (WA) and reared under experimental conditions for 1 month. The differences in density were a result of fewer fish being available from the WA stock. There were two experimental tanks per treatment for both stocks. The experimental tanks were 40 L in volume and manufactured from high-density polypropylene. Experimental tanks were covered with clear Plexiglass lids to allow light penetration, stop jumping mortalities, and minimize evaporation, thereby keeping experimental salinities constant. Light was supplied as a timer-controlled 12 h light : 12 h dark photoperiod by metal halide grow lights. Water aeration was provided constantly by filtered compressed air. Water quality was maintained during the course of the experiment by regular 50% water changes.

Fish were gradually acclimatised to experimental salinities, which were raised or lowered at a rate of 5‰ every 24 h. For the NSW stock, hypersaline solution (~75‰) was sourced from the Adelaide pilot desalination plant (Adelaide Aqua). The brine was mixed with seawater (40‰) at appropriate concentrations to produce the two hypersaline treatments (45‰ and 50‰). For the WA stock, Red Sea Salt was added to seawater (as desalination brine was unavailable) and allowed to stabilize for 24 h with aeration before use to produce a single hypersaline solution (50‰). All experiments used straight seawater for the ambient seawater (40‰) treatments. Treatments with salinities below 40‰ were achieved by diluting seawater with bore water (1‰–2‰) sourced from SARDI Aquatic Sciences.

Fish were gradually acclimatised to experimental temperatures at a rate of 2 °C over 48 h, using aquarium heaters in tanks to increase temperatures or flow-through chillers to decrease temperatures. All tanks were immersed in water baths to maintain constant temperatures. The 16 °C treatments were maintained by external chillers (Carrier), split system air conditioning, and back-up portable chillers (necessary during the hot South Australian summer). The 20 and 24 °C treatments were maintained for both experiments with water baths chilled to 18 °C and individual tanks raised by aquarium heaters to the appropriate levels.

Temperature and salinity were periodically measured in each tank using an electronic water quality unit (YSI Sonde, 556 MPS). The meter was calibrated once a week using a solution of known salinity and temperature chosen to represent the midrange of experimental salinities and temperatures.

Chemical analysis of water samples

Water samples were collected for elemental analysis at the beginning, middle, and completion of the rearing period. Water samples were collected using a 25 mL syringe, filtered (40 μm) and nitric acid preserved (pH < 2) before refrigeration. Experimental water samples were analysed for ambient elemental concentrations, which were determined using dual-view inductively coupled plasma – atomic emission spectrometer (Perkin-Elmer 3000) for the analysis of calcium (Ca) and magnesium (Mg) and a dynamic reaction cell inductively coupled plasma – mass spectrometer (Perkin-Elmer 6000) for the analysis of strontium (Sr) and barium (Ba). Water concentration data were converted to molar concentrations and ratioed to calcium.

Otolith preparation and analysis

At the end of the experiments (28 days), all fish were euthanized in an ice and seawater slurry, and then stored frozen until the otoliths were extracted. For each fish, standard lengths were recorded before dissection. The sagittal otoliths were extracted, washed in ultrapure water, and allowed to dry before being stored in microcentrifuge tubes. Otoliths were embedded in two-part epoxy "Epofix" (Struers), spiked with 40 ppm indium, and sectioned transversely through the nucleus to 0.35 ± 0.05 mm using an Isomet low-speed diamond saw (Buehler Ltd.). Otolith sections were polished using lapping film lubricated with ultrapure water to produce a surface finish appropriate for chemical analysis (~3 μm). Otolith sections from different treatments were mounted randomly on microscope slides using 40 ppm indium-spiked thermoplastic cement (Crystalbond 509). Slides were then cleaned by sonication in ultrapure water for 5 min and allowed to dry in a laminar flow hood. Otolith sections were examined under a fluorescent microscope with transmitted light (Leica model DMLB), which highlighted the alizarin mark, thereby indicating experimental growth. Rough otolith drawings indicating the alizarin mark were made to facilitate the targeting of experimental growth during elemental analysis.

Table 1. Results of permutational ANOVA comparing the Sr:Ca, Ba:Ca, and Mg:Ca elemental ratio in otoliths of New South Wales and Western Australia *Argyrosomus japonicus* among salinity (S), temperature (T), and stock (St) treatments.

		Sr:Ca			Ba:Ca			Mg:Ca		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
St	1	1.5159	38.093	0.001	3.7161×10^{-7}	0.70158	0.404	9.6017×10^{-2}	3.3236	0.095
S	3	2.9024	72.926	0.001	1.3404×10^{-5}	25.231	0.001	3.0005×10^{-2}	1.0350	0.423
T	1	0.6183	15.538	0.002	3.9604×10^{-6}	7.4770	0.014	0.6049	20.940	0.003
St × S	3	7.663×10^{-2}	1.9254	0.177	6.8038×10^{-6}	12.807	0.001	4.6681×10^{-2}	1.6101	0.225
St × T	1	0.1288	3.2357	0.084	1.0053×10^{-6}	1.8979	0.187	2.1790×10^{-4}	7.5427×10^{-3}	0.929
S × T	3	8.6913×10^{-2}	2.1838	0.138	1.8942×10^{-6}	3.5655	0.042	3.1047×10^{-2}	1.0709	0.389
St × S × T	3	0.2798	7.0301	0.004	6.0891×10^{-7}	1.1462	0.360	1.0641×10^{-2}	0.3670	0.772
Residual	212	3.9097×10^{-2}			2.266×10^{-7}			8.9821×10^{-7}		

Note: df, degrees of freedom; MS, mean squares; *F*, *F* ratio; results were considered significant at $P < 0.05$.

The concentrations of Sr, Ba, Mg, and Ca (the trace elements that record the environmental conditions) in the otolith samples were determined using an Agilent 7500cs inductively coupled plasma mass spectrometer coupled to a Merchantek UP213 (New Wave Research) Nd:YAG deep ultraviolet laser microprobe, with a pulse rate of 5.00 Hz. The outer otolith edge of all experimental fish was analysed using a 30 μm diameter laser beam. The beam was centred approximately 20 μm from the outer edge of the otolith but within the experimental growth region. Sample gases were extracted from the chamber through a smoothing manifold facilitated by a helium and argon stream. Analysis involved a 30 s background count to determine detection limits followed by a 100 s ablation of the experimental otolith growth.

To correct for machine drift a glass reference sample (National Institute of Standards and Technology, NIST 612) was analysed at the beginning and end of the sampling sessions, and after every 10–12 samples. All elements in otoliths were at least one order of magnitude greater than the background. The element (Sr, Ba, Mg, and Ca) mass count data were converted to concentrations (ppm) using Glitter software (Macquarie University; http://www.accessmq.com.au). Concentrations (ppm) were then converted to molar concentrations and standardised to calcium for statistical analysis (as per the water samples). Elements were standardised to calcium, as these elements substitute for Ca in the $CaCO_3$ matrix of the otoliths. The mean analytical accuracy of elements ranged from 99% (Mg) to 100% (Ba). The precision was <4.3% for all elements.

Statistical analysis

Univariate permutational analysis of variance (ANOVA) was used to test whether significant differences occurred in the concentration of trace elements Sr:Ca, Ba:Ca, and Mg:Ca (the response variables) between stocks and among temperatures and salinities. Four separate multifactorial ANOVAs were used to test (*i*) the influence of salinity (seven levels at one temperature) on the NSW stock, the combined effects of salinity and temperature for the (*ii*) NSW stock (four salinity levels at each of three temperatures) and the (*iii*) WA stock (four salinity levels at each of two temperatures), and (*iv*) the influence of stock for the common temperature and salinity levels (four salinities and two temperatures) across both stocks. Four separate designs were used because the same treatments of temperature and salinity were not possible for both stocks. The same response variables (Sr:Ca, Ba:Ca, Mg:Ca) were tested in each design. Salinity, temperature, and stock were treated as fixed factors and tank as a random factor nested in either salinity, temperature, and stock (comparison among stocks experiment (test 1)); salinity (NSW salinity experiment (test 2)); or salinity and temperature (other experiments (tests 3*a* and 3*b*)). If significant differences were detected ($P < 0.05$), a posteriori pairwise tests were used to determine which treatments (e.g., temperature, salinity, stock, or their interactions) differed.

For the stock comparisons between NSW and WA fish our analyses were restricted to the two temperatures (20 and 24 °C) and four salinities (10‰, 30‰, 40‰, 50‰), which overlapped between stocks. The results report outcomes of the full analyses, but restrict post hoc pairwise comparisons to between stock comparisons, since this was the main factor of interest for this analysis. In addition, the salinity and temperature results for each stock were similar to the findings of the designs discussed below (i.e., where there was overlap in statistical tests).

Results

Rearing environment and fish growth

Experimental salinities and temperatures generally conformed to the treatment conditions, with only minor variation between treatment tanks (Table S1[1]). Each treatment was significantly different for both temperature and salinity. Salinities were sometimes slightly elevated from the nominal level, which was likely due to concentration from evaporation (Table S1[1]). Trace elemental concentrations of rearing waters were not manipulated and were generally consistent between tanks (Table S1[1]). However, there was some minor fluctuation in Ba:Ca concentration in the most saline treatments (50‰) compared with the other salinity treatments. Standard lengths of experimental *A. japonicus* revealed slight (nonsignificant) variation in fish size at the time of sacrifice (Table S1[1]).

Comparison of NSW and WA stocks at different temperatures and salinities (test 1)

A significant interactive effect of salinity × temperature × stock was found for Sr:Ca concentrations in *A. japonicus* otoliths (Fig. 1*a*; Table 1). For Ba:Ca in otoliths, a significant salinity × stock plus salinity × temperature interaction was identified (Figs. 1*b*, 2*a*; Table 1). Mg:Ca in otoliths only showed a significant effect of temperature, where Mg concentration increased with an increase in temperature (Figs. 1*c*, 2*b*; Table 1). For the three-way interaction (salinity × temperature × stock) found for otolith Sr:Ca, there were three significant differences between stocks. At 24 °C, the WA stock had significantly higher Sr:Ca concentrations for 30‰ and 40‰, but not for 10‰ and 50‰ (Fig. 1*a*), than the NSW stock. At 20 °C, there were only significant differences between stocks at 50‰ with the WA stock again incorporating more Sr:Ca (Fig. 1*a*). The otolith Ba:Ca interaction between salinity and stock was due to the two stocks differing at two salinities (30‰ and 50‰), but not at the other two salinities (Figs. 1*b*, 2*a*). At 30‰, otolith Ba:Ca was significantly greater in the NSW stock, whereas at 50‰ it was significantly greater in the WA stock (Figs. 1*b*, 2*a*).

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2012-0442.

Fig. 1. Mean concentrations (± standard error) of trace elements in otoliths of New South Wales (black) and Western Australia (grey) *Argyrosomus japonicus* reared under experimental treatments of salinity (S) and temperature (T).

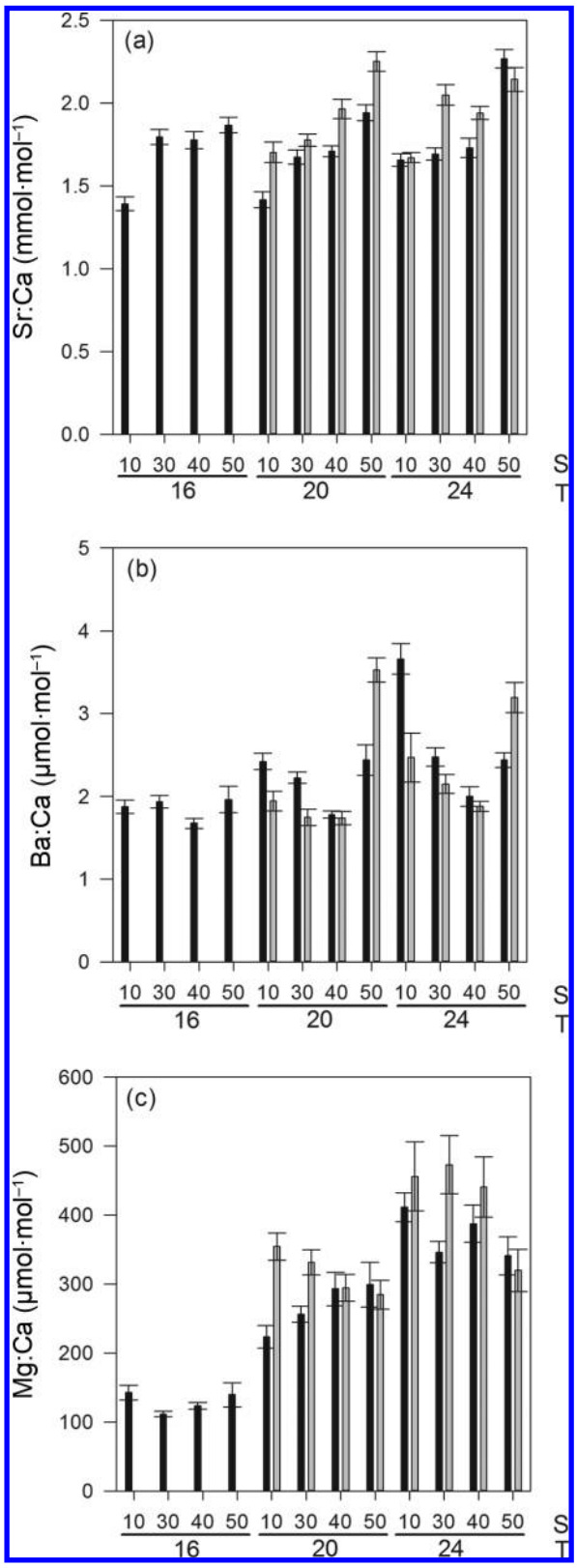
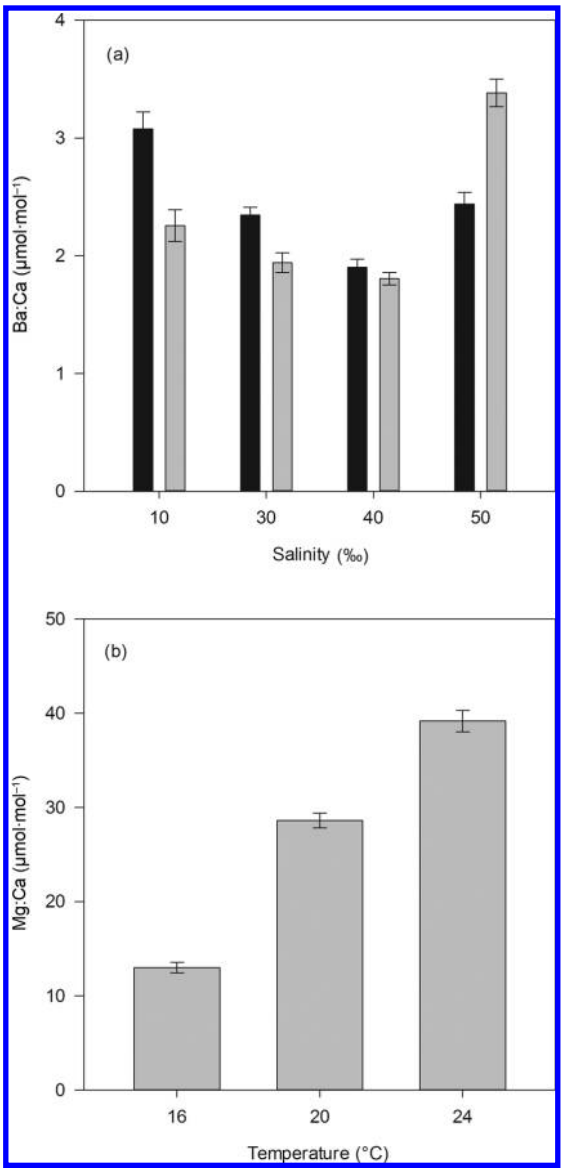


Fig. 2. (*a*) Mean concentrations (± standard error) of Ba:Ca in otoliths of New South Wales (black) and Western Australia (grey) *Argyrosomus japonicus* reared under the experimental treatment of salinity and (*b*) mean concentrations (± standard error) of Mg:Ca in otoliths of *A. japonicus* reared under the experimental treatment of temperature.



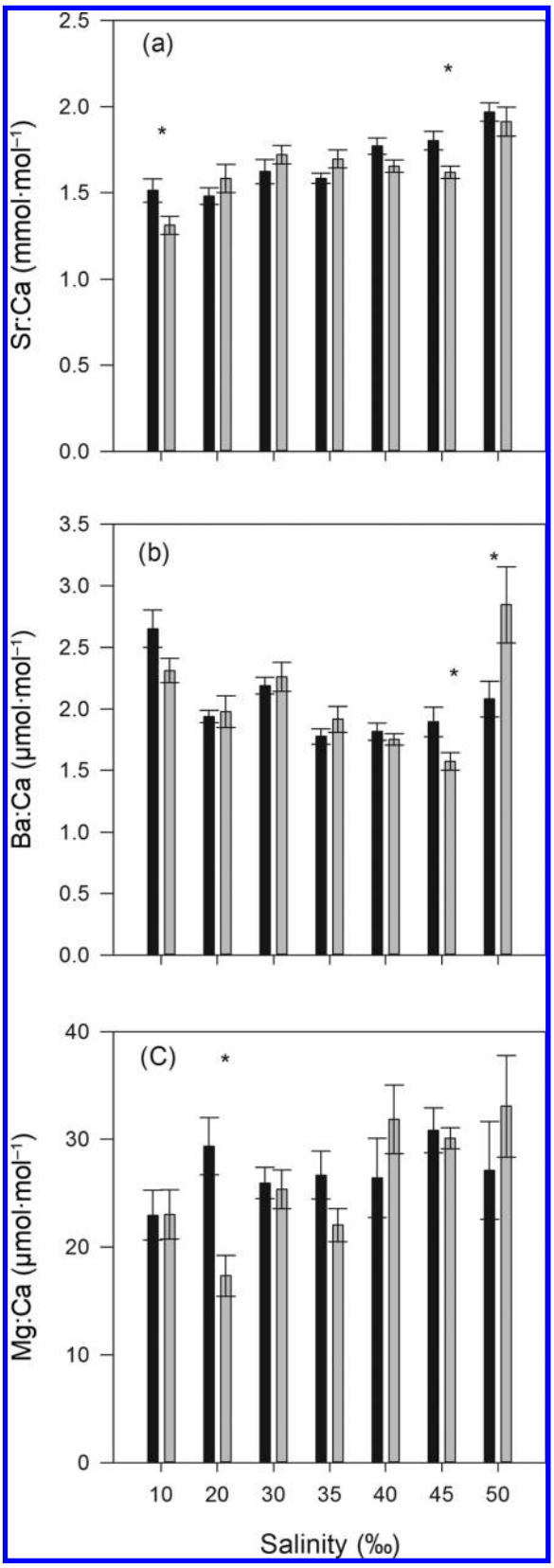
NSW stock: salinity effects (test 2)

A significant effect of salinity on the concentration of NSW *A. japonicus* otoliths was detected for Sr:Ca, but not Ba:Ca or Mg:Ca (Fig. 3; Table 2). Otolith Sr:Ca for the 50‰ salinity treatment differed from all salinities by less than 35‰. An increase in otolith Sr:Ca concentration was observed with increasing salinity (Fig. 3*a*). Significant tank effects were detected in otoliths of NSW fish for all three elemental ratios (Table 2). Pairwise tests showed that one to two treatments had significant tank effects (Fig. 3).

NSW Stock: salinity and temperature effects (test 3*a*)

A significant interactive effect of salinity and temperature was detected for Sr:Ca and Ba:Ca concentrations in *A. japonicus* otoliths, but not for Mg:Ca (Fig. 1; Table 3). At all three temperatures, Sr:Ca concentrations differed among salinities, but the same salinities did not necessarily differ for each temperature. At 16 °C, the difference in Sr:Ca otolith concentration was due to the

Fig. 3. Mean concentrations (± standard error) of trace elements in otoliths of New South Wales *Argyrosomus japonicus* reared under experimental treatments of salinity at a fixed temperature (20 °C). An asterisk (*) denotes significant ($P < 0.05$) tank effect; bars with different shading distinguish the two tanks.



low-salinity treatment (10‰), which was significantly lower than all other treatments (Fig. 1*a*). At 20 °C, Sr:Ca showed an increase with increasing salinity, but only differed among the lowest salinity treatments (10‰ and 30‰) and the highest salinity treatment (50‰) (Fig. 1*a*). At 24 °C the hypersaline treatment (50‰) was significantly greater than all other salinity treatments (Fig. 1*a*). For Ba:Ca, no differences were found between salinities at low temperature, but there was some variation at higher temperatures (Fig. 1*b*). At 20 and 24 °C the difference in Ba:Ca otolith concentration was due to the 40‰ treatment, which was significantly less than all other treatments, with the exception of the hypersaline (50‰) treatment at 20 °C (Fig. 1*b*).

Temperature differences in otolith Sr:Ca incorporation were only found at the lowest salinity (10‰) (Fig. 1*a*). At low salinity, the general trend was for an increase in otolith Sr:Ca with increasing temperature, but only the highest and lowest temperatures differed from each other (Fig. 1*a*). For Ba:Ca in otoliths, differences among temperatures were only found at the 10‰–40‰ salinities and also showed a positive increasing trend (Fig. 1*b*). For Mg:Ca in otoliths, all temperature treatments were significantly different; concentration increased with increasing temperature (Fig. 1*c*). Salinity did not influence Mg:Ca incorporation into otoliths (Fig. 1*c*; Table 3).

NSW *A. japonicus* otoliths showed a significant tank effect for two of the three elements analysed, Ba:Ca and Mg:Ca (Table 3). For Ba:Ca, pairwise tests showed that the tank effect was due to a single treatment (Fig. 1*b*). For Mg:Ca, significant tank effects were found for four treatments (Fig. 1*c*).

WA stock: salinity and temperature effects (test 3*b*)

A significant interaction between salinity and temperature was found for Sr:Ca in otoliths, whereas Ba:Ca and Mg:Ca were only significantly affected by salinity and temperature, respectively (Fig. 1; Table 4). For otolith Sr:Ca at 20 °C, the hypersaline (50‰) treatment was significantly greater than all other salinity treatments (Fig. 1*a*). The two lowest salinities were similar (10‰ and 30‰), as were the two midlevel salinities (30‰ and 40‰). This contrasted with the 24 °C treatment, where the lowest salinity (10‰) was significantly lower than all other salinities, but the 30‰–50‰ salinities had similar otolith Sr:Ca concentrations. For otolith Ba:Ca, the hypersaline treatment was significantly higher than all other salinities (Fig. 1*b*). For otolith Mg:Ca, temperature treatments were significantly different where higher concentrations were found at higher temperatures, a finding that was similar to the NSW experiment (Fig. 1*c*).

A significant tank effect was also found for the elemental composition of *A. japonicus* otoliths from the WA stock for two of the elements analysed, Ba:Ca and Mg:Ca (Table 4). Significant tank effects were due to differences among duplicate tanks for one Ba:Ca treatment and two Mg:Ca treatments.

Discussion

Our findings suggest that Sr:Ca and Ba:Ca in otoliths record not only environmental conditions, but may also be influenced by intrinsic factors such as genetics. Environmental reconstructions for *A. japonicus* are more complex than anticipated, but stock differences may enhance the method as a stock identification tool. For example, differences in otolith chemistry among stocks of wild *A. japonicus* have been attributed to different environmental conditions (e.g., salinity and temperature) and thus have been used to delineate stock structuring (Ferguson et al. 2011). Our findings indicate that differences in the otolith chemistry of *A. japonicus* may reflect both environmental and genetic differences among populations. Hence, the differences in otolith chemistry detected among populations of *A. japonicus* by Ferguson et al. (2011) may indicate highly distinct groupings. Mitochondrial DNA support the existence of genetically discrete stocks of *A. japonicus* (Farmer 2008). As such, it may be possible that subtle genetic

Table 2. Results of permutational ANOVA comparing the effects of treatment salinities (S) on otolith Sr:Ca, Ba:Ca, and Mg:Ca for New South Wales *Argyrosomus japonicus* reared at 20 °C.

		Sr:Ca			Ba:Ca			Mg:Ca		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
S	6	0.4820	5.8848	0.007	1.8454×10^{-6}	3.6077	0.072	1.9351×10^{-2}	1.2351	0.400
Tank(S)	7	8.1926×10^{-2}	2.7069	0.014	5.1169×10^{-7}	3.7067	0.001	1.5672×10^{-2}	2.4222	0.027
Residual	116	3.0266×10^{-2}			1.3805×10^{-7}			6.4703×10^{-3}		

Note: df, degrees of freedom; MS, mean squares; *F*, *F* ratio; results were considered significant at $P < 0.05$.

Table 3. Results of permutational ANOVA comparing the Sr:Ca, Ba:Ca, and Mg:Ca ratio in otoliths of New South Wales *Argyrosomus japonicus* among salinity (S) and temperature (T) treatments.

		Sr:Ca			Ba:Ca			Mg:Ca		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
S	3	2.8093	56.5300	0.001	7.0346×10^{-6}	11.3960	0.001	6.9062×10^{-3}	0.3006	0.820
T	2	0.5140	10.3440	0.003	1.1563×10^{-5}	18.7410	0.002	1.1280	49.1160	0.001
S × T	6	0.2987	6.0092	0.005	2.5557×10^{-6}	4.1360	0.022	1.6494×10^{-2}	0.7170	0.634
Tank(S × T)	12	4.9736×10^{-2}	1.2044	0.287	6.1913×10^{-7}	2.6553	0.002	2.3057×10^{-2}	3.5532	0.001
Residual	202	4.1295×10^{-2}			2.3316×10^{-7}			6.4890×10^{-3}		

Note: df, degrees of freedom; MS, mean squares; *F*, *F* ratio; results were considered significant at $P < 0.05$.

Table 4. Results of permutational ANOVA comparing the Sr:Ca, Ba:Ca, and Mg:Ca elemental ratio in otoliths of Western Australia *A. japonicus* among salinity (S) and temperature (T) treatments.

		Sr:Ca			Ba:Ca			Mg:Ca		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
S	3	1.1025	41.9470	0.001	1.0634×10^{-5}	25.1700	0.003	5.5951×10^{-2}	1.8162	0.220
T	1	7.3170×10^{-2}	2.7681	0.109	3.9038×10^{-7}	0.9319	0.338	0.25148	8.2457	0.024
S × T	3	0.1548	5.8909	0.032	5.4928×10^{-7}	1.3002	0.358	1.622×10^{-2}	0.5265	0.676
Tank(S × T)	8	2.5984×10^{-2}	0.70774	0.701	4.2951×10^{-7}	2.4308	0.022	3.1422×10^{-2}	3.3590	0.004
Residual	78	3.6714×10^{-2}			1.7670×10^{-7}			9.3548×10^{-3}		

Note: df, degrees of freedom; MS, mean squares; *F*, *F* ratio; results were considered significant at $P < 0.05$.

differences may interact with environmental variables to affect otolith trace elemental signatures, which could enhance differences among stocks.

The temperature and salinities used in our study are within those in which *A. japonicus* naturally occur. They are a euryhaline species found in subtropical to temperate nearshore, surf zone, and estuarine areas of the Southern Hemisphere. The species commonly encounter a wide range of salinities (NSW: 0‰–35‰ (Gray and McDonall 1993; Taylor et al. 2007) and WA: 2‰ to ~40‰ (Loneragan et al. 1987)). Estuaries in WA often have higher salinities than those in NSW mainly due to the effects of a lower annual rainfall (Loneragan et al. 1987; Taylor et al. 2007), exposing *A. japonicus* in WA to higher salinities. *Argyrosomus japonicus* has been recorded in hypersaline conditions (~50‰–60‰) in the Coorong Estuary, South Australia, during prolonged drought conditions (Q. Ye, SARDI Aquatic Sciences, personal communication, 2007). Their natural reported temperature ranges in NSW are 14–26 °C (Taylor et al. 2007) and 13–25 °C in WA (Loneragan et al. 1987) depending on the season. Increased somatic growth in juvenile *A. japonicus* occurs where temperatures are above 20 °C (Bernatzeder and Britz 2007). No clear optimum growth pattern with salinity was found (Bernatzeder et al. 2010; Fielder and Bardsley 1999), but juveniles are commonly found in low-salinity environments (Ferguson et al. 2008).

The significant effect of population genetics (albeit influenced by environmental factors) suggests that environmental reconstructions using otolith chemistry are complicated, particularly for euryhaline fish. We found that stock (genetics) had a significant effect on the incorporation of Sr and Ba, but not Mg in the otoliths of *A. japonicus*, but this was often influenced by salinity or temperature. For Sr, stock showed a significant interaction with salinity and temperature, while Ba showed a significant stock and salinity interaction. These results suggest that the effects of salinity and temperature on Sr and Ba are at least modified by intrinsic factors such as intraspecific genetic differences. We are only aware of one previous study that investigated genetic effects on otolith chemistry. Similar to our results, Clarke et al. (2011) found a significant effect of genetic stock on Ba:Ca. However, they also found an effect on Mg:Ca, but not Sr:Ca. The findings show that studies that directly test the effect of population genetics on otolith chemistry suggest that traditional environmental influences (salinity and temperature) are modified slightly (but significantly) by intraspecific genetic differences. The same general trends in the elemental response to extrinsic factors are evident between stocks, i.e., Ba:Ca concentration in otoliths increased as the salinity treatments departed from the ambient marine (40 ppk) to brackish or hypersaline. However the magnitude of incorporation differed, i.e., the NSW stock incorporated more Ba at the brackish treatments and the WA stock incorporated more at the hypersaline treatment.

Mg may be an important temperature recorder for the study species, since no interaction of stock or salinity was found. Increasing temperature caused an increase in Mg:Ca concentration in *A. japonicus* otoliths. Past studies have found mixed responses of Mg:Ca to temperature, with no effect of temperature (Hoff and Fuiman 1995; Elsdon and Gillanders 2002; Martin and Thorrold 2005) and a negative effect on one species (Fowler et al. 1995*a*, 1995*b*) reported. These include experiments on Sciaenids, suggesting that consistent temperature effects on otolith Mg:Ca concentrations do not occur at the family level at least over the temperature range tested. Mg thermometry is well established in calcitic skeletons of formanifera (which also display a positive relationship), but in teleosts Mg is likely under biological control (Martin and Thorrold 2005). Mg is at a higher concentration in the

blood of fish compared with that in the endolymphatic fluid bathing the otoliths and therefore controls their composition (Melancon et al. 2009). As such it is likely that physiological fractionation of Mg occurs between the blood and endolymph (Woodcock et al. 2012), and in *A. japonicus* this fractionation is possibly negatively affected by temperature. Therefore, it may be that Mg is under a temperature-dependent discrimination in *A. japonicus* that is not the case in other species of teleost, highlighting the species-specific response on some elements.

Sr and Ba in fish otoliths are commonly used markers of salinity, but our findings suggest salinity is also modified by temperature. Significant interactions between the extrinsic or environmental factors of salinity and temperature suggest that the otolith element and salinity relationship is being modified by temperatures that occur within the normal range of the study species. An interaction between salinity and temperature for Sr:Ca in otoliths of *A. japonicus* occurred in both stocks and for Ba:Ca in the NSW stock. A similar interaction has been described for another estuary-associated species, *Acanthopagrus butcheri* (Elsdon and Gillanders 2002). Most studies testing salinity and temperature differences have found no significant interaction between these variables (e.g., Martin and Wuenschel 2006). Our findings are similar to those of Elsdon and Gillanders (2002), where *A. butcheri* also showed an increasing concentration in Sr:Ca and Ba:Ca with increasing temperature at low salinities. Unfortunately, Elsdon and Gillanders (2002) did not test salinities higher than 30‰, limiting a comparison of element responses at higher salinities. Although we did not detect an interaction between temperature and salinity for Ba:Ca in WA fish otoliths, there was a significant effect of salinity. This result was also found in *Lutjanus griseus* when tested for a salinity × temperature interaction (Martin and Wuenschel 2006). Our results show that hypersalinity caused an increase in Ba:Ca, reflecting the water chemistry, and as such suggest that Ba incorporation is not just a function of bioavailability at low salinities. Even within stocks it appears that the full range of environmental conditions that fish may be exposed to should be considered when reconstructing environmental histories.

For *A. japonicus*, Sr is a useful marker of salinity changes when other variables (extrinsic and intrinsic) are taken into account. When salinity was the only factor tested, Sr:Ca increased with increasing salinity for *A. japonicus*. A positive increase in otolith Sr:Ca with increasing salinity has been reported in some studies (Radtke et al. 1988; Kalish 1990; Limburg 1995) but not in others (Hoff and Fuiman 1995). In the present study, differences in Sr:Ca concentrations were driven by the most saline treatment (50‰) that is at the upper limit of the species' natural occurrence. The few studies that have examined the affect of hypersalinity (>40‰) on Sr:Ca otolith chemistry have generally shown a trend that is consistent with these findings (but see Gillanders and Munro 2012). Martin and Wuenschel (2006) reared fish in the laboratory and included a salinity treatment level of 45‰; they found that salinity significantly affected otolith Sr:Ca but only at relatively high temperature treatments (28 and 33 °C), and they did not specify which salinity treatment levels drove the differences (their graphs suggest it could have been 45‰). In the wild, hypersalinity (>40‰) similarly increased Sr incorporation into the otolith (Diouf et al. 2006). Fish in hypersaline environments may experience physiological stress, which inhibits their ability to osmoregulate. Thus, there may be increased elemental uptake into the endolymph and otolith (Secor et al. 1995; Diouf et al. 2006) with increasing salinity. Similarly, while not statistically significant, our results suggest that Ba:Ca and Mg:Ca otolith concentrations are elevated in hypersaline environments, reflecting the surrounding water chemistry, a finding that is consistent with that of Gillanders and Munro (2012).

We have demonstrated that element incorporation is modified by intraspecifc genetic differences where the uptake of some elements into the otoliths of fish is the result of complex interactions between extrinsic and intrinsic factors. This study has shown that environmental variables (i.e., salinity and temperature) significantly influence otolith chemistry, but these are not the only influencing factors. While the influence of genetic differences on otolith chemistry may strengthen the application of otolith chemistry for delineating patterns of stock structure of wild fish, differences in otolith element incorporation among stocks and variable patterns of interaction with environmental factors will render the use of otolith chemistry for the reconstruction of environmental histories challenging (at least for the study species). However, if genetic stocks are considered and a multifactorial approach is used, reconstructions may be possible (Perrier et al. 2011). We caution against using environmental reconstructions without validating basic assumptions about the relative contributions of environmental and genetic factors of otolith element incorporation. For example, the study species is an estuary-associated marine fish that potentially encounters different levels of salinity (fresh to hypersaline) at different stages of its life history. Ideally, we could assess the animal's use of different habitats based on levels of Sr and Ba. However, inferences of movement or environmental histories based on fine-scale salinity gradients (e.g., brackish to marine) could be erroneous owing to the possible influence of factors other than salinity (e.g., temperature and genetics). This may not be an issue when studying fish that experience broad changes in salinity, such as those experienced by anadromous fish owing to salinity being the main influence and other factors slightly modifying otolith chemistry. Otolith chemistry may still be useful for aiding spatial identification of management units, but for environmental reconstructions elemental responses to extrinsic and intrinsic influences should be validated for study species. It may also be pertinent to use otolith chemistry in tandem with other techniques (e.g., genetics, morphometrics, and dart tagging).

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Chapter 5: Statement of authorship

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Principal Author

Name of Principal Author (Candidate)	Thomas C. Barnes		
Contribution to the Paper	Contributed to intellectual development of ideas, undertook all field and laboratory work, conducted analyses and wrote manuscript.		
Overall percentage (%)	90		
Signature		Date	9/09/2015

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 in 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	Bronwyn M Gillanders		
Contribution to the Paper	Contributed to intellectual development of ideas, design, implementation and also helped write the paper via commenting and providing direction on multiple drafts and helped organise funding.		
Signature		Date	9/09/2015

Chapter 5: Movement of an exploited demersal fish species (*Argyrosomus japonicus* - sciaenidae) around marine parks inferred from satellite telemetry

Tagging a very large mulloway

Movement of an exploited demersal fish species (*Argyrosomus japonicus* - sciaenidae) around marine parks inferred from satellite telemetry

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Abstract

There is a paucity of information available on the movement of demersal fish, despite many commercial fisheries targeting these species. Determining the spatial and temporal aspects of movement aids in fisheries and conservation management which is increasingly being implemented via marine protected areas (MPAs). Large mulloway (*Argyrosomus japonicus*) are a fishery revered demersal species but despite their iconic status their numbers may be in decline and relatively little is known about their movements. During the Austral summers and autumns from 2011 to 2014 we deployed 19 mini-pop-up satellite archival tags (PSATs) on mature mulloway at an aggregation site in the Great Australian Bight Marine Protected Area (GABMPA) to examine their movement patterns. Approximately 58% of the tags transmitted useful data from deployments ranging from ~8 to 136 days. Pop-up location data revealed that the fish moved up to ~550km from the tag location. Nearly all tagged mulloway remained in the vicinity of the tagging location and hence MPAs over summer; however relatively large horizontal movements were observed over autumn for most fish. Depths encountered by the deployed mulloway ranged from very shallow to relatively deep (0.0 to 56.5m). Some tag data were incomplete due to rapid beaching of detached tags as fish were relatively close to shore. We found that there were some problems with PSAT deployed on the demersal study animal (e.g. the rapid beaching), but unprecedented information on the species was acquired which could aid in their conservation.

Introduction

The nature of demersal aquatic animals (i.e. living near the sea floor) makes them particularly challenging to elucidate movement patterns (Peklova et al. 2012). Pelagic animals can readily be observed when they are near the ocean surface, allowing their movements to be tracked using Global Positioning System technology (Newlands et al. 2006, Sims et al. 2009). For large bodied demersal fish that form high value fisheries, movement information is historically acquired from fisheries data and limited scale tagging (Griffiths 1996). Natural tags (genetic and otolith techniques) and artificial tags (mark/recapture and acoustic tags) have been utilised on demersal fish to obtain some information on movements (Palumbi 2004). Predatory demersal fish form many important commercial, recreational and traditional fisheries but stocks are depleted on a global scale (Myers and Worm 2003). As such it is imperative to understand their broad scale movements to help manage and conserve fish stocks under extractive pressure and subject to habitat loss from developments and climate change.

Marine Protected Areas (MPAs) have the potential to protect fish and marine ecosystems. By restricting potentially damaging activities MPAs can aid in sustaining fish populations (e.g. via improved recruitment) (Palumbi 2004). The spatial fishing restrictions incorporated in MPAs could benefit fish populations and lead to improved catches in adjacent areas (Halpern 2003; Hilborn et al. 2004; Edgar et al. 2014; Breen et al. 2015). Unfortunately, large scale MPAs may be detrimental to small coastal communities that rely on fishing and associated tourism for economic sustainability and growth (Sale et al. 2005). Hence, there is often vocal opposition against proposed MPAs from certain sectors. As such it is imperative to underpin MPAs with quality scientific data (e.g. determining the “ocean neighbourhood” or spatial range of target species) to ensure reasonable benefits from spatial restrictions (Sale et al. 2005).

Pop-up satellite archival tags (PSAT) have been utilised to gain movement information on aquatic animals for the last decade. For example, unprecedented information has been acquired on the

movement of pelagic white sharks, tuna and billfish (Block et al. 2011) and benthic stingrays (Le Port et al. 2008) and flatfish (Loher 2008). However, there are yet no published studies where PSATs are utilised on benthopelagic demersal fish movements. This is surprising given PSAT archival properties (e.g. potential to record independent information whilst the individual is at large) and the hard to track nature of demersal fish.

Mulloway (*Argyrosomus japonicus* – sciaenidae) are an important demersal species that form fisheries across their Australian and South African range. Unfortunately in some parts of the range mulloway abundance is declining and stocks have been reported as collapsed in South Africa (Griffiths 1997; Taylor et al. 2006). Like many sciaenids, mulloway are particularly vulnerable to habitat destruction and poorly managed fishing, mainly due to reliance on certain specialised habitats but also because they mature late (Griffiths 1996). To enhance diminished fishing opportunities they are being restocked in some parts of their Australian range (Taylor et al. 2006). In other parts of their range there is potential for marine protected areas to provide some protection and allow numbers to rebuild.

Most mulloway movement studies have been undertaken in estuaries (Taylor et al. 2006; Næsje et al. 2012), despite mulloway having either a marine life stage for estuary associated populations or in some regions being purely marine (Ferguson et al. 2011; Barnes et al. 2015; Chapter 2). Information on the species entire range or "neighbourhood" is therefore required.

In the eastern section of the Great Australian Bight (GAB; the far west coast (FWC) of South Australia) there is a unique population of mulloway. In the FWC, particularly the surf beaches of the Yalata Indigenous Protected Area, large adult mulloway are thought to spawn in relatively shallow water just behind the surf-line in late spring and early summer, and the area is a year round nursery for earlier life stages (Rogers et al. 2014). The population is genetically differentiated from others (Chapter 2), exhibits different life history strategies from surrounding populations (e.g. lack of estuary association) and is faster growing than some neighbouring populations (Ferguson 2010). This

population has cultural significance and also supports a large but isolated recreational fishery (Rogers et al. 2014), as well as commercial catches via various gear types and licenses (e.g. Commonwealth and state) (Ferguson and Ward 2003). Recent anecdotal concerns from the local indigenous and fishing communities suggest that the GAB mulloway population is declining. There are two marine parks (Far West Coast and Nuyts Archipelago) in the eastern GAB that may benefit mulloway.

An important step in aiding the persistence of demersal fish populations is furthering our understanding of spatial movement patterns. It is possible that MPA boundaries and zones can be designed strategically via fishery independent movement information to more precisely achieve their objectives and hence aid in satisfying community concerns on their placement. To begin to achieve this for mulloway in the GAB we attached PSAT with three goals in mind. First, we aimed to determine the spatial scale of mulloway movements at the GAB, second, assess the amount of protection provided to the unique mulloway population via the MPAs, and third, provide assessment of the suitability of PSAT as a method to provide movement information on a coastal demersal fish species. By addressing these objectives we test the hypothesis: are mulloway non-migratory and therefore potentially protected from capture by the GAB marine parks?

Materials and Methods

Study area

The GAB is a pristine region of the south coast of Australia encompassing the south western section of South Australia and south eastern Western Australia (Fig. 1). The area is very isolated, abundant in marine life and has a variety of oceanographic influences. Within the GAB, are high energy sandy beach and reef lagoons in the Yalata Indigenous Protected Area; this zone forms critical fish habitat (Rogers et al. 2014). Two levels of MPAs are designed to protect the pristine environments and associated biota in the area. The Commonwealth level Great Australian Bight Marine Reserve (GABMR) and the South Australian State government Far West Coast Marine Park (FWCMP) (Fig. 1). The FWCMP has three zones which vary temporally throughout the year. For example, between 1 November and 30 April there are restricted access, sanctuary and habitat protection zones, whereas from 1 May to 31 October the entire marine park is a restricted access zone to protect southern right whales but with shore-based recreational line fishing permitted in four areas. The GABMR covers greater than 45,000 km^2 and includes a marine national park zone, multiple use zone and a special purpose zone.

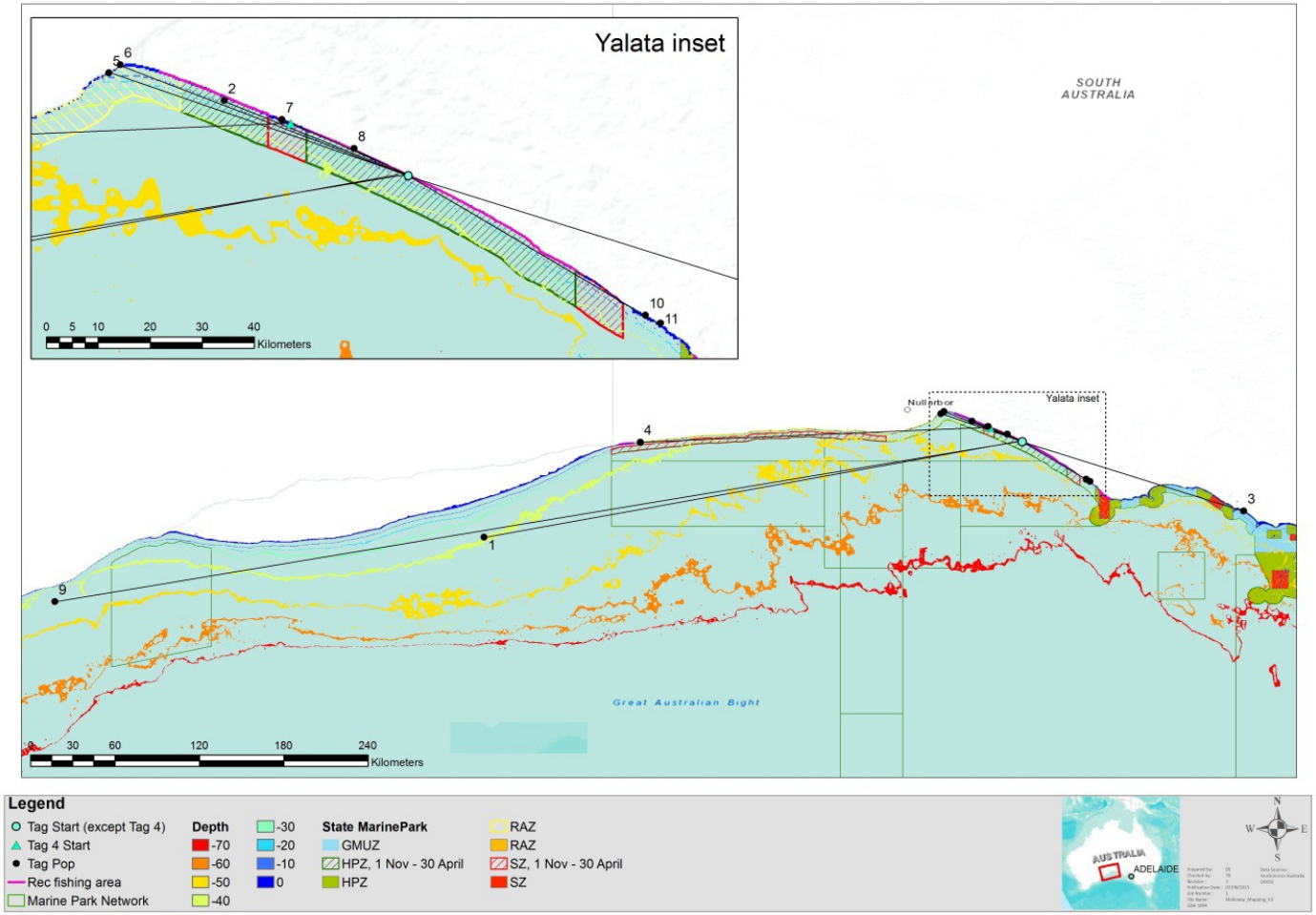

Fig. 1 Location of the site where PSATs were fitted to individual fish with black lines indicating the net displacement distance and direction. Coloured lines represent depth contours. Legend abbreviations: Rec = Recreational, GMUZ = General Management Use Zone, HPZ = Habitat Protection Zone, RAZ = Restricted Access Zone, Nov = November and SZ = Sanctuary Zone.

Mulloway capture

Adult mulloway were angled from the beach using heavy surf fishing equipment comprising line and baited hook (Table 1). This configuration was chosen because large fish could be landed relatively quickly and handling effects minimised. All work on live fish was carried out on the beach (hook removal, measuring (total length, cm), condition assessment and potentially tagging) just above the surf line, to minimise time out of water, facilitating release of the fish in the best possible condition. As the fish could not be externally sexed, differences in movement and behaviour between male and female fish were not investigated. Mulloway were targeted for tagging in the Austral spring and summer between 2011/12 and 2013/14 (Table 1).

Table 1 Deployment and biological information for mulloway fitted with PSATs in the far west coast region of South Australia. Data days = the number of days the fish was at large recording data, Transmit days = the number of days the PSAT transmitted data to satellite, Interval = programmed PSAT release, Tag 9 has (summer/autumn) deployment season as the deployment spans both seasons.

Deployment trip No.	Reporting tag no.	Argos platform transmission terminal no.	PSAT deployment date (GMT)	PSAT pop-up date (GMT)	Data days	Transmit days	Percent data decoded	Total length (cm)	PSAT release type	Hook location on fish mouth	Straight line (deployment) distance (~ km)	Austral deployment season
1	1	42951	18/02/12	28/05/12	105	9	76	98	interval	Internal	332	Autumn
1	*A	52465	20/02/12	20/05/12	81	4	28	116	No data	Internal	No data	Autumn
1		52471	20/02/12					96		Internal		
2	2**	113296	10/11/12	18/02/13	100	6	71	95	interval	Internal	33	Summer
2		52464	11/11/12					130		Internal		
2		52471	11/11/12					151		External		
2		42963	11/11/12					131		Internal (deep)		
2	3	52465	11/11/12	19/02/13	99	5	64	139	interval	Internal	143+	Summer
3		99694	8/2/13					120		External		
3	4	99691	12/2/13	23/05/13	110	9	68	128	interval	Internal	212	Autumn
3		99693	13/2/13					94		Internal		
4		60652	14/11/13					118		Internal		
4	5	99692	14/11/13	11/02/14	82	1	66	132	premature	Internal	53	Summer
5	6	134449	12/12/13	06/02/14	53	6	48	119	Premature	External	51	Summer
5	7	134445	12/12/13	10/03/14	92	9	89	135	Premature	External	23	Summer
5	8***	134444	13/13/13	23/03/14	101	13	71	120	Interval	External	10	Summer
5	9	134448	13/12/13	05/05/14	136	9	79	150	Interval	Internal (deep)	594	Summer/autumn
6	10	134447	19/02/14	30/05/14	99	9	70	105	Interval	Internal	47	Autumn
6	11	134446	17/02/14/	27/05/14	101	9	81	110	Interval	External	50	Autumn

For reporting tag information codes used were *Location quality poor (z) ,**Tag retrieved, ***Damaged data, GMT Greenwich Mean Time, +Interferes with land.

Tagging

Extensive testing of various anchoring systems (the device that penetrates the animal and attaches it for deployment) was done on deceased mulloway and live snapper at South Australian Research and Development Institute - Aquatic Sciences (West Beach). The best practice assembly (i.e. least invasive but adequate holding power) consisted of a 30 mm titanium or surgical stainless steel blade attached to the tag (Wildlife Computers - miniPAT) by 400 lb test monofilament. This assembly was attached to the animal in the field using a stainless steel applicator needle. All surgical and anchoring equipment was sterilised using 100 % ethanol and betadine before applying to the individual fish. The needle and anchor were aimed between the bony pterygiophores under the dorsal fin during the tagging process as this position produced the maximum holding strength of the anchor whilst keeping the trailing tag from interfering with the animal during swimming.

An archival-satellite type pop up tag was chosen as the most suitable method to identify movements of mulloway because opportunistic surface transmission of data is likely poor with demersal fish species (personal communication, Paul Rogers, South Australian Research and Development – Aquatic Sciences). Archival tags tend to be relatively large mainly due to the size of the battery limiting their use to large animals (personal communication, Simon Goldsworthy, South Australian Research and Development – Aquatic Sciences). Wildlife Computers miniPAT (mini-pop up archival tag) were chosen because these tags are the smallest available device of their type (12 cm long and ~ 53 g total mass), and provide minimal interference with the animal at large.

The PSATs were programmed to archive time series depth information at 5 minute intervals. Time at depth and time at temperature data were internally binned at 6 hour intervals. The deployment period was programmed at 100 days to be a trade-off between a reasonable duration to record environmental changes, minimise risk of biofouling of sensors, minimise risk of removal by other animals and to be within safe limits of the battery capacity, with all sensors sampled at 3 second intervals. Premature release was programmed to occur if the depth was constant (within 2m

± 1m) for 5 days. After the PSATs was released from the fish the data were transmitted via the ARGOS satellite network. The tags were also programmed to record sunrise and sunset times to potentially estimate the geographical position of mulloway (details below).

Horizontal movements

The straight line or net displacement distance (km) between the tag deployment location and the popup location was calculated using the path tool in the Hawth's Tools extension for ArcMap 10.1 (ESRI, Redlands, CA, USA) (Hoffmayer et al. 2014). Distance estimates were rounded to the nearest kilometre. Mulloway position at the time of pop up was calculated via the Doppler-shift service (precision <1 km) from Argos Data Collection and Location. For interpretation of straight line movements fish were classed by the migration direction (east (E), west (W) or fish that did not leave the Yalata section of the Far West Coast Marine Park which were termed non-migrant).

Daily geographical positions were estimated using the Wildlife Computers Global Position Estimator (GPE3) software. This software uses a Hidden Markov Model (time series) using the forward and backward algorithm (Thiebot and Pinaud 2010); at a 0.25 degree grid size. The Bayesian model incorporated hidden variables including: user and ARGOS locations, bathymetry (land masses), sea surface temperatures (SST), light level information and estimated maximum swimming speed of the species. The maximum swimming speed of mulloway was estimated to be 1m s^{-1}, which was calculated from acoustic telemetry data in Taylor et al. (2006). The "most likely" daily locations were determined using a spline interpolation (Thiebot and Pinaud 2010). To visualise the estimated fish positions daily locations were imported to ArcMap 10.1 (ESRI, Redlands, CA, USA) and projected using the global WGS 1984 PDC Mercator.

Results

Tag deployments

A total of 19 PSAT devices were deployed on sexually mature (based on size) mulloway ranging in total length from 94 to 151 cm (Table 1). All fish were captured in a relatively small section of the GAB (30 km stretch) in western South Australia within the Far West Coast Marine Park (Fig. 1). Twelve devices transmitted data to satellite but with variation in data days and data quality (53 to 110 data days and 28 to 89% ARGOS messages decoded). As such, each archived time series of depth data had varying amounts of missing data (e.g. Fig. 2). Satellite transmission days varied from 1 to 9 full days. Tag A did not provide any movement or habitat data other than two depth and temperature histogram days (time at depth and temperature summarised over two days) and was not further analysed. The other 11 reporting tags provided information on the pop-up location, daily position estimates via dusk and dawn light levels, time series of depth and time at depth and temperature histograms (Table 1, 2). Tag 2 was retrieved and provided a full archival record however it appears the tag was snagged on an underwater structure after 8 days attached to the host fish (see below). Some of the data from tag 8 was corrupted due to an undetermined cause and therefore no depth or temperature information could be obtained (Table 2), however, horizontal movement data were accurate and hence utilized.

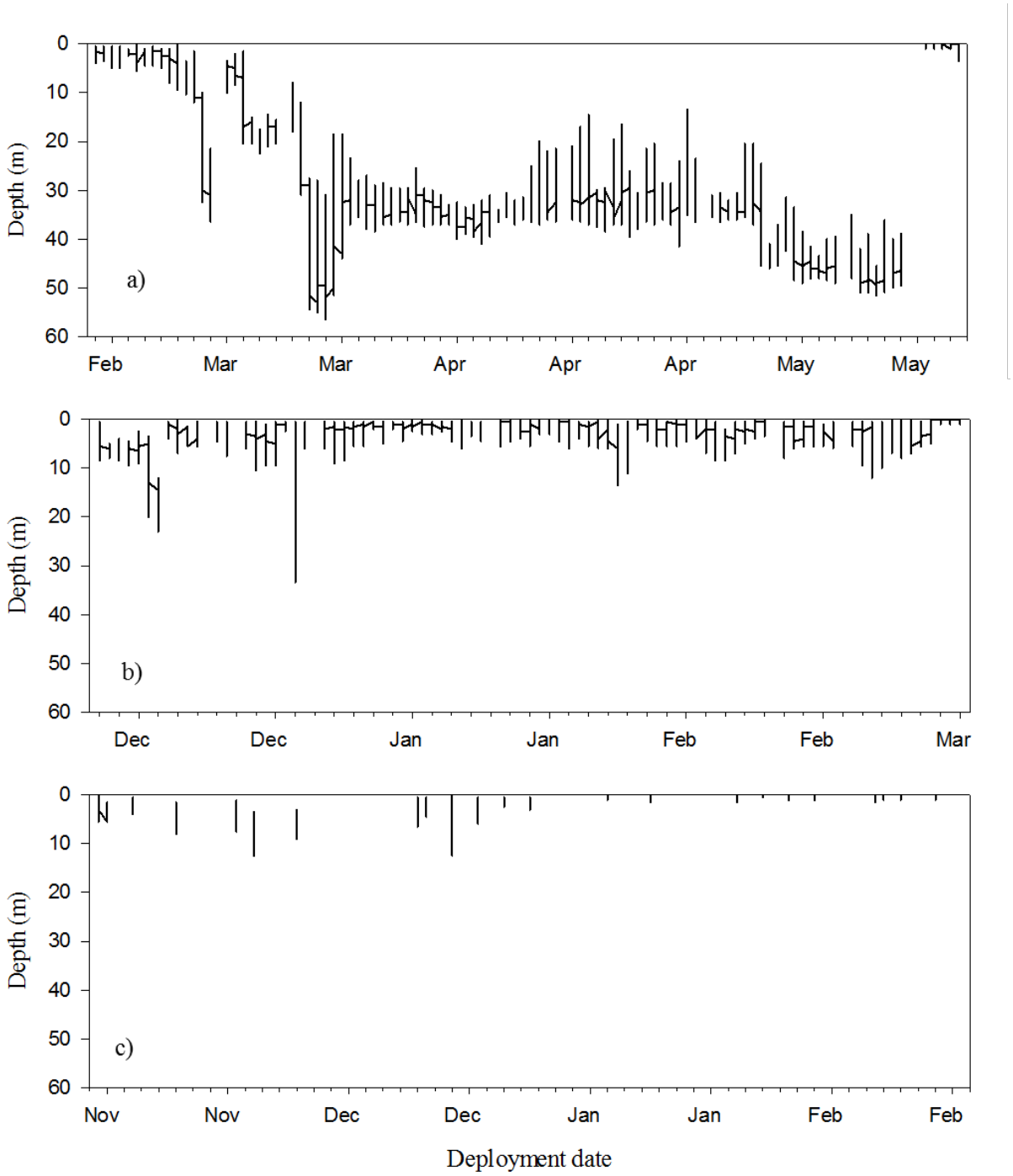

Fig. 2 Representative plots of depth (± 0.25m) time series showing data summarised at the four day level (i.e. each tick represents 4 days) for tagged fish 1 a), 7 b) and 3 c).

Table 2 Mean and range of depths (min-max), as well as number of depth data points recorded from PSAT tags placed on mulloway in the far west coast region of South Australia during autumn and summer.

Reporting Tag no.	Depth (m) Mean (± SE)	Min-Max	Data points
1	29.4 (0.10)	0.0-56.5	19968
2	4.3 (0.07)	0.0-18.0	2418
3	2.5 (0.06)	0.0-12.5	2111
4	19.5 (0. 13)	0.0-45.5	4511
5	3.2 (0.06)	0.0-15.0	2753
6	5.3 (0.26)	0.0-14.0	288
7	3.1 (0.02)	0.0-33.5	18123
8	Omitted data		
9	36.8 (0.15)	0.0-56.5	15728
10	2.3 (0.02)	0.0-8.0	3168
11	3.5 (0.02)	0.0-21.0	8419
Overall average	10.9 (4.06)		77487

Net displacement distance and direction

The minimum straight line or net displacement distance travelled by mulloway fitted with PSATs ranged from 10 to 594 km, with a mean distance of 140.7 (SE 54.1); there was a significant influence of deployment duration (8 to 136 days) on distance travelled ($P<0.05$, regression, Supplementary Figure 1). All of the tags were fitted on the shoreline and most tags popped up within 3 km of the shore except tags 1 and 9 which were ~30 and 15 km offshore respectively. Interestingly the two offshore pop ups occurred during autumn, suggesting potential for a seasonal offshore migration. There was a significant influence of season (summer and autumn pop up) on the net displacement ($P<0.05$, $F_{1,20}$ 6.6, ANOVA), with the longest transits recorded during autumn (e.g. tags 1 and 9), although not all autumn displacements were large (e.g. tags 10 and 11 travelled < 55 km). The deployment season (autumn or summer) did not significantly affect the displacement direction (east, west or non-migrant, $X^2 = 3.47$, df 2, $P = 0.176$, $n = 11$)), similarly the size of the tagged fish (TL) did not significantly affect the direction or distance of the displacements (both tests $P>0.05$, regression, Supplementary Figure 1).

Depth and temperature

The minimum and maximum depths experienced by the deployed mulloway ranged from 0.0 to 56.5 (± 0.50) m, whereas the minimum and maximum mean depth (± SE) was 2.3 (0.02) and 36.8 (0.15) m respectively (Table 2). The average mean depth (± SE) for all 10 fish (tag 8 was omitted due to unreliable data) was 10.9 (4.06) m (Table 2). Four tagged fish (1, 4, 7 and 9) showed relatively rapid (day level) and large depth changes from shallow (~ <10 m) to deep (~ >30 m) waters and then returned to shallow water (~ <10 m) (Fig. 2a and 2b for tags 1 and 7, Supp. Fig. 2 for tags 4 and 9). However, there was no obvious pattern to these large (~>20 m) depth changes. There were missing depth data points in all of the Argos downloads with between 288 (e.g. Fig. 2c) and 19 968 data points recorded depending on the tag (Table 2). A best case scenario would have 28 880 depth data points for a 100 day tag deployment.

Depth distributions suggest that fish at large over autumn spent most of their time at large at relatively deep depths (20.1 to 50 m) with the exception of two fish, tags 10 and 11 (Fig. 3). Conversely, fish at large over summer spent most of their time at relatively shallow depths (2.1 to 10.0 m), with one individual at depths of 0.1 to 2.0 m (tag 7, Fig. 3). The two autumn fish (tags 10 and 11) that were not at relatively deep depths displayed depth habitat distributions more in line with summer deployments. There was a significant influence of season on time spent at depth (X^2 = 7.33, df 2, P = 0.02, n = 10).

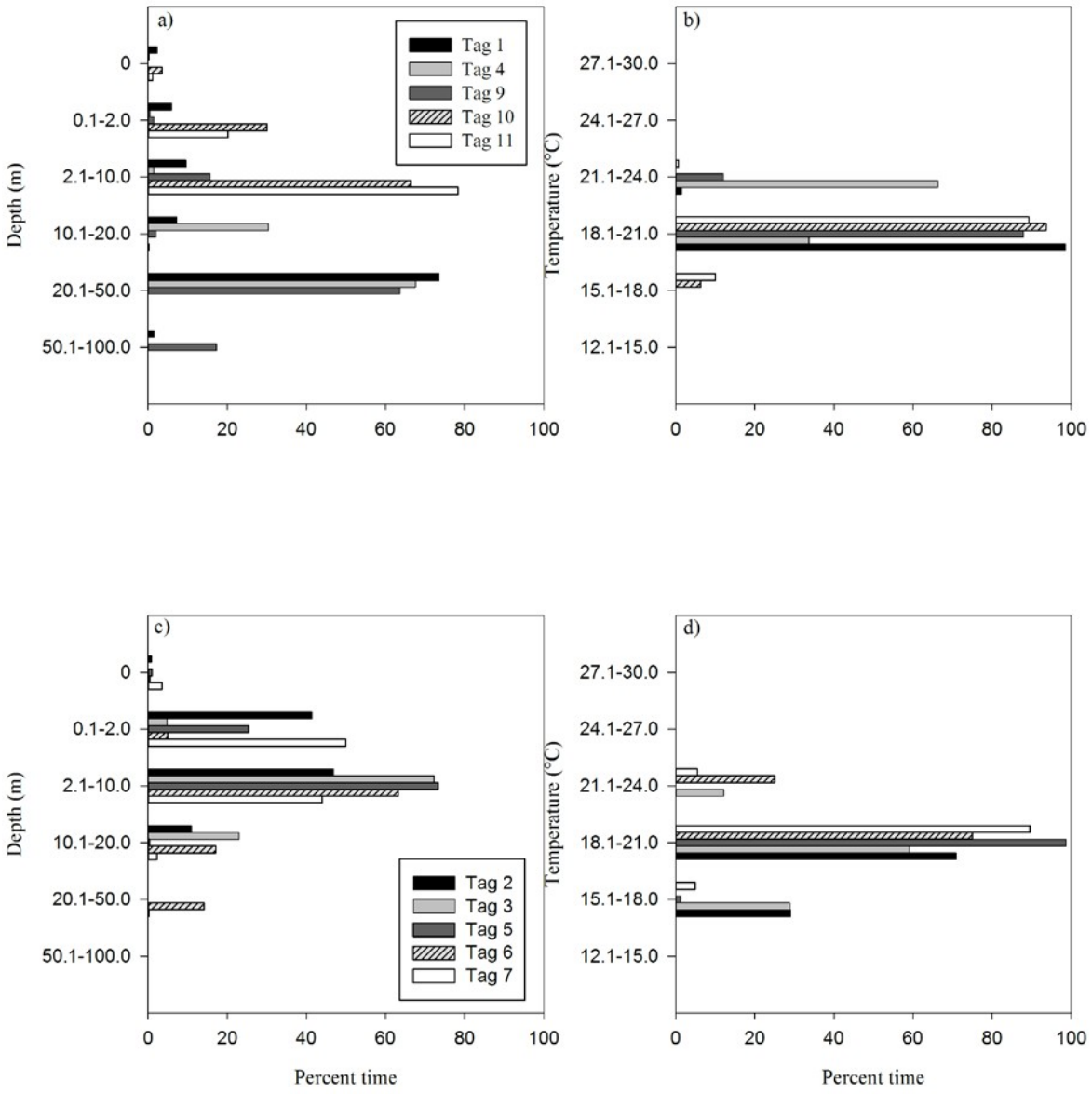

Fig. 3 Bar plots of percent time spent at different depths and temperatures for tags deployed on fish in autumn (a and b) and summer (c and d).

Temperature distributions suggest deployments over summer experienced cooler water conditions (Fig. 3). These fish exclusively spent time in cool waters of 15.1 to 18.0 ° C, with the exception again of tagged fish 10 and 11 which, despite autumn deployment, also entered cool waters. These latter fish (tag 10 and 11) also experienced temperature conditions more like summer deployed fish (i.e. a small amount of time in the cooler (15.1 to 18.0 ° C) conditions), but tag 11 also recorded a brief foray into the warmest temperature range (20.1 to 24.0 ° C), hence this individual experienced the greatest range of temperatures. All fish, regardless of the deployment season, spent the majority of time at large in water temperatures of 18.1 to 20.0 °C. However, deployments over autumn generally experienced warmer waters with time spent in the 20.1 to 24 ° C range.

Fish movement and use of the marine parks

Some pop up locations (6 out of 11) were outside of the Yalata section (where the fish were tagged), of the Far West Coast Marine Park (FWCMP) (Fig.1). However, one tag was in another section of the FWCMP but over 200 km west of the Yalata section and another tag was in another marine park (Nuyts Archipelago Marine Park). Two tags that popped up out of the marine park were only ~20 km from the FWCMP eastern boundary (Fig. 1). Two tags (1 and 9) popped up substantial distances from the marine park.

Virtually all western tag pop ups (i.e. to the west of the Yalata section of the FWCMP) occurred in autumn, although tags 10 and 11 (also autumn deployments) were east of the tagging location. All but one of the tags that were deployed in summer (tags 2, 5, 6, 7, 8 but not 3) detached within the Yalata section of the FWCMP. Thus, two cohorts of mulloway, that migrated from or resided in the Yalata portion of the FWCMP, may exist.

We reconstructed the daily position estimates from geolocation calculations for all 11 tagged mulloway that reported light location data, however, the positions appear to lack precision

(Supplementary Fig. 3). For example, according to the daily position estimates, tag 5 migrates off the continental shelf to a depth of approximately 200 m. Migration to the shelf break would be unusual for the species and the depth data recorded for this fish does not match the estimated location for a demersal species (i.e. the maximum depth recorded was <20 m, depth data not shown). Due to the apparent lack of precision the daily position estimates were not further analysed to infer fish presence or absence in the marine protected areas on a daily basis.

The offshore border of the FWCMP is between 40 and 50 m deep (Fig 1), hence fish that did not record depths of <40 m (tags 2, 3, 5, 6, 7, 10 and 11) probably did not cross the offshore marine park boundary whilst at large (Fig 2b and 2c, Supplementary Fig. 2). However, the missing depth data (in all cases) creates some uncertainty. Those tags that recorded >50m depth (tags 1 and 9; Fig. 2a and Supplementary Fig. 2) suggest they possibly crossed the offshore park boundary whilst at large. As the longitudinal FWCMP boundaries (east and west rather than offshore) do not have a cut off depth (i.e. a certain depth contour like the offshore boundary) it is possible that all the tagged mulloway crossed these boundaries whilst at large, although the pop up locations suggest this is unlikely for most deployments with the summer component (Tags 2, 5, 6, 7). Clearly, tag 3 crossed the eastern border as it popped up in the Nuyts Archipelago Marine Park. Also the autumn deployed tags 10 and 11 more than likely crossed this eastern border rather than the offshore border given their pop up location and depth data (Supplementary Fig. 2).

Discussion

The present study is the first to utilise pop-up satellite archival telemetry to investigate the movement of a commercially important demersal finfish. The results provide fishery independent movement information and environmental information on a unique fish population in one of the most isolated coastal environments in Australia. The interaction of tagged fish with the MPAs has the potential to enhance the design of parks or assess the performance of existing parks. For example, we have shown that the ocean neighbourhood is beyond the marine parks in the GAB for some large sexually mature mulloway. Therefore, at least some members of the mulloway population in the GAB are migratory, satisfying our hypothesis and providing more new information for the subpopulation.

The net displacement distance (mean 140.7 km) or movement covered by the tagged mulloway could be described as a migration of "medium" spatial scale compared to large bodied pelagic fish (Palumbi 2004). For example, large pelagic fish have travelled extreme distances (e.g ~1000km in a similar temporal period to the present study (Block et al. 2011), whilst some other fish species such as benthic fish display movements that are small scale (~5km) (Starr et al. 2002)). However, the average spatial scale movement evident in mulloway (e.g. 140 km over 100 days) is likely to be typical for large bodied demersal fish and similar to small pelagic species (Palumbi 2004).

Most mulloway movement studies have been estuary based and have utilised acoustic telemetry and thus been at a relatively small spatial scale (Taylor et al. 2006). Mulloway have been subjected to mark and recapture using conventional tags in South Australia but recaptures have been very low (4.6 %) (Hall 1986). None-the-less some fish were found to move "medium" distances (e.g. approximately 200 km) (Hall 1986). In South Africa adult fish were migratory based on tagging (mark recapture) and commercial catch records, although precise distances moved are unclear (Griffiths 1996). An Australian population genetics study suggests medium range movements (100s km) on the west coast of Australia, but potentially longer movements on the eastern seaboard (Chapter 2).

Hence it is possible different genetic populations may exhibit different life history traits including movements.

There was a seasonal aspect to tagged mulloway behaviour. The seasonal difference in movements and environments experienced by the tagged mulloway suggests a shallow water summer residency which may indicate a spawning aggregation at Yalata beginning in late spring and continuing until mid to late summer. It has previously been suggested that mulloway likely spawn in the surf zone in late spring and summer in South Australia (Ferguson and Ward 2003). This timing is similar to other areas of Australia (e.g. southeast, Gray and McDonall 1993; south Western Australia, Parsons et al. 2009), although mulloway have also been observed to spawn year round in sub- tropical regions (with seasonal peaks) (Farmer 2008). Preliminary evidence suggests that adult mulloway are aggregating at Yalata to spawn (Hall 1986; Rogers et al. 2014). Fish have been observed in spawning condition during dressing for consumption by recreational anglers (Hall 1986; Barnes pers. obs.) and groups of fish observed tailing with an oily slick around them (Barnes, pers. obs.). Also, it is unusual to catch sexually mature mulloway (TL >1m) in autumn, winter and early spring at Yalata but sub-adults and juveniles are caught year round (Barnes, pers.obs.).

Fish showed greater movements in autumn compared to summer and were potentially moving to overwintering grounds post spawning. No information is available on overwintering of mulloway in South Australia, but seasonal movements have been observed in South Africa (Griffiths 1996) and in other sciaenids (Semmens et al. 2010) including movement to overwinter (Weinstein et al. 2009). The southern shoreline of Australia is at the southern extremity of the range of mulloway (Ferguson et al. 2014). Hence, the predominantly westerly movement of tagged mulloway may have been to overwinter in warmer waters provided by the warm Leeuwin current. However, fish (tags 10 and 11) cast some doubt to an obligate autumn movement west. Different migratory behaviour has been reported for mulloway in other regions (Griffiths 1996) and other large bodied predatory scale fish including sciaenids (Semmens et al. 2010). Unfortunately, the fish could not be safely sexed in the present study to elucidate any sex differences in movement behaviour.

Mulloway at Yalata may receive some protection from the marine parks in the area, during late spring and summer. Protection of fish via MPAs is a potential outcome of implementing these areas (Dugan and Davis 1993; Nemeth 2005). The sandy beaches and reef lagoons in the Yalata area are in the FWCMP. In this area the marine park has two seasonally enforced sanctuary zones (1st November to 30th April, 7 and 10 km^2 in area), where the removal of any marine animal is prohibited, except by indigenous traditional custodians. As such, these sections of the FWCMP may provide protection to the summer (potentially spawning) aggregation of mulloway at Yalata. Another sanctuary zone is at the western end of the park where the shore line is cliffs rather than sandy beaches; as such it is unlikely that mulloway spawn in this zone. At the very least it provides a section of unimpeded access from the western overwintering grounds to the known spawning habitat. The remaining areas of the marine park have various levels of zoning protection, but all allowing some form of extraction of mulloway (in line with South Australian fisheries regulations) by commercial and recreational fishers. Fishing for large adult mulloway in the isolated Yalata area is very popular with the recreational fishing community, with a large number of fish caught over the late spring and summer season (at least 300 per season) (Rogers et al. 2014).

The GAB Commonwealth Marine Reserve surrounds the offshore boundary of the FWCMP. There are two zones which affect mulloway when they cross the offshore boundary (mainly the special purpose zone but also marine national park zone). All fishing gear types are allowed in the Commonwealth special purpose zone except demersal trawl. These gear types include commercial methods which are very effective at catching large quantities of adult mulloway (e.g. demersal gillnet and demersal long-line) (Ferguson and Ward 2003). Consequently, significant catches of mulloway are taken in the GAB by the commercial sector, although accurate numbers are hard to ascertain as there are 100 kg trip limit restrictions on the demersal gillnet fishery, hence there is potentially some discarding (Ferguson and Ward 2003). No fishing gear can be used in the

Commonwealth marine national park. Hence, this area offers protection to adult mulloway that may use offshore waters.

Mulloway leave the managed Yalata beaches during autumn. As such, this may be a period when the population is vulnerable to depletion due to capture by the commercial fishing sector. However, despite the various protected areas in the GAB not being as large as the ocean neighbourhood of the relevant mulloway population there are still very useful benefits for the species conservation, such as reduced fishing mortality and protection of key habitat types (Palumbi 2004). This is particularly relevant for mulloway at Yalata due to the degree of protection during the spawning season. However, the views of some suggest that MPAs are inadequate unless they encompass the majority of the ocean neighbourhood of target species (see Starr et al. 2002) or at least combine spatial closures with adequate fisheries regulations (Hilborn et al. 2004). Further complicating potential conservation and sustainability for mulloway in the GAB is the fact fish cross the border into WA, meaning useful management outcomes on the ocean neighbourhood scale would have to involve multiple management agencies.

Satellite telemetry via PSAT provides important information for assessing the design of marine parks. The size of the ocean neighbourhood of highly mobile fish obtained from fishery independent movement information is critical in designing the placement and size of MPAs (Palumbi 2004). Furthermore, evidence from PSATs on timing, rate and directions of movement and other behaviours is also useful particularly for implementing boundaries, buffer zones and seasonal restrictions. Information from PSATs can help promote the ability of MPAs to protect spawning biomass of fishery species and also facilitate improved capture of trophy fish outside MPAs (Blyth-Skyrme et al. 2006), which could also aid in the acceptance of spatial closures. The flow-on benefits of MPAs such as improved captures in areas adjacent the parks are often fish species specific (Blyth-Skyrme et al. 2006); however, research via PSAT deployment can answer movement questions on individual large bodied species. Also, as MPAs have conservation objectives not just restricted to

fish, it is useful that this technique can be used on other biota such as penguins (Dee Boersma et al. 2002) and turtles (Maxwell et al. 2011).

Information on mulloway movement and other related data provided valuable insight into the ecology of a unique and isolated demersal fish population; although there were a few inherent problems with the technique. The return rate of tags was reasonable (58%) and compared favourably with other demersal tagging applications such as data storage tags (DST), which can be as low as 6% (Miller and Able 2002); however, the rate was slightly lower than at least some studies on fish with different habitat preferences, such as benthic (e.g. Armsworthy et al. 2014) and pelagic (e.g. Block et al. 2011) species. The acquisition of movement information via PSATs relies on satellites rather than the presence of fishermen or acoustic receivers providing benefits in an isolated study area. The depth data allowed fish movement in relation to the MPA to be determined which proved crucial in the absence of precise daily at large positions via geolocation. The rapid beaching of tags was unfortunate as it limited download of depth data. To provide a full data set for the present study the tags would need to float upright for ~ 2 weeks, however, new technology will help alleviate this issue in the near future (pers. comm. Kevin Lay, Wildlife Computers). The daily positions (particularly longitude) from the geolocation data were not precise enough to infer interactions with MPA boundaries at relatively small scales as they were only accurate to approximately 100 km (Thiebot and Pinaud 2010). The size of MPAs in the region of tagging is much less than 100 km (e.g. the Yalata section of the FWCMPA is a rectangle approximately 10 x 70 km, Fig. 1). Including depth information in algorithms for demersal species may help improve the accuracy of geolocation data in the future.

The utilisation of PSAT technology on a demersal fish species provided information that will help ensure the sustainability of the subpopulation into the future. The return rate of devices was favourable compared to other tagging studies of demersal marine animals, but below that expected from tagging of pelagic species. The net displacement and depth data allowed assessment of the target species movement and hence the spatial scale of the ocean neighbourhood could be

compared to the scale of the MPAs. Temporal information on movements allowed elucidation of biologically important behaviours such as possible seasonal movements. As such, the direction and timing of fish movements provided from PSAT deployment could be valuable in assessing the design of MPAs.

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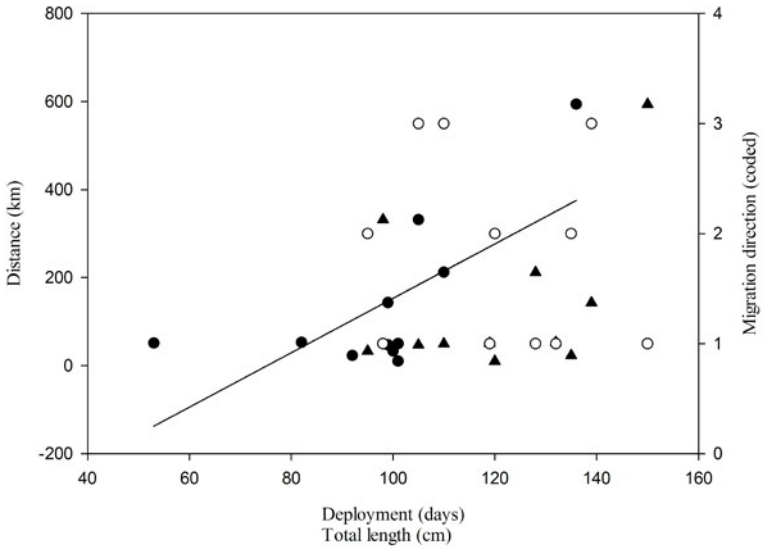
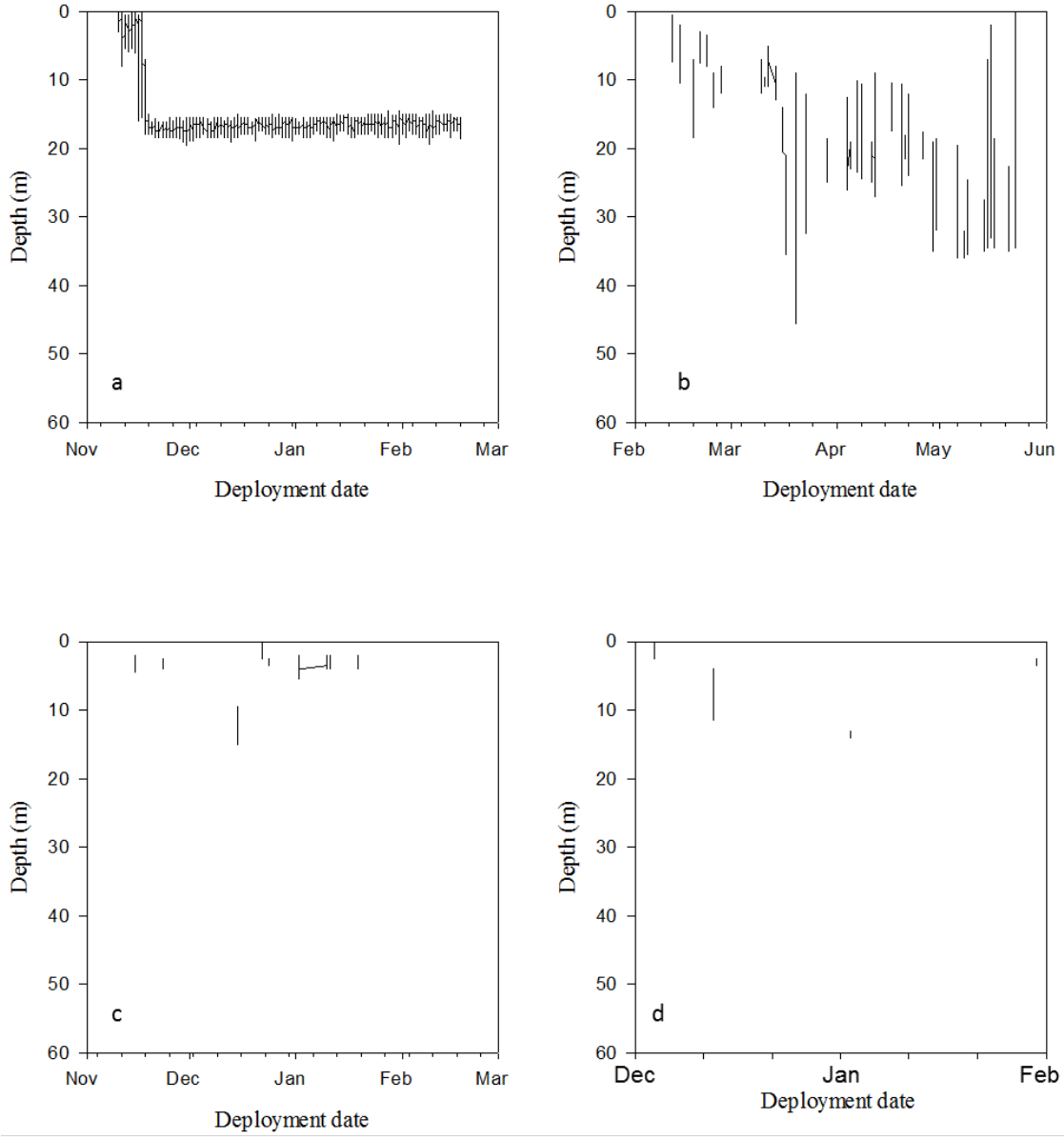
Supplementary material



Supplementary Fig. 1 Relationship between deployment duration and distance moved (solid circles), and between total length and distance moved (triangles) and migration direction (open circles). The solid line is the linear regression between deployment duration and distance moved ($y=465.20 + 6.20x$, $r^2=0.47$). Migration direction codes are 1 = west, 2 = non-migrant and 3 =east.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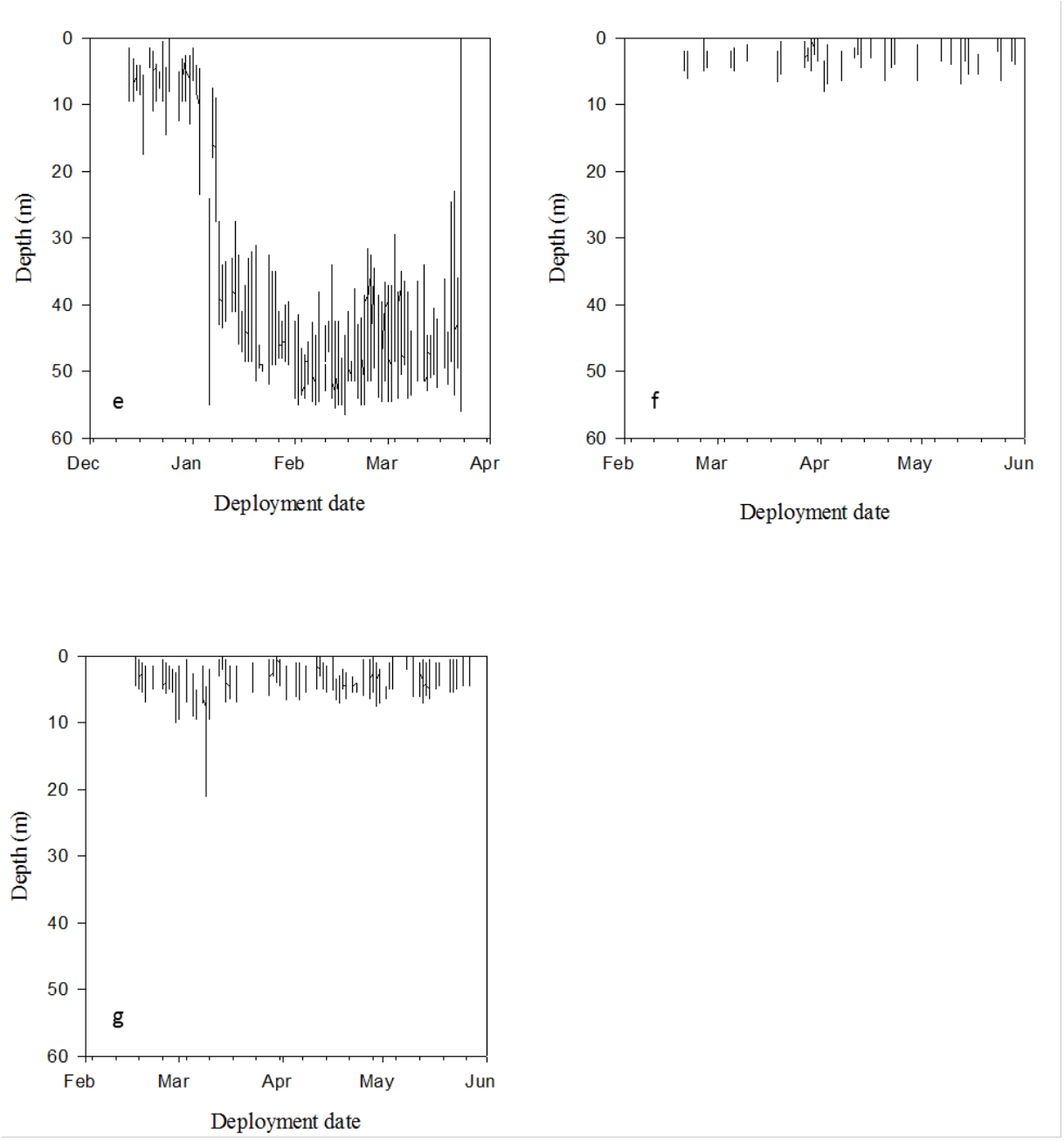
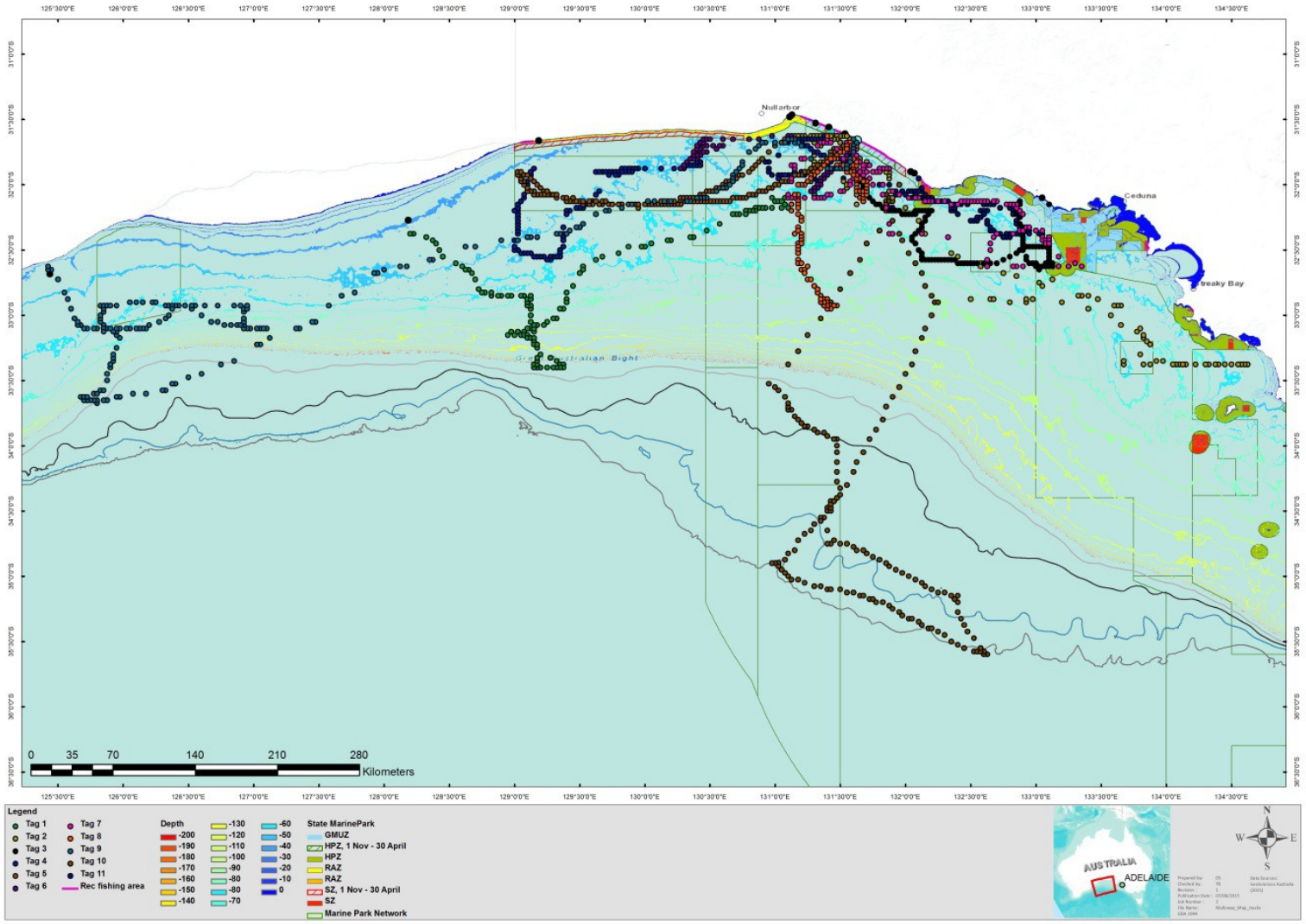


Supplementary Fig. 2 Plots of depth (± 0.25m) time series showing data summarised at the two day level (except for tagged fish 6 which is at the 10 day level) for tagged fish 2, 4, 5, and 6 (previous page - a, b, c and d respectively) and for tagged fish 9, 10 and 11 (this page – e, f and g respectively).



Supplementary Fig. 3 Daily position estimates from geolocations for the 11 tagged mulloway. Coloured lines represent depth contours. Legend abbreviations: GMUZ = General Management Use Zone, HPZ = Habitat Protection Zone, RAZ = Restricted Access Zone, Nov = November and SZ = Sanctuary Zone.

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Chapter 6 – General Discussion

Conspecific marine fish were structured into genetically different populations, despite some biological aspects suggesting that structuring is unlikely (e.g. pelagic larval phase) (Chapter 2). The results have also shown that a better understanding of the contribution of genetic drift and gene flow is achievable through new analytical techniques such as decomposed pairwise regression (DPR), therefore furthering our understanding of population connectivity. I also demonstrated that use of multiple population structure approaches can increase confidence in the results by providing a "body of evidence" (Chapter 3). Further, it showed that it is feasible to integrate three approaches and provide readily interpreted results. I also found that the influence of intrinsic (genetic) and extrinsic factors (temperature and water chemistry) on the otolith chemical tag and the response of some trace elements to these factors is species specific (e.g. magnesium) (Chapter 4). I also demonstrated that the application of satellite telemetry to gather movement, connectivity and conservation information on marine predatory demersal fish is feasible (Chapter 5). This chapter (the general discussion) explores these key points in terms of the implications and limitations for the study species (mulloway) population structure and connectivity but also puts the findings in context for predatory demersal fish in general. Finally, I discuss future research directions.

The molecular 'natural' tag differentiates mulloway populations

I found broad scale genetic population structuring between South Africa and Australia, which supports the findings of previous studies (e.g. Archangi 2008; Farmer 2008). Within Australia I found four genetically differentiated populations across the range of mulloway. Importantly, several of these populations are currently managed as one stock and a further population is currently managed independently by each of the states that it spans. Differentiation was driven by biogeographical factors, which was highlighted by a novel analytical approach (DPR). Unfortunately patchy distribution and sampling difficulties in parts of the Australian range of mulloway did not allow population boundaries to be ascertained; this remains a key area for future research.

Broadly the results of Chapter 2 have helped to further understand demersal fish population structure and connectivity. For example, I have demonstrated that genetic population structuring of demersal fish is possible. Traditionally the connectivity of the marine environment and pelagic larval stages of demersal fish had led some researchers to suggest that populations should be genetically homogeneous (e.g. Rosenblatt and Waples 1986; Doherty et al. 1995; Shulman and Bermingham 1995; Cowen et al. 2000; Palumbi 2003). More recently, researchers have questioned this traditional belief (e.g. Andreev et al. 2015). Modern genetic studies are supported by relatively new research on organismal biology which explains why structuring occurs. For example, pelagic larvae may not necessarily passively drift but actively recruit back to their source populations (Swearer et al. 2002). Furthermore, operational factors such as the lack of resolving power of early population genetic studies on demersal fish may have influenced the outcome. For example, red drum (*Sciaenops ocellatus*) have been tested for structuring by a variety of techniques with the older approaches (e.g. allozymes) suggesting panmixia and new approaches (e.g. microsatellites) suggesting differentiation, albeit weak (Rosenblatt and Waples 1986; Gold and Turner 2002). However, in some cases allozymes have found structuring (e.g. Ramsey and Wakeman 1987; Gold and Richardson 1998), which was probably due to strong population structuring evident in the target species.

Large to medium bodied sciaenids often show broad scale genetic population structuring (Ramsey and Wakeman 1987; Gold and Richardson 1998; Gold et al. 2001; Gold and Turner 2002; Leidig et al. 2015), or no structuring (Levy et al. 1998; Cordes and Graves 2003). Genetic population structuring of sciaenids is driven by geographic distance (e.g. Ramsey and Wakeman 1987; Gold and Richardson 1998; Gold et al. 2001; Gold and Turner 2002; Gold 2004), estuary association (e.g. female homing to natal estuaries (Gold and Turner 2002)) or biogeographic barriers (Cooke et al. 2012; Henriques et al. 2015); either as single factors or in combination (e.g. Gold and Turner 2002). Furthermore, particular species within the family sciaenidae have been found to have conspecific populations that demonstrate different life history traits. For example, pure marine populations are sometimes found among estuary associated populations, with such life history differences suggested to lead to

structuring (Levy et al. 1998; Gold and Turner 2002). As such the population structuring that was discovered in Chapter 2 has traits that are typical of large bodied sciaenid populations (isolation by distance (IBD), biogeographic barriers to gene flow and life history plasticity).

Factors that drive genetic population structure may be less obvious particularly in the marine environment (Ovenden 2013). However, to properly understand patterns of coastal predatory fish population structure I used DPR a relatively untested analysis in the marine environment. When I tested mulloway population differentiation for a signal of IBD it was significant. However, DPR was subsequently utilised and outliers to the IBD model were found, suggesting some populations had a greater influence from genetic drift than the genetic drift and gene flow equilibrium initially suggested (i.e. by IBD). The discovery of outlier populations allows factors driving population structure to be assessed, providing information that could lead to better management of these populations. Only one other study that I am aware of has utilised DPR on marine predatory demersal fish (Cunningham et al. 2009), whereas the DPR approach has been widely used in freshwater habitats (e.g. Huey et al. 2011).

A holistic approach to population structuring

The two genetic populations evident in South Australia were supported by the other (otolith based) approaches; however the otolith based approaches suggested finer scale structuring. Similar population structuring of mulloway has been described in South Australia before (Ferguson et al. 2011), but the present study combined molecular and otolith based approaches. The integration of different data sets could benefit further from more testing in the future, to determine the most appropriate method of integration (e.g. pre or post statistical testing).

In Chapter 3, microsatellites provided the best allocation rate of individual fish to broad collection locations, when compared to either otolith shape or otolith chemistry. The success of microsatellites suggests that the population structure and hence divergence of mulloway in South Australia is over a long term timescale. According to the literature, the better allocation success of molecular

approaches over the otolith based approaches is unusual (e.g. Thorrold et al. 2001; Wennevik et al. 2008; Smith and Campana 2010). The lack of consistency between Chapter 3 and other studies is unclear. However, it could be due to small scale environmental changes affecting the chemical tag of mulloway otoliths (at the intra genetic population level) and possibly the divergence timescale may be greater than divergence in the other studies. As the best approach (microsatellites in the present study) is likely to vary at the inter and intra species level and also at the regional level (particularly when environments are heterogeneous within fish species range), the merit of implementing a variety of approaches is clearly evident. Application of multiple tags or approaches allows results to be 'double checked' (Welch et al. 2015) and populations can have greater scrutiny applied (e.g. the search for population boundaries) (Catalano et al. 2014). However, at present there is no standard method for integrating the independent data sets which is challenging given continuous and categorical data (e.g. otolith chemical concentrations and microsatellite scores respectively) (Smith and Campana 2010). Also challenging is the simplification of complex results to a form that can be readily interpreted by end users such as fishery managers (Welch et al. 2015). My integration of data and production of readily interpreted results in Chapter 3 was achieved via utilising the population assignment proportions from the genotypes and combining all datasets before statistical testing. The results of Chapter 3 suggest it is feasible to integrate more than two data sets but in the present study otolith approaches did not always provide correct allocation of fish to sample sets and as such were not always consistent with the microsatellites.

Controlled testing of the otolith 'natural' chemical tag

The inconsistency of otolith results in Chapter 3, particularly otolith chemistry and the microsatellites highlights the need to understand the contribution of factors that form the natural chemical tag. The results of Chapter 4 suggest there is an intrinsic (genetic) influence affecting the substitution of trace elements for calcium in the otolith matrix. The genetic component to otolith chemistry has been found before (see Clarke et al. 2011). The presence of a genetic contribution to conspecific variation in otolith shape (see Campana and Casselman 1993; Vignon and Morat 2010;

Vignon 2012) suggests molecular differences are also likely to affect the chemical concentration. As well as intraspecific differences our results also form part of the growing body of evidence to suggest there are interspecific differences in otolith chemistry. For example, this study found magnesium was significantly affected by temperature and hence a good indicator of different thermal habitats experienced by mulloway, which to my knowledge has not been reported before. A review of previous studies suggests that there are interspecific differences in the levels of strontium and barium incorporated under similar environmental conditions (Elsdon and Gillanders 2003; Gillanders and Kingsford 2003; Hamer and Jenkins 2007). Hence, the use of generalised models of the otolith enviro-chemical tags remains unwise and intrinsic and extrinsic contributions require further controlled testing in the future, particularly for species which are having this natural tag applied for the first time. In particular, the preliminary evidence of a genetic contribution to the otolith chemical tag in Chapter 4 and in Clarke et al. (2011) requires further empirical testing.

The presence of a significant genetic influence on the chemical tag has implications for otolith chemistry based population studies. The finding that genetic differences may be detectable in the absence of environmental variation is of great importance, because the ability of otolith chemistry to differentiate populations is potentially more powerful than originally estimated (Clarke et al. 2011). However, the genetic influence on the natural tag means the utilisation of otolith chemistry for environmental history applications that infer movement or habitat use may be more complex than originally suggested. Also, significant interactions of water chemistry (salinity) and temperature evident in Chapter 4 and other studies (e.g. Elsdon and Gillanders 2002), the two extrinsic (environmental) parameters used in environmental history studies, further demonstrates the complex nature of the otolith chemical tag. Therefore, the concerns of some authors, about simplification of intrinsic and extrinsic factors affecting the otolith chemical tag (e.g. Thresher 1999) may be justified. Hence, controlled testing of the otolith chemical response to these factors may need to be carried out for all target species (see Elsdon and Gillanders 2002). The modification of the chemical tag by genetics and by interactions of water chemistry and temperature is probably not

enough to override the main influence on the chemical tag, namely water chemistry, in cases such as fish movement between river and sea (e.g. anadromy). However, these influences may confound environmental reconstructions when smaller changes in water chemistry are experienced by fish (e.g. movement from regions with smaller changes in water chemistry such as embayment to the open ocean).

An 'electronic' tag provides population and conservation information

The movement information from the pop-up satellite archival tags (PSATs) suggested the dimensions of the 'ocean neighbourhood' for mulloway at Yalata is supported by the population structure evident in Chapters 2 and 3. The size of the ocean neighbourhood is also supported by another study on a similar sciaenid, where the dimensions of the ocean neighbourhood (or range) of the populations were implied from the results of molecular research (Gold and Turner 2002). The movement and other environmental information provided by PSATs also allowed mulloway movement in and out of marine parks to be determined. To the best of my knowledge this has not been done on demersal finfish before but this approach has been used successfully on other types of fish (e.g. Block et al. 1998) and biota (e.g. Dee Boersma et al. 2002). Movements of mulloway have previously been assessed using acoustic (Taylor et al. 2006; Næsje et al. 2012) and mark recapture (e.g. Hall 1986) tagging.

Acoustic and mark recapture tagging requires the presence of receivers or fisheries respectively, hence, the fishery independence of PSAT renders this approach suitable for remote locations, such as the Great Australian Bight where the present study on mulloway was under taken. Other demersal fish studies have used data storage tags (DST) to gather movement and environmental data (see Godø and Michalsen 2000) but these are again dependent on fisheries to recapture tagged fish, as such the return rate is quite low (< 30%). The nature of demersal fish (i.e. living near the sea floor) makes them hard to track. The results of Chapter 5 suggest PSATs provide a means to provide movement and environmental data on predatory demersal fish. One important limitation of PSATs is

the relatively large size (approximately 175 mm and 55 grams) of even the smallest units rendering them only suitable for large individuals (> 90 cm total length). As such it is hoped that technology will reduce the size, which is due to the large battery requirements (pers. comm., Kevin Lay, Wildlife Computers), opening up their use on smaller bodied fish or different life stages of large predatory demersal fish.

Integration of the outcomes from all the experimental chapters

The results of this thesis suggest mulloway population structure is generally governed by limited dispersal of individuals. The total ocean neighbourhood or range of conspecific populations is likely within the dimensions of 500 to 1500 km around suitable habitat (e.g. Chapters 2, 3 and 5). Within the ocean neighbourhood, the habitats have been demonstrated to be heterogeneous in some cases (e.g. Chapter 3). The limited dispersal, medium sized ocean neighbourhood and types of habitat utilised appears to be typical for other similar sized sciaenids that share similar life history traits. For example, red drum (Gold and Turner 2002), black drum (Gold and Richardson 1998), silver kob (Henriques et al. 2015) are similar to mulloway (e.g. body size and life history) and have been found to have structured populations over similar spatial scales and are structured by similar mechanisms or drivers (e.g. biogeographic barriers) (Henriques et al. 2015). The limited dispersal of large sciaenids is in contrast to that of similar sized pelagic fish, whose range is much larger and hence there is likely less population structuring. Indeed, the structuring evident in our study species may be a trait typical of large bodied predatory demersal fish in general.

Future research directions

Despite the worthwhile findings of this study and other similar research there is still a great deal to be learnt about mulloway, sciaenids and predatory demersal fish in general:

I demonstrated that mulloway are differentiated into populations across their Australian range, however, I did not always locate the position of the population boundaries. Locating the boundaries could be achieved by sampling mulloway in the vicinity of the likely boundaries as suggested by

Chapter 2. The sampling at the boundaries could utilise new genomic sequencing approaches to enhance the molecular resolution and hence aid in finding the boundaries. Also the utilisation of molecular markers under selection could provide useful information on the timing of differentiation of mulloway populations.

The integration of different data sets in Chapter 3 meant that the microsatellites required transformation to assignment proportions to make them compatible with other data. Further research into the reduction from codominant to a compatible format is recommended. This will ensure that subtle variation can be retained and hence the transformation facilitates the retaining of as much information as possible. The retention of fine scale variation of molecular information was not critical to the present study due to the strong genetic differentiation across the study populations; however, in other studies it may be important to retain as much variation as possible, especially in the event of weak structuring.

A genetic influence on otolith chemistry was discovered for only the second time (according to the literature) (Chapter 4), although other authors have suggested it was likely (e.g. Thresher 1999). I do not know why a different genotype would affect the final chemical concentration in otoliths, but it could be related to growth, physiology or physical structure of the otolith. Also, more field testing of the genetic influence on otolith chemistry would be useful as thus far the genetic influence has mainly been tested in controlled 'tank' experiments.

I applied pop-up satellite archival tags (PSATs) to demersal finfish for the first time, to the best of my knowledge. The success rate was about 50% in the present study which is quite low compared to other PSAT studies on fish (pelagic and benthic). As the devices are very expensive (~ $4 000 AUD each) some controlled tag retention studies would be very useful. It may be that the slower swimming speed of demersal fish means the devices are prone to bite off by conspecifics and other predators such as sharks. Also, demersal fish may be able to rub them off on underwater structure. Testing of anchoring devices and tethers may help reduce the loss of devices.

Conclusions

The natural tags, namely microsatellites, otolith chemistry and shape, and an artificial tag, namely satellite telemetry, have provided a better understanding of the population structure of mulloway in Australia. The presence of spatial structuring revealed in this thesis provides new information for the species. This information is now available to natural resource managers and could lead to a better future for mulloway, an iconic species. Furthermore, the results of this thesis are important for understanding predatory demersal fish and fish in general. An improved biological understanding is timely given the worldwide decline of predatory fish, an important food source, ecosystem component and sport fishery. Modern approaches to delineating population structure, such as those utilised in this thesis, coupled with more research on more fish species has the potential to facilitate a sustainable future for the worlds predatory fish.

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