

Stock structure and connectivity of black bream including implications for management

Postgraduate funding

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6 January 2025

FRDC Project No 2019-012

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2025

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

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Executive Summary

The research in this report was undertaken as part of Koster Sarakinis's PhD project at the University of Adelaide supervised by Professor Bronwyn Gillanders (University of Adelaide), Dr Patrick Reis Santos (University of Adelaide), Dr Qifeng Ye (SARDI Aquatic Sciences), and Dr Jason Earl (SARDI Aquatic Sciences). The project investigated stock structure of black bream and how they were responding to changing environmental conditions for fish collected across southern Australia (stock structure) with additional work focused on south-eastern Australia. Outcomes contributed to better understanding of stock structure of black bream across southern Australia, and research training of a fisheries scientist now employed at SARDI Aquatic Sciences.

Background – The SA RAC identified defining stock structure for black bream in southern Australia, and implications for management as one of three post-graduate student projects to address a range of priority fisheries research. The aim was for the student to undertake direct applied research and gain industry experience by being co-supervised by scientists from the South Australian Research and Development Institute (SARDI), while researching an industry relevant project and be registered at a South Australian university in order to undertake their post-graduate studies. The application was there developed according to this call and after consultation with government researchers, industry and representatives from the recreational sector.

Aims/objectives – The aims included: (1) Define the stock structure of black bream in southern Australia using a multi-methods approach incorporating genetics/genomics and otolith-based techniques; (2) Review information on and determine how black bream respond to changing environmental conditions; (3) Provide recommendations for fishery managers based on results of stock structure analyses; and (4) Increase fisheries science capability in South Australia through training of the next generation of researchers.

Methodology – The genetic structure of black bream across its entire distribution range in southern Australia, as well as regional scale gene flow across south-eastern Australia was investigated by undertaking a comprehensive analysis of the populations in estuaries across the region. Genome-wide sampling of single-nucleotide polymorphism (SNP) markers generated from restriction site-associated DNA sequencing was utilised. We then utilised a range of natural markers, including genetics, otolith shape, otolith isotopic composition and otolith elemental composition, to determine the effectiveness of each marker independently and through integration. To assess how black bream responded to changing environmental conditions otolith increment measurements were obtained and a mixed effects modelling approach used to assess local-scale (water flow and temperature) and regional scale (El Nino Southern Oscillation) effects on growth.

Results/key findings – Broad scale genetic structuring, with limited gene flow among regional clusters (i.e. Western Australia, South Australia and western Victoria, and eastern Victoria, Tasmania and New South Wales) was identified. Local-scale genetic structuring was also identified across the south-eastern Australian estuaries sampled. Combining genetics and otolith-based techniques was complementary in increasing the accuracy of our results but not all marker integrations were consistently beneficial, highlighting the importance of appropriate marker selection. The most important factor explaining variations in yearly growth was regional scale, annual-lagged ENSO effects, although local scale annual-lagged effects also elicited mixed growth responses among estuaries suggesting potential for system-specific differences in environmental conditions.

Implications for relevant stakeholders – Overall, the thesis provided industry, managers and policy makers with information on the stock structure of black bream across southern Australia highlighting the importance of location-specific management. Similarly,

Keywords – black bream, *Acanthopagrus butcheri*, Australia, gene flow, philopatry, population structure, single-nucleotide polymorphism, otolith, growth, population connectivity, otolith shape, otolith isotope composition, otolith elemental composition

PhD thesis – Koster Sarakinis

The PhD thesis of Koster Sarakinis follows.

POPULATION DYNAMICS OF AN ESTUARINE FISH AND IMPLICATIONS FOR MANAGEMENT

Koster Georgien Sarakinis



School of Biological Sciences The University of Adelaide

Presented for the degree of Doctor of Philosophy on 23rd of February 2024



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Declaration

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

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Date:

Cover image: Koster Sarakinis

Acknowledgments

I am forever thankful to my mentors, colleagues, and loved ones for their support and guidance throughout this academic journey.

I could not have completed my studies if it weren't for Prof. Bronwyn Gillanders. I met Bronwyn on a study tour in southern China and later worked with her on an ecology essay topic I selected during my undergraduate studies, which coincidentally happened to be her area of expertise: otolith science. After showing much interest in working with her, Bronwyn invited me to continue my research in the Gillanders Laboratory, where I have been fortunate enough to remain for many years. Bronwyn has been a supportive and caring supervisor throughout this time, has helped me develop my research skills, and introduced me to such an amazing scientific community. I am so grateful to have met and worked with you and I hope we can continue to collaborate in the future.

Thank you to Dr Patrick Reis-Santos, my co-supervisor and friend. You have been someone I can always depend on and I am so grateful you were willing to supervise and guide me through this experience. You have always been free for a chat and have helped me through more than a few tricky situations. Thank you to my co-supervisors Dr Jason Earl and Assoc. Prof. Qifeng Ye, who have given me the opportunity to communicate my research to a broader community beyond academia. I can't wait to continue working with you both. A big thanks to my collaborators Prof. Stephen Donnellan and Assoc. Prof. John Morrongiello for helping me develop my skills in population genetics and growth chronologies, respectively.

A special thanks to my office pals Brittany Williams and Nikita Pring for the daily support over the years and the many scuba diving trips. Thank you to the Gillanders Laboratory, who I've been lucky enough to share this journey with. Namely, Nina Wootton, Sophie Dolling, Troy Rogers, Jasmin Martino (who first taught me how to process otoliths), Jackson Stockbridge, Vinuri Silva, Joseph Widdrington, Sophie Russel, Qiaz Hua, Alice Jones, and Rhiannon Van Eck. Also, thank you to the remaining Southern Seas Ecology Laboratories and the amazing support provided by all its members throughout my candidature.

Most importantly, thank you to my family and close friends for always being there for me through thick and thin. Thank you, Mum, Dad, Leticia, Peter, and Elissa, for the love and support you've provided and always believing in me. I could not have done any of this without you. A special thanks to Marcus Deluca, Daniel Settimio, John Thomolaris, and Tom Settimio; friends I can depend on to pull me out of a rut and have a laugh with even at the most stressful of times.

Funding

Funding and logistical support for this study were provided by:

- School of Biological Sciences, The University of Adelaide
- South Australian Research and Development Institute
- Fisheries Research and Development Corporation (FRDC; project #2019-012)
- Australian Research Council (ARC) Discovery grant (DP220103846)

The PhD candidate was supported by:

- Australian Government Research Training Program Scholarship

Ethics

Animal ethics for this project were obtained from The University of Adelaide Animal Ethics Committee (approval #S-2020-069).

Summary

Understanding population dynamics plays an important role in how we sustainably manage fish species, and the spatial scales at which management strategies are applied. By harnessing the power of archival tissues and hard parts (i.e., natural tags), we can access valuable biological and environmental information encoded within materials to investigate how species distributions are structured, their connectivity, as well as their future resilience and vulnerability to overexploitation and climate change. In this thesis, I used black bream *Acanthopagrus butcheri* (Munro 1949) as a case study to investigate population dynamics using a range of natural tagging techniques, including the chemistry, shape and growth of fish ear stones (i.e., otoliths), and genetics.

In Chapter 2, I investigate genetic variation and potential gene flow present among black bream in southern Australian estuaries. Through next-generation sequencing of 33,493 Single Nucleotide Polymorphism (SNP) markers, I detected continental-scale structuring, reflecting the known biogeographical provinces found across southern Australia, as well as large open water bodies, unsuitable habitats and strong ocean currents further explaining the genetic differentiation identified at this spatial scale. Furthermore, I detected local-scale genetic structuring across south-eastern Australian estuaries with limited gene flow among adjacent locations, while fish movement beyond nearby habitats was attributed to lone migrants. These findings reflect the species' estuarine dependency and highlight how neither the spatial proximity of estuaries nor black bream's ability to move into coastal waters reflects increased gene flow.

In Chapter 3, I assess the effectiveness of multiple natural tags both independently and through integration to investigate population structure and connectivity. As different markers function effectively at varying spatiotemporal ranges, I found that combining otolith-based techniques (i.e., elemental composition, isotopic composition and shape) with genetics increased the classification accuracy of fish to their estuary of capture up to an overall correct classification of 95%. However, simply increasing the number of markers integrated did not consistently increase the resolution of the findings, which highlights the importance of appropriate marker selection. Additionally, these findings further reflect the high structuring of populations both on a generational and ecological timescale, and the limited connectivity among estuaries.

In Chapter 4, I examine the vulnerability and future resilience of *A. butcheri* to changing environmental conditions by investigating growth variability among estuaries. I collected 31,006 otolith increment measurements spanning from 1981 to 2016 from 4,792 fish and used a mixed effects modelling approach to assess both local-scale effects (water flow and temperature) and regional-scale effects (El Niño Southern Oscillation, ENSO), as well as determine whether spatial synchrony in growth among locations was occurring. I found that an annual-lagged ENSO effect best

explained the variability in annual growth, with cases of spatial synchrony occurring across southeastern Australian estuaries (1000s km apart). These findings highlight the potential impact of extreme weather events on the growth of *A. butcheri* and the potential vulnerability of this estuarine fish to intensifying climatic events despite it occurring in estuaries with a diversity of environmental conditions.

Lastly, in Chapter 5, I assess fish movement patterns to determine habitat utilisation and whether this varies across the species' distribution. Using otolith chemical profiles, I identified fish that were under more stable conditions and resident within the estuaries, and others that showed large variations in environmental conditions, with potential movements outside of estuarine environments, reflecting the species' ability to exhibit partial migration. I found that the ratio of resident and migratory life histories varied among estuaries of different sizes and features, with residency found more often in larger intermittently closed systems and movement more apparent in open systems and smaller intermittently closed systems, highlighting the importance of considering management actions that extend across the salinity gradient and beyond estuaries for select populations.

In this thesis, I detected high structuring of *A. butcheri* populations across its entire distribution range with limited connectivity across both a continental-scale and local-scale. The use of natural tags provided insight into the population dynamics of this estuarine fish and was successful in delineating stocks and highlighting the unique characteristics and attributes of each population, as well as the vulnerability of this species to climate change. Overall, these findings strengthen the link between biological populations and static management boundaries and stress the importance of location-specific fisheries management, with specialised stock assessments of regions and target estuaries recommended.

Publications and contributions

Chapter 2: Strong philopatry in an estuarine-dependent fish

Authors: Sarakinis, KS, Reis-Santos, P, Donnellan, SC, Ye, Q, Earl, J, Gillanders, BM. Status: Published in *Ecology and Evolution* (vol. 14, e10989. doi:10.1002/ ece3.10989).

Chapter 3: Combining natural markers to investigate fish population structure and connectivity

Authors: Sarakinis, KS, Reis-Santos, P, Ye, Q, Earl, J, Gillanders, BM. Status: Submitted for publication in *Estuarine, Coastal and Shelf Science* and is *under review*.

Chapter 4: ENSO effects on growth of an estuarine fish

Authors: Sarakinis, KS, Reis-Santos, P, Morrongiello, J, Ye, Q, Earl, J, Gillanders, BM. Status: Written as a scientific paper for submission to a peer-reviewed journal.

Chapter 5: Fish otolith chemistry reveals varying movement patterns across estuaries Authors: Sarakinis, KS, Reis-Santos, P, Ye, Q, Earl, J, Gillanders, BM. Status: Written as a scientific paper for submission to a peer-reviewed journal.

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CHAPTER 1

General introduction

1.1 Population dynamics

Fish are essential components of a healthy and functioning aquatic ecosystem and drive a billiondollar global fishing industry, generating important socio-economic and cultural values. However, overexploitation, extreme weather events, and climate change are rapidly changing our environments and have led to species' habitat range shifts and increasing cases of local extinction (Nikolaou and Katsanevakis 2023; Pecl *et al.* 2014; Booth *et al.*, 2011). Moreover, increasing anthropogenic and climate stressors can alter ecosystems and their biodiversity, impacting population resilience while increasing the risk of population collapse (Martino *et al.*, 2019; Frank *et al.*, 2016; Ong *et al.*, 2016; Pinsky and Byler 2015).

Understanding population dynamics, including changes in structure, movement, connectivity, and productivity (e.g., growth), is crucial for assessing and sustainably managing commercially and recreationally important species. In particular, connectivity plays a fundamental part in population dynamics, linking geographically separate populations through the exchange of genes and organisms (larvae, juveniles, and adults) (Secor 2015; Cowen *et al.*, 2007). Furthermore, the structure of populations, comprised of various attributes including size, age, sex, genetics, and spatial distribution (Sbordoni *et al.*, 2012; Lowe and Allendorf 2010) is important to understand in order to assess change over time and why this occurred (e.g., interannual recruitment variability, removal of specific size classes by fishing).

1.2 Management implications

For managers of exploited fish populations, a key challenge is to separate the impacts of fishing and other processes that influence the size and demographics of populations. This requires a comprehensive understanding of the population dynamics of individual species, including the spatial scales over which key demographic processes operate (e.g., egg and larval dispersal, recruitment, movement of individuals). This information is also fundamental for delineating biological stock structure and determining appropriate spatial scales for management and scientific assessment (Cadrin *et al.*, 2023). Fisheries are often managed at regional or jurisdictional scales, and the pre-established static boundaries of such management units sometimes do not align with those of the biological population(s), which may impact the effectiveness of management strategies (Berger *et al.*, 2021; Reiss *et al.*, 2009; Coetzee *et al.*, 2008). Determining population structure and understanding how this can be influenced by animal movements and connectivity between populations is required to determine the appropriate spatial scale for scientific assessment and enable the development of sustainable management strategies (Avigliano *et al.* 2017; Walters and Martell 2004). Such

information is also necessary to foster and reliably evaluate cross-jurisdictional or transnational management efforts and can be optimised using a range of tagging techniques.

1.3 Tagging techniques

Tags that are artificially applied to or naturally occurring in animals can provide a suite of valuable information on population dynamics. For example, artificial tags can be used to reveal the movement patterns of fish (e.g., acoustic telemetry) (Williams *et al.*, 2017; Gannon *et al.*, 2015; March *et al.* 2011), as well as the physical and biological characteristics of the habitats they occupy (e.g., archival tags) (Goetz *et al.*, 2018; Afonso *et al.* 2008; Block *et al.*, 2001). These applied approaches aid in understanding the spatial scale and habitat utilisation of fish populations by providing high-quality data, although are temporally limited to the date of tag deployment and require individual handling of fish, with potential impacts on behaviour, growth, or survival (Jepsen *et al.*, 2015; Calò *et al.* 2013).

Alternatively, natural tags (i.e., markers) have the benefit of encoding information throughout an individual's life history from birth to capture, which can be harnessed through various approaches such as chemistry (Morales-Nin et al., 2022; Sarakinis et al., 2022), morphometry (Khan et al., 2022; Dwivedi 2022), genetics (Amoutchi et al., 2023; Luo et al., 2022), or identifying unique external markings (Magson et al., 2022; Castillo et al., 2018). In particular, the hard parts of animals are commonly analysed to identify location-specific signatures, including vertebrae in elasmobranchs (Frazier et al., 2023; McMillan et al., 2018), shells in molluscs (Dong et al., 2018; Nielsen et al., 2008), and otoliths (i.e., ear stones) in fish (Reis-Santos et al., 2023; Campana 1999). Spatial and temporal variability in marker signatures can characterise populations and resolve fish movement of individuals by reflecting signatures of previously occupied environments, as well as elucidate the degree of population connectivity across a species' distribution (Reis-Santos et al., 2023; Elsdon et al. 2008). Each marker has its strengths and limitations, reflecting different biological, environmental, or genetic conditions and functioning at different spatiotemporal resolutions (Tanner et al., 2016). Combining tagging techniques allows the utilisation of information from different sources and can mitigate marker-specific limitations while potentially strengthening estimates of population connectivity. However, our understanding of the best approaches and the power of such a 'multimarker' approach for assessing population connectivity is limited. Studies have previously integrated molecular and non-molecular tagging techniques (e.g., chemistry and morphometry), given they function effectively at different spatiotemporal ranges (Izzo et al., 2017b; Abaunza et al., 2008). For example, molecular approaches can provide information beyond an individual's life history (Reis-Santos et al., 2018; Tanner et al., 2016).

1.4 Population genetics

Genetic markers function on a generational to evolutionary timescale where genetic variation and gene flow (i.e., connectivity) among populations can be investigated (Garvin *et al.*, 2010; Morin *et al.*, 2004; Reis-Santos *et al.*, 2018). Nuclear DNA and mitochondrial DNA markers are commonly used for inferring population structure. Single Nucleotide Polymorphisms (SNPs) are powerful nuclear DNA markers, abundant and widely distributed across genomes (1000s to 100,000s of markers), and are effective at detecting population structure and connectivity in marine and freshwater fishes with high levels of dispersal, fish movement and gene flow across broad spatial scales (Bernatchez *et al.*, 2017; Hall and Beissenger 2014; Anderson and Garza 2006; Biesack *et al.*, 2020; Attard *et al.*, 2017). While only a few migrants per generation can decrease genetic variation between populations and increase connectivity to form larger populations (Wang 2004; Mills and Allendorf 1996), decreased gene flow reflected by high genetic variation can reflect reproductive isolation among populations (Secor 2015), and subsequently influence the appropriate spatial scale for management. Although a powerful tagging technique, genetic markers function effectively beyond the ecological timescales (i.e., an individual's life history) that can be investigated when harnessing non-molecular tags.

1.5 Fish otoliths

Otoliths are polycrystalline structures located in the inner ear of teleost (ray-finned) fish that provide valuable information relating to a fish's life history, such as growth rate, individual movement, habitat use, and natal origin (Rogers *et al.*, 2019; Morrongiello and Thresher 2015; Elsdon *et al.*, 2008). This archival hard part continuously accretes new material over time to form daily and annual increments, thereby reflecting age and growth (Reis-Santos *et al.*, 2023; Campana 2001; Panella 1971). Otolith accretion also incorporates minor and trace elements and isotopes found in the ambient water to form metabolically inert chemical signatures that reflect habitat use throughout an individual's life history, such as the location of birth (otolith core) to the location of capture (otolith edge) (Reis-Santos *et al.*, 2023; Campana *et al.*, 2023; Campana *et al.*, 2017a). However, this accretion process can also be physiologically influenced, which can dampen these environmental signals (Grammer *et al.*, 2017; Sturrock *et al.*, 2015). The shape of otoliths can also provide location-specific information, with shape variations within species occurring among populations, influenced by environmental and genetic factors (Smoliński *et al.*, 2020; Moreira *et al.*, 2019; Campana and Casselman 1993).

The rate of otolith accretion (i.e., size of increment widths) can be assessed to determine variability in growth among populations and growth responses to changing environmental conditions (Rountrey *et al.* 2014; Morrongiello *et al.* 2012; Stock *et al.* 2011). This otolith-based technique can also predict future growth responses of fish to extrinsic effects, as well as evaluate their vulnerability

and future resilience (e.g., decreased fish growth with increasing drought/flooding). In particular, otolith growth can be used to assess correlations in growth dynamics among populations and evaluate the occurrence of 'spatial synchrony', a phenomenon that can impact long-term resilience and vulnerability to stochastic events (Campana *et al.*, 2022; Morrongiello *et al.*, 2021; Tanner *et al.*, 2020).

1.6 An Australian sparid case study

The black bream Acanthopagrus butcheri (Munro, 1949) is a long-lived and slow-growing species distributed across southern Australia, from Western Australia to New South Wales (Izzo et al., 2017a; Norriss et al., 2002). Acanthopagrus butcheri inhabit estuaries, coastal waters and the lower sections of rivers, reflecting its ability to tolerate dramatic shifts in water salinity and temperature, although is considered an estuarine-dependent species as it requires estuaries to complete its life cycle (Doubleday et al., 2015; Sakabe et al., 2010; Partridge and Jenkins 2002). Estuaries are complex, highly dynamic, and biologically productive aquatic environments located where freshwater from land mixes with saline waters of the ocean. They provide important habitat for key life history stages of A. butcheri, including as breeding and nursery grounds. Movement of individuals across these estuarine systems can vary within and among locations, with the coexistence of both migratory and resident life cycles known to occur within the same A. butcheri population (i.e., partial migration; Tracey et al., 2020; Gillanders et al., 2015). However, whether migratory A. butcheri that leave their estuary of natal origin, move to other estuaries, and/or return is not known. Our understanding of their movement across salinity gradients within estuaries, and among marine, estuarine, and freshwater environments, as well as the proportions of migratory and resident individuals among estuaries, is limited but important to understanding the degree of population connectivity across the species' distribution.

Throughout its distribution range across the Australian continent, *A. butcheri* inhabits estuaries with highly variable environmental conditions that are influenced by seasonal variations in rainfall and runoff, and extreme weather events (Jaffrés *et al.*, 2022; Wetz and Yoskowitz 2013; Norriss *et al.*, 2002), with sub-tropical regions prone to flooding and dry Mediterranean type climate regions often affected by droughts. Changing environmental conditions may impact the future resilience of this species, with an ecological risk assessment suggesting that *A. butcheri* was highly sensitive to the impacts of climate change (i.e., associated changes to water flow, temperature, and salinity), and predicted to undergo a future range contraction (Pecl *et al.*, 2014). Little is known of the long-term effects of such environmental impacts across this species' distribution nor if these are driving environmentally induced synchronised growth along a broad longitudinal and latitudinal

gradient. Moreover, regional-scale, climatic events that pose risks in amplifying such environmental effects include the El Niño Southern Oscillation (ENSO), an interannual climatic phenomenon that occurs in the tropical Pacific and oscillates between a warm phase (El Niño) and cool phase (La Niña) (Lovelock *et al.*, 2017; Jiménez-Muñoz *et al.*, 2016; Meuser *et al.*, 2013). Understanding of how local-scale and regional-scale effects interplay and influence this species' growth, and how these impacts may vary across its distribution, is limited.

Acanthopagrus butcheri supports important commercial and recreational fisheries across southern Australia, with current management of these fisheries mostly applied at a management unit scale (i.e., fisheries-specific) (SAFS 2020). However, the extent of the biological stocks that occur within management units, as well as the demographic processes that operate at local scales and potentially across management boundaries are poorly understood across the continent. For example, *A. butcheri* stocks that support geographically adjacent fisheries in South Australia, the State-wide Marine Scalefish Fishery and the Lakes and Coorong Fishery (which share a management boundary) are currently classified as 'sustainable' (Smart *et al.*, 2023) and 'depleted' (Earl 2023), respectively. However, our understanding of the structure and spatial extent of the biological population(s) that support these fisheries is limited, which limits the capacity to effectively evaluate whether the current management strategies are appropriate (Earl 2023; Smart *et al.*, 2023; Elsdon and Gillanders 2006).

Thesis aims and scope

This thesis investigates population dynamics and connectivity of A. butcheri across southern Australian estuaries, including small and large intermittently closed and open systems, using a range of natural tagging techniques. These data provide information that facilitate our understanding of this estuarine species' structuring, growth, movement and habitat use across its distribution, as well as the implications for the management of exploited populations. The thesis is organised into four data chapters (Chapters 2 to 5), which are written as scientific papers. Hence, each chapter has the structure of a stand-alone study with its own targeted introduction and discussion that also draw back on findings in earlier chapters (Fig. 1.1). First, I investigated the genetic structure of A. butcheri populations spanning the species' entire distribution range using next-generation sequencing techniques to determine the degree of gene flow (i.e., connectivity) (Chapter 2). The next chapter combined molecular and non-molecular natural tagging techniques to determine the effectiveness of each marker for resolving population structure, both independently and through integration (Chapter 3). Following the assessment of population structure and connectivity, I then assessed the variability in the growth dynamics of A. butcheri to past and present environmental conditions (Chapter 4). Lastly, I investigated A. butcheri movement patterns within estuaries and whether this varied across southern Australia (Chapter 5). Chapter 6 synthesises my main findings, the subsequent implications for fisheries management, and directions for future research.





An overview of each chapter and its objectives is detailed below.

Chapter 1 - General introduction

This chapter provides an overview of the importance of understanding the population dynamics of fish species for their sustainable management, and the techniques that can be used to obtain this information. It also provides a brief synthesis of information on the distribution and life history of the species I used as a case study *Acanthopagrus butcheri*, including its strong association with estuaries.

Chapter 2 - Strong philopatry in an estuarine-dependent fish

Objective: Investigate the genetic structure of *A. butcheri* populations across southern Australian estuaries and determine the degree of gene flow present.

In this chapter, I collected over 400 *A. butcheri* samples from 25 estuaries across the species' entire distribution range. I investigated the genetic differentiation among estuaries, applying a genome-wide sampling of 33,493 Single-Nucleotide Polymorphism (SNP) markers generated from next-generation sequencing. Local-scale gene flow was also investigated across south-eastern Australia, where sampling of estuaries was geographically comprehensive.

This chapter has been accepted for publication in *Ecology and Evolution* (vol. 14, e10989, doi:10.1002/ ece3.10989).

Chapter 3 - Combining natural markers to investigate fish population structure and connectivity

Objective: Assess the integrative power of multiple tagging techniques for determining structure and connectivity in *A. butcheri*.

In this chapter, I assessed the population structure and connectivity of *A. butcheri* across southeastern Australian estuaries using a range of natural tagging techniques that function at different ecological and evolutionary timescales. Markers included otolith elemental composition, otolith isotopic composition, otolith shape, and genetics. Overall, I assessed the discriminatory power and effectiveness of each marker at resolving population structure, both independently and through integrative techniques.

This chapter has been submitted and is under review in Estuarine, Coastal and Shelf Science.

Chapter 4 - ENSO effects on growth of an estuarine fish

Objective: Assess the variability of *A. butcheri* growth across south-eastern Australia and their growth response to past and present environmental conditions.

In this chapter, I analysed 4,792 *A. butcheri* otoliths from historical collections across south-eastern Australia to assess the variability of growth among populations. I collected growth data from otolith increment measurements of each fish (31,006 measurements spanning from 1981 to 2016) and used a mixed effects modelling approach that assessed both local-scale effects (water flow and temperature) and regional-scale effects (El Niño Southern Oscillation, ENSO) on growth. I then assessed possible spatial synchrony in growth among populations.

This chapter has been written as a scientific paper for submission to a peer-reviewed journal.

Chapter 5 - Fish otolith chemistry reveals varying movement patterns across estuaries

Objective: Investigate movement patterns of *A. butcheri* and their variability within and among estuaries.

In this chapter, I sampled over 200 *A. butcheri* from eight estuaries, including large open systems and small intermittently closed systems across southern Australia. Using otolith chemical signals that spanned entire life histories from otolith core to otolith edge, I interpreted fish movement and grouped fish into similar patterns within each estuary using cluster analyses. Furthermore, I characterised the ratio of different movement patterns and how this varied among estuaries.

This chapter has been written as a scientific paper for submission to a peer-reviewed journal.

Chapter 6 - General Discussion

This chapter provides a broad, integrated discussion and synthesis of the key results of this thesis, and their subsequent implications for management, as well as options for future research directions.

References

- Abaunza, P, Murta, AG, Campbell, N, Cimmaruta, R, Comesaña, AS, Dahle, G, García Santamaría, MT, Gordo, LS, Iversen, SA, MacKenzie, K, Magoulas, A, Mattiucci, S, Molloy, J, Nascetti, G, Pinto, AL, Quinta, R, Ramos, P, Sanjuan, A, Santos, AT, Stransky, C, Zimmermann, C (2008) Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. *Fisheries Research* 89, 196-209. doi:10.1016/j.fishres.2007.09.022
- Afonso, P, Fontes, J, N. Holland, K, Santos, R (2008) Social status determines behaviour and habitat usage in a temperate parrotfish: implications for Marine Reserve design. *Marine Ecology Progress Series* 359, 215-227. doi:10.3354/meps07272
- Amoutchi, AI, Kersten, P, Vogt, A, Kohlmann, K, Kouamelan, EP, Mehner, T (2023) Population genetics of the African snakehead fish *Parachanna obscura* along West Africa's water networks: Implications for sustainable management and conservation. *Ecology and Evolution* 13, e9724. doi:10.1002/ece3.9724
- Anderson, EC, Garza, JC (2006) The power of single-nucleotide polymorphisms for large-scale parentage inference. *Genetics* 172, 2567-82. doi:10.1534/genetics.105.048074
- Attard, CRM, Brauer, CJ, Sandoval-Castillo, J, Faulks, LK, Unmack, PJ, Gilligan, DM, Beheregaray, LB (2018) Ecological disturbance influences adaptive divergence despite high gene flow in golden perch (*Macquaria ambigua*): Implications for management and resilience to climate change. *Molecular Ecology* 27, 196-215. doi:10.1111/mec.14438
- Avigliano, E, Leisen, M, Romero, R, Carvalho, B, Velasco, G, Vianna, M, Barra, F, Volpedo, AV (2017) Fluvio-marine travelers from South America: Cyclic amphidromy and freshwater residency, typical behaviors in *Genidens barbus* inferred by otolith chemistry. *Fisheries Research* 193, 184-194. doi:10.1016/j.fishres.2017.04.011
- Berger, AM, Deroba, JJ, Bosley, KM, Goethel, DR, Langseth, BJ, Schueller, AM, Hanselman, DH, Bartolino, V (2021) Incoherent dimensionality in fisheries management: consequences of misaligned stock assessment and population boundaries. *ICES Journal of Marine Science* 78, 155-171. doi:10.1093/icesjms/fsaa203
- Bernatchez, L, Wellenreuther, M, Araneda, C, Ashton, DT, Barth, JMI, Beacham, TD, Maes, GE, Martinsohn, JT, Miller, KM, Naish, KA, Ovenden, JR, Primmer, CR, Young Suk, H, Therkildsen, NO, Withler, RE (2017) Harnessing the Power of Genomics to Secure the Future of Seafood. *Trends in Ecology & Evolution* 32, 665-680. doi:10.1016/j.tree.2017.06.010
- Biesack, EE, Dang, BT, Ackiss, AS, Bird, CE, Chheng, P, Phounvisouk, L, Truong, OT, Carpenter, KE (2020) Evidence for population genetic structure in two exploited Mekong River fishes across a natural riverine barrier. *Journal of Fish Biology* 97, 696-707. doi:10.1111/jfb.14424
- Block, BA, Dewar, H, Blackwell, SB, Williams, TD, Prince, ED, Farwell, CJ, Boustany, A, Teo, SLH, Seitz, A, Walli, A, Fudge, D (2001) Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* 293, 1310-1314. doi:DOI 10.1126/science.1061197
- Booth, DJ, Bond, N, Macreadie, P (2011) Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research* 62, 1027-1042. doi:10.1071/Mf10270
- Cadrin, SX, Friedland, KD (1999) The utility of image processing techniques for morphometric analysis and stock identification. *Fisheries Research* **43**, 129-139. doi:10.1016/S0165-7836(99)00070-3
- Calò, A, Félix-Hackradt, F, Garcia, J, Hackradt, CW, Rocklin, D, Treviño Otón, J, García-Charton, J, García Charton, A (2013) A review of methods to assess connectivity and dispersal between fish populations in the Mediterranean Sea. In 'Advances in Oceonography and Limnology.' Vol. 4 pp. 150-175.
- Campana, SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* 188, 263-297. doi:DOI 10.3354/meps188263
- Campana, SE, Casselman, JM (1993) Stock discrimination using otolith shape-analysis. Canadian

Journal of Fisheries and Aquatic Sciences 50, 1062-1083. doi:DOI 10.1139/f93-123

- Campana, SE, Smolinski, S, Black, BA, Morrongiello, JR, Alexandroff, SJ, Andersson, C, Bogstad, B, Butler, PG, Denechaud, C, Frank, DC, Geffen, AJ, Godiksen, JA, Gronkjaer, P, Hjorleifsson, E, Jonsdottir, IG, Meekan, M, Mette, M, Tanner, SE, van der Sleen, P, von Leesen, G (2022)
 Growth portfolios buffer climate-linked environmental change in marine systems. *Ecology* 104, e3918. doi:10.1002/ecy.3918
- Campana, SE, Thorrold, SR (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences* 58, 30-38. doi:10.1139/f00-177
- Castillo, GC, Sandford, ME, Hung, TC, Tigan, G, Lindberg, JC, Yang, WR, Van Nieuwenhuyse, EE (2018) Using Natural Marks to Identify Individual Cultured Adult Delta Smelt. *North American Journal of Fisheries Management* 38, 698-705. doi:10.1002/nafm.10066
- Coetzee, JC, van der Lingen, CD, Hutchings, L, Fairweather, TP (2008) Has the fishery contributed to a major shift in the distribution of South African sardine? *ICES Journal of Marine Science* 65, 1676-1688. doi:10.1093/icesjms/fsn184
- Cowen, R, Gawarkiewicz, G, Pineda, J, Thorrold, S, Werner, F (2007) Population Connectivity in Marine Systems: An Overview. *Oceanography* **20**, 14-21. doi:10.5670/oceanog.2007.26
- Dong, ZG, Chen, YH, Ge, HX, Li, XY, Wu, HL, Wang, CH, Hu, Z, Wu, YJ, Fu, GH, Lu, JK, Che, H (2018) Response of growth and development of the Pacific oyster (*Crassostrea gigas*) to thermal discharge from a nuclear power plant. *BMC Ecology* 18, 31. doi:10.1186/s12898-018-0191-y
- Doubleday, ZA, Izzo, C, Haddy, JA, Lyle, JM, Ye, Q, Gillanders, BM (2015) Long-term patterns in estuarine fish growth across two climatically divergent regions. *Oecologia* 179, 1079-90. doi:10.1007/s00442-015-3411-6
- Dwivedi, AK (2022) Detection of body shape variations in vulnerable snow trout, *Schizothorax richardsonii* (Gray, 1832) from rivers of the Indian Himalayan Region. *Limnologica* 97,doi:10.1016/j.limno.2022.126025
- Elsdon, TS, Gillanders, BM (2006) Identifying migratory contingents of fish by combining otolith Sr:Ca with temporal collections of ambient Sr:Ca concentrations. *Journal of Fish Biology* 69, 643-657. doi:10.1111/j.1095-8649.2006.01136.x
- Elsdon, TS, Wells, BK, Campana, SE, Gillanders, BM, Jones, CM, Limburg, KE, Secor, DH, Thorrold, SR, Walther, BD (2008) Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. *Oceanography and Marine Biology: An Annual Review* 46, 297-330. doi:DOI 10.1201/9781420065756.ch7
- Frank, KT, Petrie, B, Leggett, WC, Boyce, DG (2016) Large scale, synchronous variability of marine fish populations driven by commercial exploitation. *Proceedings of the National Academy of Sciences of the United States of America* 113, 8248-53. doi:10.1073/pnas.1602325113
- Frazier, BS, Vinyard, EA, Fields, AT, Driggers, WB, Grubbs, RD, Adams, DH, Drymon, JM, Gardiner, JM, Hendon, JM, Hoffmayer, E, Hueter, RE, Wells, RJD, Wiley, TR, Portnoy, DS (2023) Age, growth and maturity of the bonnethead *Sphyrna tiburo* in the U.S. Gulf of Mexico. *Environmental Biology of Fishes* 106, 1597-1617. doi:10.1007/s10641-023-01439-5
- Gannon, R, Payne, NL, Suthers, IM, Gray, CA, van der Meulen, DE, Taylor, MD (2015) Fine-scale movements, site fidelity and habitat use of an estuarine dependent sparid. *Environmental Biology of Fishes* 98, 1599-1608. doi:10.1007/s10641-015-0385-5
- Garvin, MR, Saitoh, K, Gharrett, AJ (2010) Application of single nucleotide polymorphisms to nonmodel species: a technical review. *Molecular Ecology Resources* 10, 915-34. doi:10.1111/j.1755-0998.2010.02891.x
- Gillanders, BM, Izzo, C, Doubleday, ZA, Ye, Q (2015) Partial migration: growth varies between resident and migratory fish. *Biology Letters* 11, doi:10.1098/rsbl.2014.0850
- Goetz, FW, Jasonowicz, AJ, Roberts, SB (2018) What goes up must come down: Diel vertical migration in the deep-water sablefish (*Anoplopoma fimbria*) revealed by pop-up satellite archival tags. *Fisheries Oceanography* 27, 127-142. doi:10.1111/fog.12239

- Grammer, GL, Morrongiello, JR, Izzo, C, Hawthorne, PJ, Middleton, JF, Gillanders, BM (2017) Coupling biogeochemical tracers with fish growth reveals physiological and environmental controls on otolith chemistry. *Ecological Monographs* 87, 487-507. doi:10.1002/ecm.1264
- Hall, LA, Beissinger, SR (2014) A practical toolbox for design and analysis of landscape genetics studies. *Landscape Ecology* 29, 1487-1504. doi:10.1007/s10980-014-0082-3
- Izzo, C, Doubleday, ZA, Grammer, GL, Disspain, MCF, Ye, QF, Gillanders, BM (2017a) Seasonally resolved environmental reconstructions using fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 74, 23-31. doi:10.1139/cjfas-2016-0055
- Izzo, C, Ward, TM, Ivey, AR, Suthers, IM, Stewart, J, Sexton, SC, Gillanders, BM (2017b) Integrated approach to determining stock structure: implications for fisheries management of sardine, *Sardinops sagax*, in Australian waters. *Reviews in Fish Biology and Fisheries* 27, 267-284. doi:10.1007/s11160-017-9468-z
- Jaffrés, JBD, Cuff, B, Cuff, C, Knott, M, Rasmussen, C (2022) Hydrological characteristics of Australia: national catchment classification and regional relationships. *Journal of Hydrology* 612,doi:10.1016/j.jhydrol.2022.127969
- Jepsen, N, Thorstad, EB, Havn, T, Lucas, MC (2015) The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. *Animal Biotelemetry* 3. doi:10.1186/s40317-015-0086-z
- Jiménez-Muñoz, JC, Mattar, C, Barichivich, J, Santamaria-Artigas, A, Takahashi, K, Malhi, Y, Sobrino, JA, Schrier, G (2016) Record-breaking warming and extreme drought in the Amazon rainforest during the course of El Nino 2015-2016. *Scientific Reports* 6, 33130. doi:10.1038/srep33130
- Khan, U, Bal, H, Battal, ZS, Seyhan, K (2022) Using otolith and body shape to discriminate between stocks of European anchovy (Engraulidae: *Engraulis encrasicolus*) from the Aegean, Marmara and Black Seas. *Journal of Fish Biology* 101, 1452-1465. doi:10.1111/jfb.15216
- Lovelock, CE, Feller, IC, Reef, R, Hickey, S, Ball, MC (2017) Mangrove dieback during fluctuating sea levels. *Scientific Reports* 7, 1680. doi:10.1038/s41598-017-01927-6
- Lowe, WH, Allendorf, FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology* 19, 3038-51. doi:10.1111/j.1365-294X.2010.04688.x
- Luo, YT, Fang, DA, Zhou, YF, Xu, DP, Peng, YX, Zhang, MY, Mao, CC, Tang, XM, Xu, J, You, Y (2022) Genetic diversity, habitat relevance and conservation strategies of the silver carp in the Yangtze River by simple sequence repeat. *Frontiers in Ecology and Evolution* 10,doi:10.3389/fevo.2022.850183
- Magson, K, Monacella, E, Scott, C, Buffat, N, Arunrugstichai, S, Chuangcharoendee, M, Pierce, SJ, Holmberg, J, Araujo, G (2022) Citizen science reveals the population structure and seasonal presence of whale sharks in the Gulf of Thailand. *Journal of Fish Biology* 101, 540-549. doi:10.1111/jfb.15121
- March, D, Alos, J, Grau, A, Palmer, M (2011) Short-term residence and movement patterns of the annular seabream *Diplodus annularis* in a temperate marine reserve. *Estuarine Coastal and Shelf Science* 92, 581-587. doi:10.1016/j.ecss.2011.02.015
- Martino, JC, Fowler, AJ, Doubleday, ZA, Grammer, GL, Gillanders, BM (2019) Using otolith chronologies to understand long-term trends and extrinsic drivers of growth in fisheries. *Ecosphere* 10, e02553. doi:10.1002/ecs2.2553
- McMillan, MN, Huveneers, C, Semmens, JM, Gillanders, BM (2018) Natural tags reveal populations of conservation dependent school shark use different pupping areas. *Marine Ecology Progress Series* 599, 147-156. doi:10.3354/meps12626
- Meuser, E, Mooers, AØ, Cleary, DFR (2013) El Niño and biodiversity. In 'Encyclopedia of Biodiversity.' (Ed. SA Levin.) pp. 155-163. (Academic Press: Waltham)
- Mills, LS, Allendorf, FW (1996) The One-Migrant-per-Generation Rule in Conservation and Management. *Conservation Biology* 10, 1509-1518. doi:10.1046/j.1523-1739.1996.10061509.x

Morales-Nin, B, Perez-Mayol, S, MacKenzie, K, Catalan, IA, Palmer, M, Kersaudy, T, Mahe, K (2022) European hake (*Merluccius merluccius*) stock structure in the Mediterranean as assessed by otolith shape and microchemistry. *Fisheries Research* 254,doi:10.1016/j.fishres.2022.106419

- Moreira, C, Froufe, E, Vaz-Pires, P, Correia, AT (2019) Otolith shape analysis as a tool to infer the population structure of the blue jack mackerel, *Trachurus picturatus*, in the NE Atlantic. *Fisheries Research* 209, 40-48. doi:10.1016/j.fishres.2018.09.010
- Morin, PA, Luikart, G, Wayne, RK, Grp, SW (2004) SNPs in ecology, evolution and conservation. *Trends in Ecology & Evolution* 19, 208-216. doi:10.1016/j.tree.2004.01.009
- Morrongiello, JR, Horn, PL, C, OM, Sutton, PJH (2021) Synergistic effects of harvest and climate drive synchronous somatic growth within key New Zealand fisheries. *Global Change Biology* 27, 1470-1484. doi:10.1111/gcb.15490
- Morrongiello, JR, Thresher, RE (2015) A statistical framework to explore ontogenetic growth variation among individuals and populations: a marine fish example. *Ecological Monographs* 85, 93-115. doi:10.1890/13-2355.1
- Morrongiello, JR, Thresher, RE, Smith, DC (2012) Aquatic biochronologies and climate change. *Nature Climate Change* 2, 849-857. doi:10.1038/Nclimate1616
- Nielsen, JK, Helama, S, Schöne, B (2008) Shell growth history of geoduck clam (*Panopea generosa*) in parry passage, british columbia, canada: temporal variation in annuli and the pacific decadal oscillation. *Journal of Oceanography* 64, 951-960. doi:DOI 10.1007/s10872-008-0078-1
- Nikolaou, A, Katsanevakis, S (2023) Marine extinctions and their drivers. *Regional Environmental Change* 23,doi:10.1007/s10113-023-02081-8
- Norriss, JV, Tregonning, JE, Lenanton, RCJ, Sarre, GA, 2002. Biological synopsis of the black bream, *Acanthopagrus butcheri* (Munro) (Teleostei: Sparidae) in Western Australia with reference to information from other southern states. Department of Fisheries, Perth, Western Australia.
- Ong, JJ, Rountrey, AN, Zinke, J, Meeuwig, JJ, Grierson, PF, O'Donnell, AJ, Newman, SJ, Lough, JM, Trougan, M, Meekan, MG (2016) Evidence for climate-driven synchrony of marine and terrestrial ecosystems in northwest Australia. *Global Change Biology* 22, 2776-86. doi:10.1111/gcb.13239
- Panella, G (1971) Fish otoliths: daily growth layers and periodical patterns. *Science* 173, 1124-7. doi:10.1126/science.173.4002.1124
- Partridge, GJ, Jenkins, GI (2002) The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). *Aquaculture* 210, 219-230. doi:10.1016/S0044-8486(01)00817-1
- Pecl, GT, Ward, TM, Doubleday, ZA, Clarke, S, Day, J, Dixon, C, Frusher, S, Gibbs, P, Hobday, AJ, Hutchinson, N, Jennings, S, Jones, K, Li, XX, Spooner, D, Stoklosa, R (2014) Rapid assessment of fisheries species sensitivity to climate change. *Climatic Change* 127, 505-520. doi:10.1007/s10584-014-1284-z
- Pinsky, ML, Byler, D (2015) Fishing, fast growth and climate variability increase the risk of collapse. *Proceedings of the Royal Society B: Biological Sciences* 282, 20151053. doi:10.1098/rspb.2015.1053
- Reiss, H, Hoarau, G, Dickey-Collas, M, Wolff, WJ (2009) Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries* 10, 361-395. doi:10.1111/j.1467-2979.2008.00324.x
- Reis-Santos, P, Gillanders, BM, Sturrock, AM, Izzo, C, Oxman, DS, Lueders-Dumont, JA, Hüssy, K, Tanner, SE, Rogers, T, Doubleday, ZA, Andrews, AH, Trueman, C, Brophy, D, Thiem, JD, Baumgartner, LJ, Willmes, M, Chung, M-T, Charapata, P, Johnson, RC, Trumble, S, Heimbrand, Y, Limburg, KE, Walther, BD (2023) Reading the biomineralized book of life: expanding otolith biogeochemical research and applications for fisheries and ecosystem-based management. *Reviews in Fish Biology and Fisheries* 33, 411-449. doi:10.1007/s11160-022-09720-z
- Reis-Santos, P, Tanner, SE, Aboim, MA, Vasconcelos, RP, Laroche, J, Charrier, G, Perez, M, Presa, P, Gillanders, BM, Cabral, HN (2018) Reconciling differences in natural tags to infer demographic and genetic connectivity in marine fish populations. *Scientific Reports* 8, 10343.

doi:10.1038/s41598-018-28701-6

- Rogers, TA, Fowler, AJ, Steer, MA, Gillanders, BM (2019) Discriminating natal source populations of a temperate marine fish using larval otolith chemistry. *Frontiers in Marine Science* 6,doi:10.3389/fmars.2019.00711
- Rountrey, AN, Coulson, PG, Meeuwig, JJ, Meekan, M (2014) Water temperature and fish growth: otoliths predict growth patterns of a marine fish in a changing climate. *Global Change Biology* 20, 2450-8. doi:10.1111/gcb.12617
- SAFS (2020), 'Black bream' Available at https://fish.gov.au/report/366-Black-Bream-2020 [Accessed 16/01/24]
- Sakabe, R, Lyle, JM (2010) The influence of tidal cycles and freshwater inflow on the distribution and movement of an estuarine resident fish *Acanthopagrus butcheri*. *Journal of Fish Biology* 77, 643-60. doi:10.1111/j.1095-8649.2010.02703.x
- Sarakinis, KG, Taylor, MD, Johnson, DD, Gillanders, BM (2022) Determining population structure and connectivity through otolith chemistry of stout whiting, *Sillago robusta*. *Fisheries Management and Ecology* 29, 760-773. doi:10.1111/fme.12577
- Sbordoni, V, Allegrucci, G, Cesaroni, D (2012) Population Structure. In 'Encyclopedia of Caves.' (Eds WB White, DC Culver.) pp. 608-618. (Academic Press: Amsterdam)
- Secor, DH (2015) 'Migration Ecology of Marine Fishes.' (Johns Hopkins University Press:
- Smoliński, S, Schade, FM, Berg, F (2020) Assessing the performance of statistical classifiers to discriminate fish stocks using fourier analysis of otolith shape. *Canadian Journal of Fisheries and Aquatic Sciences* 77, 674-683. doi:10.1139/cjfas-2019-0251
- Stocks, J, Stewart, J, Gray, CA, West, RJ (2011) Using otolith increment widths to infer spatial, temporal and gender variation in the growth of sand whiting. *Fisheries Management and Ecology* 18, 121-131. doi:10.1111/j.1365-2400.2010.00761.x
- Sturrock, AM, Hunter, E, Milton, JA, Johnson, RC, Waring, CP, Trueman, CN, Leder, E (2015) Quantifying physiological influences on otolith microchemistry. *Methods in Ecology and Evolution* 6, 806-816. doi:10.1111/2041-210x.12381
- Tanner, SE, Giacomello, E, Menezes, GM, Mirasole, A, Neves, J, Sequeira, V, Vasconcelos, RP, Vieira, AR, Morrongiello, JR (2020) Marine regime shifts impact synchrony of deep-sea fish growth in the northeast Atlantic. *Oikos* 129, 1781-1794. doi:10.1111/oik.07332
- Tanner, SE, Reis-Santos, P, Cabral, HN (2016) Otolith chemistry in stock delineation: A brief overview, current challenges and future prospects. *Fisheries Research* 173, 206-213. doi:10.1016/j.fishres.2015.07.019
- Tracey, SR, Hartmann, K, McAllister, J, Lyle, JM (2020) Home range, site fidelity and synchronous migrations of three co-occurring, morphologically distinct estuarine fish species. *Science of The Total Environment* 713, 136629. doi:10.1016/j.scitotenv.2020.136629
- Walters, C, Martell, S (2004) 'Fisheries ecology and management.' (Princeton University Press: Princeton, NJ)
- Wang, JL (2004) Application of the one-migrant-per-generation rule to conservation and management. *Conservation Biology* 18, 332-343. doi:10.1111/j.1523-1739.2004.00440.x
- Wetz, MS, Yoskowitz, DW (2013) An 'extreme' future for estuaries? Effects of extreme climatic events on estuarine water quality and ecology. *Marine Pollution Bulletin* 69, 7-18. doi:10.1016/j.marpolbul.2013.01.020
- Williams, J, Hindell, JS, Jenkins, GP, Tracey, S, Hartmann, K, Swearer, SE (2017) The influence of freshwater flows on two estuarine resident fish species show differential sensitivity to the impacts of drought, flood and climate change. *Environmental Biology of Fishes* **100**, 1121-1137. doi:10.1007/s10641-017-0632-z

CHAPTER 2

Strong philopatry in an estuarine-dependent fish

	Strong philopatry in an estuarine-d	spendent f	ish	
Publication Status	Published	X	Accepted for P	ublication
	Submitted for Publication		Unpublished ar manuscript styl	nd Unsubmitted work written in e
Publication Details	Sarakinis, KG, Reis-Santos, P, Dc Strong philopatry in an estuarine- doi: 10.1002/ece3.10989	nnellan, So lependent	G, Ye, Qifeng, E fish.	arl, J (2024)
Principal Author				
Name of Principal Author (Candidate)	Koster Georgien Sarakinis			
Contribution to the Paper	Conceptualization (Equal); Data c Methodology (Equal); Project adm Writing – original draft (Lead); Wri	uration (Le inistration ing – revie	ead); Formal ana (Lead); Softwar ew & editing (Eq	alysis (Equal); Investigation (Lead); e (Equal); Visualization (Lead); ual).
Overall percentage (%)	70%			
Certification:	This paper reports on original res Research candidature and is not s party that would constrain its inclu	earch I co ubject to a sion in this	nducted during ny obligations o thesis. I am the	the period of my Higher Degree by r contractual agreements with a third e primary author of this paper.
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By signing the Statement of Authorship	o, each author certifies that:			
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Name of Co-Author	Patrick Reis-Santos			
Contribution to the Paper	Conceptualization (Equal); Data c Supervision (Equal); Visualization	uration (Eq (Equal); W	qual); Formal an /riting – review a	alysis (Equal); Investigation (Equal) & editing (Equal).
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Name of Co-Author	Stephen Donnellan			
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2.1 Abstract

Understanding fish movement is critical in determining the spatial scales in which to appropriately manage wild populations. Genetic markers provide a natural tagging approach to assess the degree of gene flow and population connectivity across a species distribution. We investigated the genetic structure of black bream Acanthopagrus butcheri across its entire distribution range in Australia, as well as regional scale gene flow across south-eastern Australia by undertaking a comprehensive analysis of the populations in estuaries across the region. We applied genome-wide sampling of Single-Nucleotide Polymorphism (SNP) markers generated from restriction site-associated DNA sequencing. Genetic structure and potential gene flow was assessed using principal component analyses and admixture analyses (STRUCTURE). Using 33,493 SNPs, we detected broad-scale genetic structuring, with limited gene flow among regional clusters (i.e., Western Australia, South Australia and western Victoria; and eastern Victoria, Tasmania and New South Wales). This is likely the result of unsuitable habitats, strong ocean currents (e.g., the Leeuwin Current and the East Australian Current), large water bodies (e.g., Bass Strait) and known biogeographical provinces across the continent. Local-scale genetic structuring was also identified across the south-eastern Australian estuaries sampled, reflecting that the coexistence of both migratory and resident individuals within populations (i.e., partial migration), and the movement of fish into coastal waters, still results in strong philopatry across the region. Instances of movement among estuaries at this spatial scale were primarily found between adjacent estuaries and were likely attributed to lone migrants utilising inshore coastal currents for movement beyond nearby habitats. Targeting SNP markers in A. butcheri at this continental scale highlighted how neither spatial proximity of estuaries nor black bream's ability to move into coastal waters reflects increased gene flow. Overall, our findings highlight the importance of location-specific management.

Keywords: population structure; *Acanthopagrus butcheri*; single-nucleotide polymorphisms; philopatry; gene flow; Australia

2.2 Introduction

Animal movement that leads to effective reproduction is a critical migratory process that can structure populations and influence gene flow across a species distribution. As only a few migrants per generation can decrease genetic differentiation between populations (Wang 2004; Mills and Allendorf 1996), a change in conditions that leads to an increase in migrants could over time increase gene flow (i.e., connectivity) between two locations to form a single distinct population. Inversely, multiple generation return of populations to a breeding site that leads to reproductive isolation (i.e., philopatry; Secor 2015) can result in decreased gene flow forming multiple, genetically isolated populations. The potential of such animal movement can have major implications on how we manage overexploited or conservation interest species. Yet, whilst many marine species have large-scale distributions, we generally lack an understanding of the structuring and movement patterns of all or large parts of populations, but such information is necessary to reliably evaluate broad-scale changes in ecological conditions and support cross-jurisdictional or transnational management efforts. Overall, historical and contemporary movement among locations, and any resulting gene flow determines the spatial and temporal scales at which management regulations should be implemented.

Genetic markers are natural tags that play a key role in determining population structure and connectivity, providing an alternative, or complement, to artificial tagging techniques such as conventional and telemetry tagging (Cooke and Cowx 2006). While applied tags can be used to reconstruct individual movements, they are restricted temporally to an individual's life span and the date of tag deployment, whereas genetic markers function on a generational to evolutionary timescale documenting gene flow and genetic differences within and among locations and enable investigating the evolutionary factors that drive this variation (Garvin *et al.*, 2010; Morin *et al.*, 2004; Reis-Santos *et al.*, 2018).

Nuclear DNA and mitochondrial DNA markers are commonly used for inferring genetic population structure. With the advent of next-generation sequencing technologies and complexity reduction approaches, genome-wide sampling of nuclear SNPs (Single-Nucleotide Polymorphisms) can now be readily applied to model organisms (i.e., extensively studied) and non-model organisms (Garvin *et al.*, 2010; Aitken *et al.*, 2004). The power of SNP markers lies in their abundance and distribution across genomes (1000s to 100,000s of markers), while also being effective at detecting population structure and connectivity in marine fishes with high levels of dispersal, fish movement and gene flow across broad spatial scales (Bernatchez *et al.*, 2017; Hall and Beissenger 2014; Anderson and Garza 2006). Additionally, the power of an increased number of markers can increase the resolution of genetic structure (Sunde *et al.*, 2020).

The black bream, Acanthopagrus butcheri, is distributed across southern Australia, ranging from Western Australia in the west to New South Wales in the east, including Tasmania (Norriss et al., 2002). It supports important commercial and recreational fisheries across this range. A long-lived and slow-growing species, this sparid matures at approximately 2-4 years of age (28-34 cm total length [TL]) and has a longevity of 32 years (55 cm TL) (Izzo et al., 2017; Ye et al., 2013; Gray 2008). Due to its tolerance of dramatic shifts in water salinity and temperature, A. butcheri can inhabit coastal waters, estuaries, and rivers, although it is regarded as an estuarine-dependent species, requiring estuaries to complete its life cycle (Doubleday et al., 2015; Partridge and Jenkins 2002). Movement out of estuarine systems can vary among locations, with the coexistence of both migratory and resident life cycles known to occur within the same population (i.e., partial migration; Gillanders et al., 2015; Lack 1944). However, we are unsure whether fish are moving among estuaries or returning to their estuary of origin. Given the large geographic distribution, spawning periods can vary among estuaries but typically occur during austral spring and summer (Jenkins et al., 2018). Spawning occurs in the upper reaches of streams feeding estuaries (Sakabe et al., 2011; Williams et al., 2012), with eggs hatching ~36-48 hours after fertilisation followed by a larval duration of approximately 20-30 days, during which they recruit within estuaries (Roberts et al., 2010). Hybridisation is known to occur with Acanthopagrus australis (yellowfin bream) in the species' eastern Australian distribution and is a possible threat to the persistence of southern New South Wales A. butcheri. Genetic swamping has been found to occur in coastal lagoons where only ~5% of fish were A. butcheri, with the remainder either hybrids or A. australis (Roberts and Ayre 2020; Roberts et al., 2009, 2010; Farrington et al., 2000).

Our understanding of the population structure and gene flow of this estuarine-dependent species is limited. Population structure studies on *A. butcheri* have focussed mainly on specific sections of either the western or eastern coast of Australia (Gardner *et al.*, 2013; Roberts *et al.*, 2011, 2010; Chaplin *et al.*, 1998). Only one study has covered the species' distribution range but it focused on hybridisation and interspecific gene flow between *A. butcheri* and *A. australis* (Roberts *et al.*, 2009). Therefore, importantly, characterising the population structure of *A. butcheri* across its distribution can provide valuable information on movement among estuaries spread across a continent that is characterised by highly variable climate, rainfall and runoff patterns, as well as fishing effort. Geographic differences in climates and biomes are likely to play a role in the population structure of *A. butcheri* when comparing the connectivity among large, nearby estuaries in subtropical-temperate south-eastern Australia to smaller, more geographically isolated, and temporarily open systems found across the Mediterranean climate regions of Western Australia and South Australia. The potential influence of large biogeographic breaks may also be evident (Waters *et*
al., 2003; Bennet and Pope 1953), such as the absence of estuaries across the Great Australian Bight separating Western Australia and south-eastern Australian estuaries, and the Bass and Investigator Straits separating Tasmanian and Kangaroo Island estuaries respectively from those on the mainland.

Population structure, gene flow and inter-specific hybridisation research on A. butcheri has targeted a range of genetic markers, including microsatellites (Roberts et al., 2010, 2011; Gardner et al., 2014; Yap et al., 2000), allozymes (Farrington et al., 2000; Chaplin et al., 1998), and a combination of both nuclear DNA and mitochondrial DNA (Roberts et al., 2009; Burridge et al., 2004; Burridge and Versace 2006). Inference of population structure has varied based on the sample distribution and genetic marker. Allozymes have reflected a panmictic relationship across Victorian estuaries (~800 km sampling distribution), while mitochondrial DNA and microsatellites highlighted a higher degree of genetic structuring across the same region (Farrington et al., 2000; Burridge and Versace 2006; Burridge et al., 2004). Allozyme markers also detected genetically distinct populations along the west coast of Australia (Chaplin et al., 1998). Unique marker characteristics may be why our understanding of A. butcheri genetic structuring is inconsistent, with allozymes generally having lower resolution compared to mitochondrial DNA and microsatellites (Amiteye 2021; Liu and Cordes 2004). A more comprehensive and contemporary approach across the entire distribution range and using genome wide markers is expected to shed light on the population structure and degree of gene flow, together with insights into the impacts of environmental and biogeographical boundaries at both regional and continental scales.

Here we use genome-wide sampling of SNP markers generated from a complexity reduction method – restriction site-associated DNA sequencing, to infer the population structure of *A. butcheri* on both a broad and local scale. Specifically, we harnessed the power of this cost-effective nextgeneration sequencing to investigate (1) how *A. butcheri* is genetically structured across its distribution range, and (2) the degree of gene flow at the local scale in south-eastern Australia where sampling of individual estuaries was geographically comprehensive. We hypothesise that *A. butcheri* would show limited gene flow at a regional scale, reflecting the species' estuarine dependency. Furthermore, we hypothesise that the inconsistent freshwater outflow across southern Australia, along with known biogeographical boundaries will further limit movement of *A. butcheri* at both regional and continental scales.

2.3 Materials and Methods

Field sampling and sample preparation

Samples of *Acanthopagrus butcheri* were collected from estuaries across the species' entire distribution range (i.e., from Western Australia to New South Wales) between March 2020 and January 2022 (Fig. 2.1, Table 2.1). Samples were collected from key estuaries in South Australia and across southern Australia through collaborative efforts with interstate government agencies and angling associations, with rod and line the primary method of capture but also seine and gill netting in permitted locations (The University of Adelaide animal ethics, approval S-2020-069). Whole fish were collected, aiming for 20 fish per estuary, although a non-fatal tail clipping technique was used where community-enforced catch-and-release regulations applied (e.g., Port Lincoln and Onkaparinga River, South Australia). To evaluate potential genetic variation over time, a collection of historical frozen liver samples from Western Australia dating back to 1996 were included in our analyses (Chaplin *et al.*, 1998). Where possible, each fish was measured for total length to the nearest millimetre and weighed to the nearest gram. A sample of soft muscle tissue (~ 1 cm³) and a tail clipping (2-5 cm long) were collected from each fish and individually preserved in 100% ethanol.

Table 2.1 *Acanthopagrus butcheri* capture locations with corresponding Australian state, regional cluster and sub-cluster classifications identified using Principal Component Analyses (PCA), along with collection year and sample sizes.

state	capture location		collection	sample	regional	sub-
			year	size	cluster	cluster
Western Australia (WA)	1	Swan River	2020	19	western	-
	2	Collie River	1996	16		
	3	Margaret River	1996	3		
	4	Walpole-Nornalup	2020	19		
	5	Albany	2020	19		
	6	Pallinup River	1996	15		
	7	Bremer Bay	2020	19		
South Australia (SA)	8	Western River	2020	18	southern	northKI
	9	Middle River	2020	16		
	10	Stun Sail Boom	2020	18		southKI
	11	Harriet River	2020	21		
	12	Eleanor River	2020	18		
	13	Chapman River	2020	9		
	14	Port Lincoln	2021	15		northSA
	15	Tumby Bay	2021	11		
	16	Port River	2021	8		
	17	West Lakes	2021	25		
	18	Onkaparinga River	2021	7		
	19	Coorong	2020-21	21		southSA
	20	Robe	2020-21	20		
Victoria (VIC)	21	Glenelg River	2021	22		westVIC
	22	Hopkins River	2021	21		
	23	Lake Tyers	2021-22	31	eastern	-
	24	Snowy River	2021	4		
Tasmania (TAS)	25	Port Huon	2021	21		
New South Wales (NSW)	26	Swan Lake	2020	23		



Fig. 2.1 Map of South Australia, with an inset of Australia showing all 27 *A. butcheri* and *A. australis* capture locations. Samples were collected from Western Australia (orange), South Australia (blue), Queensland (black), New South Wales (purple), Victoria (green), and Tasmania (yellow). Map of South Australia also highlights Kangaroo Island (KI), Spencer Gulf (SG) and Gulf Saint Vincent (GSV).

SNP genotyping and data processing

Tissue samples were submitted for DNA extraction and DArTseq[™] 1.0 genotyping at Diversity Arrays Technology PL, Canberra, Australian Capital Territory, Australia (medium-density, 1.2 million reads per sample). DArTseq[™] represents a combination of DArT genome complexity reduction methods and next generation sequencing platforms, targeting the predominately active areas in the genome (low copy fragments) containing the most useful information (Kilian *et al.*, 2012). DNA samples were processed in restriction enzyme digestion/ligation reactions using a combination of the *Pstl/Sph*I restriction enzymes. Ligated fragments were PCR amplified as described by Kilian *et al.*, (2012) and Mahony *et al.* (2020) for single end sequencing for 77 cycles on an Illumina Hiseq2500. A matrix of SNP genotypes for each individual was received from DArT as a 1-row binary score for each locus.

The raw SNP data underwent a filtering pipeline similar to Junge *et al.* (2019). Four filters were applied, namely (1) removal of loci with <95% reproducibility, (2) retaining loci with <20% missing data, (3) removing secondary loci (e.g., retaining one of two or more separately recorded SNP loci for the same fragment), and (4) removing monomorphic loci (*DArTr* package in *R*; Gruber *et al.*, 2018). We retained loci with <20% missing data, given the large number of loci, with no individuals removed due to missing data.

To identify potential hybrids that could influence our interpretations of genetic structuring, the closely related species *A. australis* was sampled from the Nerang River in southern Queensland (n = 10, Fig. 2.1, Appendix Table 2.A1), north of the zone of hybridisation with *A. butcheri* (Roberts *et al.*, 2010). Potential hybrids between *A. butcheri* and *A. australis* were identified using a Principal Component Analysis (PCA). This was undertaken for samples from all capture locations to enable the detection of potential expansion of the hybridisation zone via range shifts of *Acanthopagrus spp.* (Pecl *et al.*, 2014). PCA scores were generated from the filtered SNP data and plotted (*adegenet* and *ggplot2* packages in *R*; Jombart 2008; Wickham 2016). Hybrids were identified as falling between the two species clusters, either evenly positioned between (F₁ hybrid) or partially (F₂ or backcrossed hybrid).

With many fisheries-based studies relying on trawling and manual netting procedures to collect samples (Sarakinis *et al.,* 2022; Reis-Santos *et al.,* 2018), assessing relatedness within sample sets is essential to accurately infer population structure and connectivity. There is the possibility that fish caught within an estuary on the same day or from the same net haul are closely related. As a mixture of collection types were used in this study (e.g., net and/or rod, Appendix Table 2.A1), and relatedness can influence the inference of population structure, the assumption of random sampling was validated using an identity-by-descent analysis (*SNPRelate* package in *R*; Zheng *et al.,* 2012). Kinship coefficient values were generated using the KING (robust) method of moment for each possible pairing of *A. butcheri*, both within and among capture locations; with a kinship coefficient of ≥ 0.25 representing a sibling-sibling or parent-offspring relationship (Sun and Dimitromanolakis 2014). Closely related individuals were removed, retaining only a single individual from highly related groups. All analyses were performed in *R* (R Core team 2021).

Population structure

Population structure was investigated with two clustering approaches, PCA and admixture analysis to determine whether *A. butcheri's* distribution comprises isolated breeding populations. Ten PCA scores were generated to determine which combination of PCs identified genetic clusters. Pairwise fixation index (FST) values were also calculated as a genetic distance matrix to assess genetic variance between locations (*StAMPP* package in *R*; Pembleton *et al.*, 2013). Clusters identified by both approaches that were indicative of potential genetic population structuring were independently refiltered through the pipeline for subsequent analyses to retain as many SNPs as possible (i.e., SNP filters 1 to 4 applied to selected individuals using a subset of the raw data) (Appendix Table 2.A2).

For admixture modelling, *STRUCTURE* 2.3.4 (Pritchard *et al.*, 2000) was used to determine the true number of genetic populations (*k*). Model parameters included a burn-in of 50,000 iterations, 100,000 Markov Chain Monte Carlo (MCMC) simulations, and 10 replicates for each predicted *k*. The range of predicted *k* tested was based on the number of capture locations present in the particular dataset analysed (Table 2.1). Initial output variance determined whether model parameters were adjusted. The true number of genetic populations detected for each model was based on the log likelihood of all predicted *k* (InP(*k*)), the highest delta *k* (Δk) value determined using the Evanno method (Evanno *et al.*, 2005), and investigating structure plots for each predicted *k* using *StructureSelector* (Li and Liu 2017). When structure analysis results (i.e., Δk values, InP(*k*) values, and structure plots) revealed inconsistencies in predicted *k*, additional admixture models were run in a hierarchical approach to validate finer-scale structuring and *k*.

As sampling sites outside of South Australia were not as exhaustive (lower number of capture locations and absence of sampling of adjacent estuaries), population sub-structuring detected may also be consistent with isolation by distance. Therefore, we tested for isolation by distance within each of the Western Australian capture locations and the southern New South Wales and eastern Victoria locations. Pairwise distance values (km) were measured manually as the closest distance by water between each capture location, using *Google My Maps*. Using the FST pairwise matrix generated for each *A. butcheri* capture location, the relationship between the geographic distance matrix and genetic distance matrix was assessed using a Mantel test in *GenAlEx 6.5* (Peakall and Smouse 2012), where a positive correlation indicated geographic isolation by distance. *Acanthopagrus butcheri* from Tasmania were excluded from this test due to the large oceanic barrier formed by Bass Strait separating them from the mainland locations (York *et al.*, 2008).

2.4 Results

A total of 469 samples were submitted for genotyping, including 459 *A. butcheri* and 10 *A. australis*. Sample extract quality control at DArT allowed for 439 *A. butcheri* and 10 *A. australis* to be retained for SNP genotyping (Appendix Table 2.A1). The 20 low-quality samples not genotyped were historical liver material that had been in prolonged storage. The genotype matrix contained 33,493 SNP loci with all individuals and 15,012 loci retained after data filtering (Appendix Table 2.A2). We were unable to secure contemporary samples from the same locations as the historical samples (Appendix Table 2.A1), but our findings showed genetic similarities between historical and contemporary *A. butcheri* from adjacent sites in WA. Considering the lack of differences among samples collected in 1996 and 2020-21, all samples were subsequently analysed together for the FST analysis.

Species hybridisation and relatedness

In the PCA performed to identify potential hybrids, no hybrids were detected with no individuals falling between the two distinct species clusters (Appendix Fig. 2.A1). *Acanthopagrus australis* samples were not included in any subsequent analyses and the raw SNP data were refiltered through the pipeline.

In terms of relatedness between individuals, the average kinship coefficient was -0.279 ± 0.228. Four pairs of samples showed kinship coefficient values greater than 0.25 (Appendix Table 2.A3, Appendix Fig. 2.A2). Each of the four pairs comprised individuals captured from the same location, namely two individuals each from Port Huon (Tasmania), Harriet River (South Australia), Chapman River (South Australia) and Swan Lake (New South Wales). An individual from each pair was removed from all subsequent analyses.

Broad scale population structure

The PCA for *A. butcheri* from across the sampling range identified three regional genetic clusters (Fig. 2.2a, Table 2.1). The western cluster, which included all fish from Western Australia was separated by PC1 from the remaining samples from eastern Australia. Among the remaining samples, three clusters were separated by PC2, with (1) an eastern cluster comprising samples from eastern Victoria, New South Wales and Tasmania, (2) a southern cluster comprising samples from mainland South Australia and western Victoria, and (3) another southern cluster of *A. butcheri* from Kangaroo Island. However, given their spatial proximity, both southern clusters were refiltered through the pipeline independently and subject to subsequent PCAs. Both the western cluster (Western Australia) and the eastern cluster (eastern Victoria, New South Wales, Tasmania) showed further separation driven by capture locations (Fig. 2.2b, 2.2c). Isolation by distance tests (between individuals) were run for each

of the western and eastern clusters (excluding Tasmania), both of which revealed positive correlations in both Mantel tests ($R^2 = 0.48$ and $R^2 = 0.51$, respectively).

As the southern cluster comprised the most geographically close estuaries sampled, we investigated population structure within this region in further detail. Pairwise fixation indices for the southern cluster showed a clear pattern of higher genetic differentiation between mainland South Australia and Kangaroo Island (all FST >0.10), as well as among the Kangaroo Island capture locations (Appendix Table 2.A4). Fish from Stun'Sail Boom (Kangaroo Island) exhibited consistent higher genetic differentiation (FST >0.15, and up to 0.22 between Stun'Sail Boom and Port Lincoln), except for the two closest estuaries (i.e., Harriet River and Eleanor River, FST=0.09 and 0.10, respectively). FST values among the mainland capture locations from Port River (South Australia) to Hopkins River (Victoria) were all ≤0.05 and down to 0.0017 (Port River and West Lakes, South Australia). The southern regional cluster PCA revealed discrete genetic clusters comprising A. butcheri from mainland Australia, northern Kangaroo Island, and southern Kangaroo Island capture locations (Fig. 2.3). Eight of ten PCs showed evidence of subtle, finer scale structure, which further separated these capture locations into five sub-clusters (Fig. 2.4). There was separation between the north and south coast of Kangaroo Island (sub-clusters northKI and southKI), as well as between the two major gulfs of South Australia and the eastern South Australian coastline (sub-clusters northSA and southSA, respectively). The fifth sub-cluster comprised A. butcheri from the western Victorian capture locations (sub-cluster westVIC) (Table 2.1). Subsequent analyses for admixture at local scales were conducted on the southern regional cluster.



Fig. 2.2 Principal component analysis (PCA) of the (a) entire *A. butcheri* sample distribution, (b) western cluster, and (c) eastern cluster. Data are grouped by Australian states, including Western Australia (WA, orange), South Australia (SA, blue), New South Wales (NSW, purple), Victoria (VIC, green), and Tasmania (TAS, yellow). Data are represented as bivariate plots using PCA1 and PCA2 scores as the axes.



Fig. 2.3 Structure plot (above) and Principal Component Analysis [PCA] plots (below) for the southern regional cluster of *A. butcheri*. Capture locations identifiable by colour, shape, and number both in the legend and on the map. Colour assignments are based on the dominant cluster each location is predicted to be a part of in their corresponding structure and PCA plot (i.e., unique to each sub-cluster).

Local scale population structure

An admixture model was run on the entire southern cluster, with the exploratory parameters producing acceptable output statistics. By applying the Evanno method (i.e., highest Δk) (Evanno *et al.*, 2005), five distinct populations (*k*=5) were identified within the southern regional cluster that matched those identified using PC scores though there was also a secondary Δk peak at *k*=8 (Fig. 2.3,

Appendix Fig. 2.A3a). Furthermore, InP(*k*) values plateau at *k*=8 (Appendix Fig. 2.A3b), with the structure plots of both predicted *k* showing similar grouping of capture locations, with additional clusters between Spencer Gulf and Gulf St Vincent (i.e., sub-cluster northSA) and a single individual from West Lakes appearing to have ancestry unique from all capture locations (Appendix Fig. 2.A4). This inconsistency suggests there may be hierarchical structure and therefore further admixture models were run for all five sub-clusters previously identified, including the additional clusters predicted here that are within sub-cluster northSA.

Admixture modelling for the sub-clusters reinforced the patterns observed in the PCAs, with k=2 detected for sub-cluster southKI (Fig. 2.4a) based on both Δk (Appendix Fig. 2.A5a) and InP(k) values (Appendix Fig. 2.A5b) with a clear separation between capture locations in the corresponding structure plots (Appendix Fig. 2.A6). Three populations were identified for sub-cluster southKI (k=3, Fig. 2.4b, Appendix Fig. 2.A7), with structure plots consistently identifying one individual from Harriet River as a likely migrant from Stun'Sail Boom (approximately 24 km apart) (Appendix Fig. 2.A8). Three populations (k=3) were detected for sub-cluster northSA based on InP(k) values (Fig. 2.4c, Appendix Fig. 2.A9b), showing a separation within Spencer Gulf (between Port Lincoln and Tumby Bay, with some Tumby Bay samples showing Port Lincoln ancestry and Gulf St Vincent ancestry), and between Spencer Gulf and Gulf St Vincent (Port River, West Lakes, Onkaparinga River; see Fig. 2.1). However, Δk detected k=4 (Appendix Fig. 2.A9a), with the corresponding structure plots identifying the same individual from West Lakes previously identified that appeared to have ancestry unique from all capture locations (Appendix Fig. 2.A10). Two populations were detected in sub-cluster southSA based on Δk values (Appendix Fig. 2.A11a), with structure plots revealing some Coorong samples with Robe ancestry (Fig. 2.4d, Appendix Fig. 2.A12). However, InP(k) indicated k=1 (Appendix Fig. 2.A11b) and considering the Evanno method cannot predict k=1, as well as the similarities of both capture locations using PC scores, it is likely that this is a single population (Fig. 2.4d). Two populations were identified for sub-cluster westVIC (Fig. 2.4e) based on both Δk values (Appendix Fig. 2.A13a) and InP(k) values (Appendix Fig. 2.A13b), with structure plots revealing a lone migrant sampled from the Glenelg River that had an ancestry consistent with it originating from the Hopkins River (Fig. 2.4e, Appendix Fig. 2.A14).



Fig. 2.4 Structure plots (above) and Principal Component Analysis [PCA] plots (below) for the five sub-clusters of the southern regional cluster of *A. butcheri* collected in South Australia and eastern Victoria, including sub-clusters (a) northKI, (b) southKI, (c) northSA, (d) southSA, and (e) westVIC. Each capture location is identifiable by colour, shape, and number both in the legend and on the map. Colour assignments are based on the dominant cluster each location is predicted to be a part of in their corresponding structure and PCA plot (i.e., unique to each sub-cluster). PCA plots were created using two out of the ten PC scores generated that best separated the sub-clusters.

2.5 Discussion

Using a continental-scale comprehensive sampling of *A. butcheri* across its 3,200 km longitudinal range, we highlight the power of genome wide SNP data to understand population structure and connectivity patterns for estuarine-dependent species. Genetic patterns identified in this study across *A. butcheri's* distribution range are likely the result of the interplay between the species' life history and large-scale marine biogeographic barriers and local-scale habitat distribution.

Broad scale structure

The genetic differentiation among regional clusters is likely attributable to geographic barriers. The absence of suitable habitats for A. butcheri between Western Australia and South Australia (i.e., absence of freshwater outflows and estuaries) would explain the genetic isolation between the western and southern clusters (~2,150 km apart) (Fig. 2.1, 2.2a). Studies that targeted allozyme and microsatellite markers have shown similar isolation across both Western Australia (Chaplin et al., 1998) and South Australia (Burridge et al., 2004). In particular, barriers such as the Great Australian Bight limit movement of estuarine-dependent species (e.g., Hammer et al., 2021) and support our findings on the low likelihood of movement or gene flow at this distance (Hammer et al., 2021). Gene flow at such a broad geographic scale is expected to be relatively low compared to marine fish that utilise ocean currents for egg and larval dispersal (Rakeb-Ui et al., 2022; Islam et al., 2017). It is unlikely that A. butcheri could utilise such a dispersal strategy given that they spawn in the upper reaches of estuaries, with spawning events dependent on freshwater flow and occurring away from any coastal currents (Williams et al., 2012; Roberts et al., 2010). Gene flow across marine habitats associated to individual movements is not however ruled out for mature A. butcheri and may be contributing to the albeit limited gene flow seen at larger distances (i.e., 100s kms), as seen in other species (Tan et al., 2022; Cheng et al., 2015). Overall, the need for estuarine conditions for spawning and subsequent egg and larval survival (Jenkins et al., 2010; Williams et al., 2020) is reflected in the overall philopatry we have identified, with connectivity among estuaries most likely a result of adult fish movements. The absence of suitable habitats limiting gene flow has also been found in reef fish,

where open water between rocky or coral reefs restricts gene flow and drives population structuring (Ducret *et al.,* 2022; Torres Hernández *et al.,* 2022). Furthermore, as *A. butcheri* have been shown to move out of estuarine systems through tagging, telemetry, and otolith chemistry (Gillanders *et al.,* 2015, Hindell *et al.,* 2008, Hoeksema *et al.,* 2006), the high genetic structuring found best reflects philopatry (i.e., individuals returning to capture locations) rather than year-round residency.

The genetic distinction between A. butcheri sampled from western Victoria and eastern Victoria aligns with the recognised boundaries of marine biogeographical provinces in southern Australia, namely the Flindersian and Peronian provinces, respectively (Li et al., 2013; Waters et al., 2003; Bennet and Pope 1953). This boundary coincides with species distribution limits, as well as population structuring for several species (Colgan 2016), including an east-west divergence previously identified for A. butcheri and other sparids such as snapper, Chrysophrys auratus (Burridge and Versace 2007; Bertram et al., 2023). The genetic differentiation present across Victorian locations is explained by the presence and convergence of strong ocean currents found across southeastern Australia (i.e., Leeuwin Current and East Australian Current [EAC]) creating a break in gene flow. Similar barriers reducing gene flow include the Kuroshio current along southern China (Islam et al., 2022; Gu et al., 2022), the currents along eastern and southern Africa (Reid et al., 2016; Gaylord and Gaines 2000), as well as the lower Congo River rapids (Kurata et al., 2022; Markert et al., 2010). Ocean currents would also explain the genetic similarities found within biogeographical provinces, such as the eastern cluster (Peronian province, Fig. 2.2a), where the EAC has previously been known to assist gene flow and movement of marine species, including oysters (Saccostrea glomerata, O'Hare et al., 2021), reef fish (Parma microlepis, Curley and Gillings 2009) and snapper (C. auratus, Sumpton et al., 2008). However, when the eastern cluster is isolated from the remaining capture locations, there is clear genetic differentiation across A. butcheri from each Australian state (Fig. 2.2c). This differentiation was also shown within the regional clusters, with capture locations mirroring their geographic orientation within both the western cluster (Fig. 2.2b) and southern cluster (Fig. 2.3). Genetic structure mirroring geography has also been shown in populations of water fleas (Daphnia magna, Fields et al., 2015), scallops (Pecten maximus, Vendrami et al., 2019), and terrestrial mammals (de Jong et al., 2020; Kominakis et al., 2021).

Individual-scale tests show isolation by distance within both the western cluster and eastern cluster, suggesting connectivity among nearby locations and restricted gene flow with increasing distances at this spatial scale. However, given the geographically discontinuous sampling across both regional clusters, fish movement may still occur between capture locations in adjacent estuaries. Therefore, increased sampling effort (i.e., sampling adjacent estuaries) is recommended to further

resolve population structure and investigate the degree of gene flow at the local scale along the eastern and western Australian coastlines.

Admixture modelling of the southern cluster revealed two genetically distinct populations: *A. butcheri* captured from mainland Australia and those from Kangaroo Island. The clear separation of locations is likely attributed to the water bodies between them (i.e., Investigator Strait and Backstairs Passage) limiting fish movement and gene flow. The absence of coastline and/or suitable depth range between habitats has been shown previously as a form of genetic isolation (Volk *et al.*, 2021; Nordahl *et al.*, 2019; Spies 2012), and coupled with the strong ocean currents and habitat suitability, is likely to be driving genetic differentiation at this spatial scale.

Local scale structure

Independent admixture models of southern sub-clusters revealed genetic differentiation and subtle, fine-scale structuring. Gene flow was present between spatially close collection locations (i.e., adjacent estuaries), including Harriet River and Eleanor River identified as a single genetic population (Fig. 2.4b), with estuary mouths only ~2 km apart. 'Estuary-hopping' has been observed previously for A. butcheri (Gillanders et al., 2015; Chaplin et al., 1998; Burridge and Versace 2007) and shows their ability to inhabit coastal waters while still reflecting some form of estuarine dependence. Nonetheless, cases of genetic differentiation were found between adjacent locations, including Middle River and Western River on Kangaroo Island (9 km apart) (Fig. 2.4a). Relative to capture locations on mainland Australia, Kangaroo Island has many small estuaries that undergo seasonally driven flushing events with intermittent openings to the sea that likely limit local scale fish movement (Rumbelow et al., 2010). Genetic differentiation at a local scale shows that the proximity of locations is not a consistent factor driving connectivity in this species. Reduced gene flow influenced by differences among habitats (e.g., freshwater inflow, water temperature and salinity) has been found previously in other species that use estuaries throughout their life cycles (Hollenbeck et al., 2019; O'Leary et al., 2021). Although estuary-hopping may be common in some locations, appropriate assessments of estuary geography and characteristics (e.g., systems closed for extended periods) are required before generally assuming gene flow between adjacent locations (Le Moan et al., 2016; Lassauce et al., 2022). Overall, the predicted increase in estuary closures in response to climate change across Western Australia and South Australia relative to eastern Australia (Hallet et al., 2017) is likely to further contribute to the limited gene flow present within the western and southern clusters. Furthermore, in light of the expected increase in estuarine closures associated with global change in southern Australia, evaluations of gene flow and population structure of estuarine populations over time are recommended.

In some cases, adjacent estuaries that were genetically distinct, showed low-level connectivity in the form of lone migrants, where an individual caught from one location was shown to have the genetic ancestry of *A. butcheri* captured elsewhere. This small number of migrants further demonstrates the variation in this species' movement and ability to partially migrate (Gillanders *et al.*, 2015). Migrant exchange via unidirectional flow has been shown in freshwater species, particularly from rivers into lake populations (Bernaś *et al.*, 2021; Erin *et al.*, 2019; Roman *et al.*, 2018), as well as between discrete marine populations (Lassauce *et al.*, 2022; Valesco-Montoya *et al.*, 2022,). Investigating genetic variation on a temporal scale across the southern cluster could determine the influence of these lone migrants on the stability of population structuring and connectivity.

Gene flow at greater distances across the southern cluster was revealed between collection locations >200 km apart (e.g., Coorong and Robe), with structure analyses and PC scores predicting a single genetic population (Fig. 2.4d). The presence of seasonally driven inshore currents along the coastline between Robe and the Coorong may have assisted A. butcheri movement between these locations (Middleton and Bye 2007). Seasonal currents may have contributed also to the movements of a lone migrant from Glenelg River to Hopkins River (~191 km apart), considering the southeasterly flow of the Leeuwin Current during winter, and partial reversal over summer (Li et al., 2013; Waters et al., 2003), as used by other sparids (Gardner et al., 2022). Both estuary proximity and inshore currents likely have influenced gene flow in A. butcheri from Tumby Bay. This capture location, although identified as a distinct genetic population included migrants originating from Port Lincoln (~57 km away) and similarities with the Gulf St Vincent population (~923 km away from Port River) (Fig. 2.4c). Movement between the two Spencer Gulf locations would not be surprising, given their spatial proximity, although potential movement between the South Australian gulfs would likely be attributed to seasonally driven inshore currents (Middleton and Platov 2003). Estuaries are present along the coastline of both gulfs, although few are spatially close (e.g., no viable habitats or freshwater outflows along the western coastline of Yorke peninsula) (Rumbelow et al., 2010). Therefore, even with A. butcheri estuary-hopping to adjacent locations, long distance movement would be required for fish to migrate between both South Australian gulfs. Additional sampling along the coastline between Tumby Bay and Gulf St Vincent may be needed to provide greater insight into their connectivity and gene flow associated with regional oceanographic conditions and predicted residual currents between both South Australian gulfs (Rogers et al., 2020).

The presence of interspecific hybrids can influence inferences of population structure. While we tested our data for the presence of hybrids both outside and within the known hybridisation zone at Swan Lake, New South Wales (Roberts and Arye 2020, Roberts *et al.,* 2009, Farrington *et al.,* 2000),

their absence was not consistent with previous findings of potential genetic swamping within *A*. *butcheri* populations, as well as predicted range shifts of *Acanthopagrus spp*. (Roberts *et al.*, 2010; Pecl *et al.*, 2014). Further investigations into the extent of hybridisation with increased sampling efforts across New South Wales could explore if the geography of *Acanthopagrus* interspecific hybridisation is changing and whether the impacts of genetic swamping may have stabilised over time (Mandeville *et al.*, 2017).

Our study highlights strong philopatry in this estuarine-dependent species, with cases of gene flow more present between adjacent estuaries and the limited movement identified at greater distances likely influenced by lone migrants and/or assistance from inshore currents. The geographic isolation found at a broader scale suggests the presence of geographic barriers, most likely in the form of a lack of suitable habitat, ocean currents, and large water bodies that drive the formation of biogeographic provinces across southern Australia. Strong philopatry at both a broad and local scale stresses the importance of location-specific management. Targeting SNPs in *A. butcheri* provides an example of how neither spatial proximity of estuaries nor a fish's movement into coastal waters (i.e., partial migration) is a reflection of gene flow among estuaries.

References

- Aitken, N, Smith, S, Schwarz, C, Morin, PA (2004) Single nucleotide polymorphism (SNP) discovery in mammals: a targeted-gene approach. *Mol Ecol* **13**, 1423-31. doi:10.1111/j.1365-294X.2004.02159.x
- Anderson, EC, Garza, JC (2006) The power of single-nucleotide polymorphisms for large-scale parentage inference. *Genetics* **172**, 2567-82. doi:10.1534/genetics.105.048074
- Amiteye, S (2021) Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon* **7**, e08093. doi:10.1016/j.heliyon.2021.e08093
- Bennett, I, Pope, EC (1953) Intertidal zonation of the exposed rocky shores of Victoria, together with a rearrangement of the biogeographical provinces of temperate australian shores. *Marine and Freshwater Research* **4**, 105-159. doi:10.1071/mf9530105
- Bennett, RH, Reid, K, Gouws, G, Bloomer, P, Cowley, PD (2017) Genetic stock structure of white steenbras Lithognathus lithognathus (Cuvier, 1829), an overexploited fishery species in South African waters. African Journal of Marine Science **39**, 27-41. doi:10.2989/1814232x.2017.1303398
- Bernas, R, Was-Barcz, A, Arnyasi, M, Debowski, P, Radtke, G, Pocwierz-Kotus, A, Berrebi, P (2021) Evidence of unidirectional gene flow in a fragmented population of *Salmo trutta* L. *Scientific Reports* **11**, 23417. doi:10.1038/s41598-021-02975-9
- Bernatchez, L, Wellenreuther, M, Araneda, C, Ashton, DT, Barth, JMI, Beacham, TD, Maes, GE, Martinsohn, JT, Miller, KM, Naish, KA, Ovenden, JR, Primmer, CR, Young Suk, H, Therkildsen, NO, Withler, RE (2017)
 Harnessing the Power of Genomics to Secure the Future of Seafood. *Trends in Ecology & Evolution* 32, 665-680. doi:10.1016/j.tree.2017.06.010
- Bertram, A, Bell, J, Brauer, C, Fowler, A, Hamer, P, Sandoval-Castillo, J, Stewart, J, Wellenreuther, M, Beheregaray, LB (2023) Bioregional boundaries and genomically-delineated stocks in snapper (*Chrysophrys auratus*) from southeastern Australia. *Life Science Weekly* 492. doi:10.1101/2023.01.16.524335
- Burridge, CP, Hurt, AC, Farrington, LW, Coutin, PC, Austin, CM (2004) Stepping stone gene flow in an estuarine-dwelling sparid from south-east Australia. *Journal of Fish Biology* **64**, 805-819. doi:10.1111/j.1095-8649.2004.0347.x
- Burridge, CP, Versace, VL (2006) Population genetic structuring in *Acanthopagrus butcheri* (*Pisces: Sparidae*): does low gene flow among estuaries apply to both sexes? *Marine Biotechnology* **9**, 33-44. doi:10.1007/s10126-006-6023-7
- Chaplin JA, BG, Gill HS, McCullock R, Potter IC (1998) Are assemblages of black bream (*Acanthopagrus butcheri*) in different estuaries genetically distinct? *International Journal of Salt Lake Research* **6**, 303-321.
- Cheng, J, Yanagimoto, T, Song, N, Gao, TX (2015) Population genetic structure of chub mackerel *Scomber japonicus* in the Northwestern Pacific inferred from microsatellite analysis. *Molecular Biology Reports* 42, 373-82. doi:10.1007/s11033-014-3777-2
- Colgan, DJ (2016) Marine and estuarine phylogeography of the coasts of south-eastern Australia. *Marine and Freshwater Research* **67**, 1597-1610. doi:10.1071/Mf15106
- Cooke, SJ, Cowx, IG (2006) Contrasting recreational and commercial fishing: Searching for common issues to promote unified conservation of fisheries resources and aquatic environments. *Biological Conservation* **128**, 93-108. doi:10.1016/j.biocon.2005.09.019
- Curley, BG, Gillings, MR (2009) Population connectivity in the temperate damselfish *Parma microlepis*: analyses of genetic structure across multiple spatial scales. *Marine Biology* **156**, 381-393. doi:10.1007/s00227-008-1090-0
- de Jong, JF, van Hooft, P, Megens, H-J, Crooijmans, RPMA, de Groot, GA, Pemberton, JM, Huisman, J, Bartoš, L, Iacolina, L, van Wieren, SE, Ydenberg, RC, Prins, HHT (2020) Fragmentation and translocation distort the genetic landscape of ungulates: red deer in the Netherlands. *Frontiers in Ecology and Evolution* **8**, doi:10.3389/fevo.2020.535715

- Doubleday, ZA, Izzo, C, Haddy, JA, Lyle, JM, Ye, Q, Gillanders, BM (2015) Long-term patterns in estuarine fish growth across two climatically divergent regions. *Oecologia* **179**, 1079-90. doi:10.1007/s00442-015-3411-6
- Ducret, H, Timm, J, Rodriguez-Moreno, M, Huyghe, F, Kochzius, M (2022) Strong genetic structure and limited connectivity among populations of Clark's Anemonefish (*Amphiprion clarkii*) in the centre of marine biodiversity. *Coral Reefs* **41**, 599-609. doi:10.1007/s00338-021-02205-8
- Erin, NI, Benesh, DP, Henrich, T, Samonte, IE, Jakobsen, PJ, Kalbe, M (2019) Examining the role of parasites in limiting unidirectional gene flow between lake and river sticklebacks. *Journal of Animal Ecology* 88, 1986-1997. doi:10.1111/1365-2656.13080
- Evanno, G, Regnaut, S, Goudet, J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611-20. doi:10.1111/j.1365-294X.2005.02553.x
- Farrington, LW, Austin, CM, Coutin, PC (2000) Allozyme variation and stock structure in the black bream, Acanthopagrus butcheri (Munro) (Sparidae) in southern Australia: implications for fisheries management, aquaculture and taxonomic relationship with (Gunther). Fisheries Management and Ecology 7, 265-279. doi:10.1046/j.1365-2400.2000.00178.x
- Fields, PD, Reisser, C, Dukic, M, Haag, CR, Ebert, D (2015) Genes mirror geography in *Daphnia magna*. *Molecular Ecology* **24**, 4521-36. doi:10.1111/mec.13324
- Gardner, MJ, Chaplin, JA, Fairclough, DV, Potter, IC (2022) Microsatellite-based assessment of the genetic structure of snapper, *Chrysophrys auratus*, in Australasia. *Estuarine Coastal and Shelf Science* **274**, doi:10.1016/j.ecss.2022.107932
- Gardner, MJ, Chaplin, JA, Phillips, NM (2014) Isolation and characterisation of novel microsatellite markers from *Pagrus auratus* and cross amplification in *Acanthopagrus butcheri*. *Conservation Genetics Resources* **6**, 155-157. doi:10.1007/s12686-013-0031-5
- Gardner, MJ, Cottingham, A, Hesp, SA, Chaplin, JA, Jenkins, GI, Phillips, NM, Potter, IC (2013) Biological and Genetic Characteristics of Restocked and Wild *Acanthopagrus butcheri* (Sparidae) in a Southwestern Australian Estuary. *Reviews in Fisheries Science* **21**, 441-453. doi:10.1080/10641262.2013.796804
- Garvin, MR, Saitoh, K, Gharrett, AJ (2010) Application of single nucleotide polymorphisms to non-model species: a technical review. *Molecular Ecology Resources* **10**, 915-34. doi:10.1111/j.1755-0998.2010.02891.x
- Gaylord, B, Gaines, SD (2000) Competition and the effect of spatial resource heterogeneity on evolutionary diversification. *The American Naturalist* **155**, 769-789. doi:10.1086/303357
- Gillanders, BM, Izzo, C, Doubleday, ZA, Ye, Q (2015) Partial migration: growth varies between resident and migratory fish. *Biology Letters* **11**, doi:10.1098/rsbl.2014.0850
- Gray, C, 2008. Life History and Biology of Black Bream in southern NSW. NSW Recreational Fishing Trust, Cronulla, New South Wales.
- Gruber, B, Unmack, PJ, Berry, OF, Georges, A (2018) dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources* **18**, 691-699. doi:10.1111/1755-0998.12745
- Gu, S, Yan, YR, Yi, MR, Luo, ZS, Wen, H, Jiang, CP, Lin, HD, He, XB (2022) Genetic pattern and demographic history of cutlassfish (*Trichiurus nanhaiensis*) in South China Sea by the influence of Pleistocene climatic oscillations. *Scientific Reports* **12**, 14716. doi:10.1038/s41598-022-18861-x
- Hall, LA, Beissinger, SR (2014) A practical toolbox for design and analysis of landscape genetics studies. Landscape Ecology **29**, 1487-1504. doi:10.1007/s10980-014-0082-3
- Hallett, CS, Hobday, AJ, Tweedley, JR, Thompson, PA, McMahon, K, Valesini, FJ (2017) Observed and predicted impacts of climate change on the estuaries of south-western Australia, a Mediterranean climate region. *Regional Environmental Change* **18**, 1357-1373. doi:10.1007/s10113-017-1264-8
- Hammer, MP, Adams, M, Unmack, PJ, Hassell, KL, Bertozzi, T (2021) Surprising Pseudogobius: Molecular systematics of benthic gobies reveals new insights into estuarine biodiversity (Teleostei: Gobiiformes). *Molecular Phylogenetics and Evolution* 160, 107140. doi:10.1016/j.ympev.2021.107140

- Hindell, JS, Jenkins, GP, Womersley, B (2008) Habitat utilisation and movement of black bream Acanthopagrus butcheri (Sparidae) in an Australian estuary. Marine Ecology Progress Series **366**, 219-229. doi:10.3354/meps07519
- Hoeksema, SD, Chuwen, BM, Potter, IC (2006) Massive mortalities of the black bream *Acanthopagrus butcheri* (Sparidae) in two normally-closed estuaries, following extreme increases in salinity. *Journal of the Marine Biological Association of the United Kingdom* **86**, 893-897. doi:10.1017/s002531540601383x
- Hollenbeck, CM, Portnoy, DS, Gold, JR (2019) Evolution of population structure in an estuarine-dependent marine fish. *Ecology and Evolution* **9**, 3141-3152. doi:10.1002/ece3.4936
- Islam, MRU, Tachihara, K, Imai, H (2022) Cryptic lineage and genetic structure of Acanthopagrus pacificus populations in a natural world heritage site revealed by population genetic analysis. Diversity-Basel 14, doi:10.3390/d14121117
- Izzo, C, Doubleday, ZA, Grammer, GL, Disspain, MCF, Ye, QF, Gillanders, BM (2017) Seasonally resolved environmental reconstructions using fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 74, 23-31. doi:10.1139/cjfas-2016-0055
- Jenkins, GP, Kent, JA, Woodland, RJ, Warry, F, Swearer, SE, Cook, PLM (2018) Delayed timing of successful spawning of an estuarine dependent fish, black bream *Acanthopagrus butcheri*. *Journal of Fish Biology* **93**, 931-941. doi:10.1111/jfb.13806
- Jombart, T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-5. doi:10.1093/bioinformatics/btn129
- Junge, C, Donnellan, SC, Huveneers, C, Bradshaw, CJA, Simon, A, Drew, M, Duffy, C, Johnson, G, Cliff, G,
 Braccini, M, Cutmore, SC, Butcher, P, McAuley, R, Peddemors, V, Rogers, P, Gillanders, BM (2019)
 Comparative population genomics confirms little population structure in two commercially targeted
 carcharhinid sharks. *Marine Biology* 166, 1-15 doi:10.1007/s00227-018-3454-4
- Kilian, A, Wenzl, P, Huttner, E, Carling, J, Xia, L, Blois, H, Caig, V, Heller-Uszynska, K, Jaccoud, D, Hopper, C, Aschenbrenner-Kilian, M, Evers, M, Peng, K, Cayla, C, Hok, P, Uszynski, G (2012) Diversity arrays technology: a generic genome profiling technology on open platforms. *Methods in Molecular Biology* 888, 67-89. doi:10.1007/978-1-61779-870-2_5
- Kominakis, A, Tarsani, E, Hager-Theodorides, AL, Mastranestasis, I, Hadjigeorgiou, I (2021) Clustering patterns mirror the geographical distribution and genetic history of Lemnos and Lesvos sheep populations. *PLoS ONE* **16**, e0247787. doi:10.1371/journal.pone.0247787
- Kurata, NP, Hickerson, MJ, Hoffberg, SL, Gardiner, N, Stiassny, MLJ, Alter, SE (2022) Riverscape genomics of cichlid fishes in the lower Congo: Uncovering mechanisms of diversification in an extreme hydrological regime. *Molecular Ecology* **31**, 3516-3532. doi:10.1111/mec.16495
- Lack, D (1944) The problem of partial migration. British Birds 37, 122 130.
- Lassauce, H, Dudgeon, CL, Armstrong, AJ, Wantiez, L, Carroll, EL (2022) Evidence of fine-scale genetic structure for reef manta rays *Mobula alfredi* in New Caledonia. *Endangered Species Research* **47**, 249-264. doi:10.3354/esr01178
- Le Moan, A, Gagnaire, PA, Bonhomme, F (2016) Parallel genetic divergence among coastal-marine ecotype pairs of European anchovy explained by differential introgression after secondary contact. *Molecular Ecology* **25**, 3187-202. doi:10.1111/mec.13627
- Liu, ZJ, Cordes, JF (2004) DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* **238**, 1-37. doi:10.1016/j.aquaculture.2004.05.027
- Li, J, Foighil, DO, Park, JK (2013) Triton's trident: cryptic Neogene divergences in a marine clam (*Lasaea australis*) correspond to Australia's three temperate biogeographic provinces. *Molecular Ecology* **22**, 1933-46. doi:10.1111/mec.12220
- Li, YL, Liu, JX (2018) StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Mol Ecol Resour* **18**, 176-177. doi:10.1111/1755-0998.12719
- Mahony, M, Moses, B, Mahony, SV, Lemckert, FL, Donnellan, S (2020) A new species of frog in the Litoria ewingii species group (Anura: Pelodryadidae) from south-eastern Australia. *Zootaxa* **4858**, zootaxa 4858 2 3. doi:10.11646/zootaxa.4858.2.3

- Mandeville, EG, Parchman, TL, Thompson, KG, Compton, RI, Gelwicks, KR, Song, SJ, Buerkle, CA (2017) Inconsistent reproductive isolation revealed by interactions between Catostomus fish species. *Evolution Letters* **1**, 255-268. doi:10.1002/evl3.29
- Markert, JA, Schelly, RC, Stiassny, M (2010) Genetic isolation and morphological divergence mediated by high-energy rapids in two cichlid genera from the lower Congo rapids. *BMC Ecology and Evolution* **10**, 149. doi:10.1186/1471-2148-10-149
- Middleton, JF, Bye, JAT (2007) A review of the shelf-slope circulation along Australia's southern shelves: Cape Leeuwin to Portland. *Progress in Oceanography* **75**, 1-41. doi:10.1016/j.pocean.2007.07.001
- Middleton, JF, Platov, G (2003) The mean summertime circulation along Australia's southern shelves: A numerical study. *Journal of Physical Oceanography* **33**, 2270-2287. doi:10.1175/1520-0485(2003)033<2270:Tmscaa>2.0.Co;2
- Mills, LS, Allendorf, FW (1996) The One-Migrant-per-Generation Rule in Conservation and Management. *Conservation Biology* **10**, 1509-1518. doi:10.1046/j.1523-1739.1996.10061509.x
- Morin, PA, Luikart, G, Wayne, RK, Grp, SW (2004) SNPs in ecology, evolution and conservation. *Trends in Ecology & Evolution* **19**, 208-216. doi:10.1016/j.tree.2004.01.009
- Nordahl, O, Koch-Schmidt, P, Sunde, J, Yildirim, Y, Tibblin, P, Forsman, A, Larsson, P (2019) Genetic differentiation between and within ecotypes of pike (*Esox lucius*) in the Baltic Sea. *Aquatic Conservation-Marine and Freshwater Ecosystems* **29**, 1923-1935. doi:10.1002/aqc.3196
- Norriss, JV, Tregonning, JE, Lenanton, RCJ, Sarre, GA, 2002. Biological synopsis of the black bream, *Acanthopagrus butcheri* (Munro) (Teleostei: Sparidae) in Western Australia with reference to information from other southern states. Department of Fisheries, Perth, Western Australia.
- O'Leary, SJ, Hollenbeck, CM, Vega, RR, Portnoy, DS (2021) Disentangling complex genomic signals to understand population structure of an exploited, estuarine-dependent flatfish. *Ecology and Evolution* 11, 13415-13429. doi:10.1002/ece3.8064
- O'Hare, JA, Momigliano, P, Raftos, DA, Stow, AJ (2021) Genetic structure and effective population size of Sydney rock oysters in eastern Australia. *Conservation Genetics* **22**, 427-442. doi:10.1007/s10592-021-01343-4
- Partridge, GJ, Jenkins, GI (2002) The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). *Aquaculture* **210**, 219-230. doi:10.1016/S0044-8486(01)00817-1
- Peakall, R, Smouse, PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**, 2537-9. doi:10.1093/bioinformatics/bts460
- Pecl, GT, Ward, TM, Doubleday, ZA, Clarke, S, Day, J, Dixon, C, Frusher, S, Gibbs, P, Hobday, AJ, Hutchinson, N, Jennings, S, Jones, K, Li, XX, Spooner, D, Stoklosa, R (2014) Rapid assessment of fisheries species sensitivity to climate change. *Climatic Change* **127**, 505-520. doi:10.1007/s10584-014-1284-z
- Pembleton, LW, Cogan, NO, Forster, JW (2013) StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology Resources* **13**, 946-52. doi:10.1111/1755-0998.12129
- Pritchard, JK, Stephens, M, Donnelly, P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-59. doi:10.1093/genetics/155.2.945
- R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
- Reid, K, Hoareau, TB, Graves, JE, Potts, WM, Dos Santos, SM, Klopper, AW, Bloomer, P (2016) Secondary contact and asymmetrical gene flow in a cosmopolitan marine fish across the Benguela upwelling zone. *Heredity* **117**, 307-315. doi:10.1038/hdy.2016.51
- Reis-Santos, P, Tanner, SE, Aboim, MA, Vasconcelos, RP, Laroche, J, Charrier, G, Perez, M, Presa, P, Gillanders, BM, Cabral, HN (2018) Reconciling differences in natural tags to infer demographic and genetic connectivity in marine fish populations. *Scientific Reports* 8, 10343. doi:10.1038/s41598-018-28701-6
- Roberts, DG, Ayre, DJ (2010) Panmictic population structure in the migratory marine sparid *Acanthopagrus australis* despite its close association with estuaries. *Marine Ecology Progress Series* **412**, 223-230. doi:10.3354/meps08676

- Roberts, DG, Gray, CA, West, RJ, Ayre, DJ (2009) Evolutionary impacts of hybridization and interspecific gene flow on an obligately estuarine fish. *Journal of Evolutionary Biology* **22**, 27-35. doi:10.1111/j.1420-9101.2008.01620.x
- Roberts, DG, Gray, CA, West, RJ, Ayre, DJ (2010) Marine genetic swamping: hybrids replace an obligately estuarine fish. *Molecular Ecology* **19**, 508-20. doi:10.1111/j.1365-294X.2009.04501.x
- Roberts, DG, Gray, CA, West, RJ, Ayre, DJ (2011) Temporal stability of a hybrid swarm between the migratory marine and estuarine fishes *Acanthopagrus australis* and *A. butcheri*. *Marine Ecology Progress Series* 421, 199-+. doi:10.3354/meps08901
- Rogers, TA, Rodriguez, AR, Fowler, AJ, Doubell, MJ, Drew, MJ, Steer, MA, Matthews, D, James, C, Gillanders, BM (2021) Using a biophysical model to investigate connectivity between spawning grounds and nursery areas of King George whiting (*Sillaginodes punctatus*: Perciformes) in South Australia's gulfs. *Fisheries Oceanography* **30**, 51-68. doi:10.1111/fog.12502
- Roman, I, Bourgeois, Y, Reyes-Velasco, J, Jensen, OP, Waldman, J, Boissinot, S (2018) Contrasted patterns of divergence and gene flow among five fish species in a Mongolian rift lake following glaciation. Biological Journal of the Linnean Society **125**, 115-125. doi:10.1093/biolinnean/bly083
- Rumbelow, K, Speziali, A, Bloomfield, A, 2010. Working Towards a Statewide Inventory of Estuaries: Advancing the Inventory of Estuaries in Five NRM Regions of South Australia. Adelaide.
- Sakabe, R, Lyle, JM, Crawford, CM (2011) The influence of freshwater inflows on spawning success and early growth of an estuarine resident fish species, *Acanthopagrus butcheri*. *Journal of Fish Biology* **78**, 1529-44. doi:10.1111/j.1095-8649.2011.02959.x
- Sarakinis, KG, Taylor, MD, Johnson, DD, Gillanders, BM (2022) Determining population structure and connectivity through otolith chemistry of stout whiting, *Sillago robusta*. *Fisheries Management and Ecology* **29**, 760-773. doi:10.1111/fme.12577
- Secor, DH (2015) 'Migration Ecology of Marine Fishes.' (Johns Hopkins University Press:
- Spies, I (2012) Landscape Genetics Reveals Population Subdivision in Bering Sea and Aleutian Islands Pacific Cod. Transactions of the American Fisheries Society 141, 1557-1573. doi:10.1080/00028487.2012.711265
- Sumpton, WD, Ovenden, JR, Keenan, CP, Street, R (2008) Evidence for a stock discontinuity of snapper (*Pagrus auratus*) on the east coast of Australia. *Fisheries Research* **94**, 92-98. doi:10.1016/j.fishres.2008.07.001
- Sun, L, Dimitromanolakis, A (2014) PREST-plus identifies pedigree errors and cryptic relatedness in the GAW18 sample using genome-wide SNP data. *BMC Proceedings* **8**, S23. doi:10.1186/1753-6561-8-S1-S23
- Sunde, J, Yildirim, Y, Tibblin, P, Forsman, A (2020) Comparing the performance of microsatellites and RADseq in population genetic studies: analysis of data for pike (*Esox lucius*) and a synthesis of previous studies. *Frontiers in Genetics* **11**, 218. doi:10.3389/fgene.2020.00218
- Tan, MP, Sammogam, R, Nur, NFM, Yusoff, NISM, Jaafar, TNAM, Nor, SAM, Sung, YY, Danish-Daniel, M, Megarajan, S (2022) Genetic polymorphism and phylogenetic relationships of the brushtooth lizardfish (*Saurida undosquamis*) (Aulopiformes: Synodontidae) based on mitochondrial DNA markers. *Marine Biodiversity* 52, 13. doi:10.1007/s12526-021-01250-4
- Torres-Hernandez, E, Betancourt-Resendes, I, Angulo, A, Robertson, DR, Barraza, E, Espinoza, E, Diaz-Jaimes, P, Dominguez-Dominguez, O (2022) A multi-locus approach to elucidating the evolutionary history of the clingfish *Tomicodon petersii* (Gobiesocidae) in the Tropical Eastern Pacific. *Molecular Phylogenetics and Evolution* **166**, 107316. doi:10.1016/j.ympev.2021.107316
- Velasco-Montoya, DA, Millan-Marquez, AM, Tavera, J (2022) Genetic connectivity in *Sparisoma aurofrenatum* (redband parrotfish): an unexpected journey. *Hydrobiologia* **849**, 1727-1741. doi:10.1007/s10750-022-04806-y
- Vendrami, DLJ, De Noia, M, Telesca, L, Handal, W, Charrier, G, Boudry, P, Eberhart-Phillips, L, Hoffman, JI (2019) RAD sequencing sheds new light on the genetic structure and local adaptation of European scallops and resolves their demographic histories. *Scientific Reports* **9**, 7455. doi:10.1038/s41598-019-43939-4

- Volk, DR, Konvalina, JD, Floeter, SR, Ferreira, CEL, Hoffman, EA (2021) Going against the flow: Barriers to gene flow impact patterns of connectivity in cryptic coral reef gobies throughout the western Atlantic. *Journal of Biogeography* **48**, 427-439. doi:10.1111/jbi.14010
- Wang, JL (2004) Application of the one-migrant-per-generation rule to conservation and management. *Conservation Biology* **18**, 332-343. doi:DOI 10.1111/j.1523-1739.2004.00440.x
- Waters, JM, Roy, MS (2003) Marine biogeography of southern Australia: phylogeographical structure in a temperate sea-star. *Journal of Biogeography* **30**, 1787-1796. doi:DOI 10.1046/j.0305-0270.2003.00978.x
- Wickham H (2016) ggplot2: elegant graphics for data analysis. *Springer-Verlag New York*. ISBN 978-3-319-24277-4, <u>https://ggplot2.tidyverse.org</u>.
- Williams, J, Cottingham, A, Denham, A, Hall, NG, Potter, IC (2020) Relationship between spawning and egg and larval stages of a unique estuarine-resident species and environmental variables and prey. *Estuarine Coastal and Shelf Science* **246**, doi:10.1016/j.ecss.2020.107039
- Williams, J, Hindell, JS, Swearer, SE, Jenkins, GP (2012) Influence of freshwater flows on the distribution of eggs and larvae of black bream *Acanthopagrus butcheri* within a drought-affected estuary. *Journal of Fish Biology* **80**, 2281-301. doi:10.1111/j.1095-8649.2012.03283.x
- Yap, ES, Spencer, PBS, Chaplin, JA, Potter, IC (2000) The estuarine teleost, *Acanthopagrus butcheri* (Sparidae), shows low levels of polymorphism at five microsatellite loci. *Molecular Ecology* **9**, 2225-2226.
- Ye, Q, Earl, J, Bucater, L, Cheshire, K, McNeil, D, Noell, C, Short, D (2013) Flow related fish and fisheries ecology in the Coorong, South Australia. FRDC Project 2006/45 Final Report. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2009/000014-2. SARDI Research Report Series No. 698.
- York, KL, Blacket, MJ, Appleton, BR (2008) The Bassian Isthmus and the major ocean currents of southeast Australia influence the phylogeography and population structure of a southern Australian intertidal barnacle *Catomerus polymerus* (Darwin). *Molecular Ecology* **17**, 1948-61. doi:10.1111/j.1365-294X.2008.03735.x
- Zheng, X, Levine, D, Shen, J, Gogarten, SM, Laurie, C, Weir, BS (2012) A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* **28**, 3326-8. doi:10.1093/bioinformatics/bts606

Supplementary Material



Fig. 2.A1 Principal Component Analysis (PCA) of the entire sampling distribution, including both *A. butcheri* and *A. australis*. Data are grouped by Australian states, including Western Australia (WA, orange), South Australia (SA, blue), Queensland (QLD, black inverted triangle , *A. australis*), New South Wales (NSW, purple), Victoria (VIC, green), and Tasmania (TAS, yellow). Data are represented as a bivariate plot using PCA1 and PCA2 scores.



Fig. 2.A2 Identity by descent analysis of *A. butcheri* and *A. australis* samples using the KING method of moment. Each data point represents a comparison test between two individuals, with the proportion of SNPs with zero identity-by-state (IBS) plotted against the estimated kinship coefficient. Plots include (a) the entire sample distribution and (b) sample pairings with estimated kinship coefficient values greater than 0.



Fig. 2.A3. Structure analysis of *A. butcheri* from the southern regional cluster. Plots include (a) delta k (Δk) values for each predicted k and (b) the log likelihood of each predicted k (Mean LnP(k) ± Stdev).







K=3



K=4



K=5









K=8



K=9



K=10









K=13



K=14



K=15



Fig. 2.A4. Structure plots for each predicted k (K=X) of *A. butcheri* from the southern regional cluster. Values below plots indicate capture location ID (refer to Table 2.1). Plot colours represent the predicted genetic cluster in each plot independently.



Fig. 2.A5. Structure analysis of *A. butcheri* from sub-cluster northKI. Plots include (a) delta k (Δk) values for each predicted k and (b) the log likelihood of each predicted k (Mean LnP(k) ± Stdev).



K=2



K=3



Fig. 2.A6. Structure plots for each predicted k (K=X) of *A. butcheri* from sub-cluster southKI. Values below plots indicate capture location ID (refer to Table 2.1). Plot colours represent the predicted genetic cluster in each plot independently.



Fig. 2.A7. Structure analysis of *A. butcheri* from sub-cluster southKI. Plots include (a) delta k (Δk) values for each predicted k and (b) the log likelihood of each predicted k (Mean LnP(k) ± Stdev).















K=5



Fig. 2.A8. Structure plots for each predicted k (K=X) of *A. butcheri* from sub-cluster southKI. Values below plots indicate capture location ID (refer to Table 2.1). Plot colours represent the predicted genetic cluster in each plot independently.



Fig. 2.A9. Structure analysis of *A. butcheri* from sub-cluster northSA. Plots include (a) delta k (Δk) values for each predicted k and (b) the log likelihood of each predicted k (Mean LnP(k) ± Stdev).





K=4



K=5



K=6



Fig. 2.A10. Structure plots for each predicted k (K=X) of *A. butcheri* from sub-cluster northSA. Values below plots indicate capture location ID (refer to Table 2.1). Plot colours represent the predicted genetic cluster in each plot independently.



Fig. 2.A11. Structure analysis of *A. butcheri* from sub-cluster southSA. Plots include (a) delta k (Δk) values for each predicted k and (b) the log likelihood of each predicted k (Mean LnP(k) ± Stdev).


Fig. 2.A12. Structure plots for each predicted k (K=X) of *A. butcheri* from sub-cluster southSA. Values below plots indicate capture location ID (refer to Table 2.1). Plot colours represent the predicted genetic cluster in each plot independently.



Fig. 2.A13. Structure analysis of *A. butcheri* from sub-cluster westVIC. Plots include (a) delta k (Δk) values for each predicted k and (b) the log likelihood of each predicted k (Mean LnP(k) ± Stdev).

K=1



K=2



Fig. 2.A14. Structure plots for each predicted k (K=X) of *A. butcheri* from sub-cluster westVIC. Values below plots indicate capture location ID (refer to Table 2.1). Plot colours represent the predicted genetic cluster in each plot independently.

Table 2.A1 Summary of genotyped samples from each capture location for both *A. butcheri* and *A. australis* (n = 10). Table includes method of collection (collection type), coordinates, as well as fish length and weight averaged by capture location.

state		capture location	latitude	longitude	collection type	total length (mm)	body weight (g)
	1	Swan River	-32.056440	115.729268	Net	99	17
Western	2	Collie River	-33.302336	115.667427	Mix	-	-
Australia	3	Margaret River	-33.971249	114.985882	Mix	-	-
(WA)	4	Walpole-Nornalup	-35.005578	116.729803	Net	174	86
	5	Albany	-35.025274	117.937966	Rod	298	492
	6	Pallinup River	-34.469649	118.905147	Mix	-	-
	7	Bremer Bay	-34.391443	119.399468	Net	273	305
	8	Western River	-35.674364	136.971267	Net	124	35
South	9	Middle River	-35.664786	137.073251	Net	150	66
Australia	10	Stun Sail Boom	-36.020961	137.015023	Net	35	2
(SA)	11	Harriet River	-35.985951	137.181116	Mix	144	79
	12	Eleanor River	-35.975754	137.201555	Mix	118	59
	13	Chapman River	-35.787261	138.071934	Mix	150	55
	14	Port Lincoln	-34.740203	135.885176	Rod	279	355
	15	Tumby Bay	-34.376744	136.121845	Rod	323	611
	16	Port River	-34.786495	138.466801	Net	367	858
	17	West Lakes	-34.853334	138.498547	Net	228	195
	18	Onkaparinga River	-35.164248	138.466842	Rod	276	-
	19	Coorong	-35.564694	138.873332	Mix	362	697
	20	Robe	-37.159471	139.775441	Rod	323	512
	21	Glenelg River	-38.063221	140.988287	Rod	312	-
Victoria	22	Hopkins River	-38.404822	142.509158	Rod	346	-
(VIC)	23	Lake Tyers	-37.860443	148.091705	Rod	365	-
	24	Snowy River	-37.806754	148.558078	Rod	370	-
Tasmania (TAS)	25	Port Huon	-43.305684	147.120303	Rod	355	729
New South Wales (NSW)	26	Swan Lake	-35.201748	150.562453	Net	302	-
Queensland (QLD)	27	Nerang River	-27.933361	153.434958	Net	-	-

Table 2.A2 Summary of number of loci retained after each quality control filter for the entire sampling distribution for both species, the *A. butcheri* distribution and the three regional clusters. Sample sizes included. Data for *A. butcheri* distribution and regional clusters excludes influential samples removed during data processing.

step	action	A. butcheri and A. australis	A. butcheri distribution	western cluster	eastern <i>cluster</i>	southern <i>cluster</i>
0	Raw data	33,493	33,493	33,493	33,493	33,493
1	Remove loci <95% reproducibility	19,991	20,613	18,677	21,116	21,800
2	Keep SNPs with data missing <20% overall individuals	19,991	20,613	18,677	21,116	21,800
3	Remove ambiguous SNPs - i.e., same position with more than two alleles	15,012	15,337	13,656	16,123	16,181
4	Remove monomorphic loci, including those with all NAs	15,012	12,096	6,796	10,458	8,893
	sample size	449	435	110	77	248

Table 2.A3 Pairwise comparison of *A. butcheri* samples with kinship coefficient values \geq 0.20. Table includes both samples for each pairing, the proportion of SNPs with zero identity-by-state (IBSO) and kinship coefficient value (kinship). Samples removed indicated by '*'.

pair	sample ID 1	sample ID 2	IBSO	kinshin
pan			0.0000	0.4602
1	ABPhF11-11*	ABPhF11-13	0.0000	0.4692
2	ABHa3FT8-08*	ABHa3FT8-04	0.0001	0.4659
3	ABCh2FT8-01*	ABCh1FT8-01	0.0003	0.4593
4	ABSIFT1-19*	ABSIFT1-20	0.0009	0.4492
5	ABCoFT1-15	ABCoFT1-17	0.0087	0.2385
6	ABCoFT1-17	ABCoFT2-02	0.0063	0.2320
7	ABCoFT2-02	ABPaFT1-17	0.0100	0.2044

Table 2.A4 A pairwise comparison of each capture location in the southern cluster based on genetic distance (FST). Locations are grouped by Australian state, including South Australia (blue) and Victoria (green), with further separation of South Australia into Kangaroo Island (light blue) and mainland Australia (dark blue). Colour key for FST range indicated below table.

Western River	Middle River	Stun'Sail Boom	Harriet River	Eleanor River	Chapman River	Port Lincoln	Tumby Bay	Port River	West Lakes	Onkaparinga River	Coorong	Robe	Glenelg River	Hopkins River		
0															Western River	
0.0490	0														Middle River	
0.1898	0.1630	0													Stun'Sail Boom	
0.1273	0.1007	0.0854	0												Harriet River	
0.1315	0.1039	0.0956	0.0049	0											Eleanor River	
0.1440	0.1172	0.1524	0.0700	0.0709	0										Chapman River	
0.1866	0.1593	0.2177	0.1555	0.1602	0.1836	0									Port Lincoln	
0.1575	0.1287	0.1850	0.1253	0.1312	0.1505	0.0448	0								Tumby Bay	
0.1399	0.1105	0.1703	0.1067	0.1123	0.1345	0.0672	0.0325	0							Port River	
0.1262	0.1011	0.1512	0.0975	0.1015	0.1202	0.0604	0.0296	0.0023	0						West Lakes	
0.1393	0.1095	0.1673	0.1045	0.1104	0.1321	0.0710	0.0353	0.0074	0.0054	0					Onkaparinga River	
0.1295	0.1015	0.1522	0.0989	0.1017	0.1183	0.0847	0.0509	0.0267	0.0264	0.0261	0				Coorong	
0.1281	0.0998	0.1525	0.0991	0.1026	0.1186	0.082	0.0496	0.0248	0.0242	0.0242	0.0065	0			Robe	
0.1335	0.1074	0.1532	0.1032	0.1057	0.1219	0.0973	0.0657	0.0423	0.0419	0.042	0.0334	0.0311	0		Glenelg River	
0.1328	0.1084	0.1541	0.1035	0.1068	0.1229	0.1006	0.0686	0.0473	0.0468	0.0469	0.0400	0.0367	0.0132	0	Hopkins River	

FST 0 0.1

0.2

CHAPTER 3

Combining natural markers to investigate fish population structure and connectivity

Statement of Authorship

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Title of Paper	Combining natural markers to investigate fi	sh population stru	ucture and connectivity						
Dubling Oblig	Published	Accepted for F	Publication						
Publication Status	Submitted for Publication Unpublished and Unsubmitted work written in manuacrist style								
Publication Details	Submitted to Estuarine, Coastal and Shelf reivew'.	Science. Manuscr	ript is currently 'under						
Principal Author									
Name of Principal Author (Candidate)	Koster Georgien Sarakinis								
Contribution to the Paper	Conceptualization (Equal); Data curation (Methodology (Equal); Project administratic Writing – original draft (Lead); Writing – re	Lead); Formal an on (Lead); Softwa view & editing (Ed	alysis (Lead); Investigation (Lead); re (Equal); Visualization (Lead); qual).						
Overall percentage (%)	75%								
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Signature	Juntin	Date	3/02/2024						
Co-Author Contributions									
By signing the Statement of Authorship,	each author certifies that:	iled choye);							
i. permission is granted for the	candidate in include the publication in the th	ned above); nesis: and							
iii. the sum of all co-author con	ributions is equal to 100% less the candidate	e's stated contrib	ution.						
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3.1 Abstract

Understanding population connectivity via fish movement and the appropriate scale for management is amplified by the use of tagging techniques. Natural fish markers provide unique signatures that reflect distinct environmental and physiological characteristics of populations, allowing us to determine the degree of connectivity among them. Different markers function within defined spatiotemporal ranges with specific advantages and limitations. We tested whether applying multiple markers would increase the power of assessing population structure and connectivity of black bream Acanthopagrus butcheri from 12 estuaries across southern Australia. We utilised a range of natural markers, including genetics, otolith shape, otolith isotopic composition, and otolith elemental composition, to determine the effectiveness of each marker independently and through integration. For this estuarine-dependent species, combining genetics and otolith-based techniques was complementary in increasing the accuracy of our results, but not all marker integrations were consistently beneficial, highlighting the importance of appropriate marker selection. The maximum classification accuracy to collection site of 95% (a combination of genetics, otolith shape, and isotopic composition) emphasised the species' estuarine dependency and limited connectivity across the sampling range, suggesting location-specific management is needed for this commercially important species.

Keywords: population dynamics; *Acanthopagrus butcheri*; otolith chemistry; single-nucleotide polymorphisms; otolith shape; multi-marker

3.2 Introduction

Understanding animal movements and connectivity between populations is fundamental for determining the appropriate spatial scales for resource and conservation management. Effective management of highly connected populations requires broader spatial approaches, whereas location-specific strategies are likely to be more appropriate for isolated populations with minimal connectivity. Population structure and connectivity can be determined using natural markers, i.e., signatures that are naturally present in organisms, such as genetic markers (Amoutchi *et al.*, 2023; Luo *et al.*, 2022), bone and tissue chemistry (Morales-Nin *et al.*, 2022; Sarakinis *et al.*, 2022), morphometrics (Khan *et al.*, 2022; Dwivedi 2022) or unique external markings (Magson *et al.*, 2022; Castillo *et al.*, 2018). Variability in marker signatures resolve movements (or isolation) of individuals among habitats and elucidate the degree of population connectivity across a species' distribution. Each marker has its strengths and limitations, reflects different biological, environmental, or genetic conditions, and functions at different spatiotemporal resolutions (Tanner *et al.*, 2016). Therefore, understanding the benefits and limitations of specific markers in the context of available biological information is fundamental for adequately responding to different research questions.

Otoliths (fish ear stones) are polycrystalline structures that provide a suite of valuable information relating to a fish's life (e.g., growth rate, movement, habitat use, or natal origin). Otoliths continuously accrete over time to form daily and annual increments, thereby reflecting age and growth (Panella 1971). This accretion incorporates minor and trace elements found within the ambient water, forming metabolically inert chemical signatures that reflect an individual's life history, such as the location of birth (otolith core) and location of capture (otolith edge) (Reis-Santos *et al.*, 2022; Campana *et al.*, 1999). Otolith chemistry is influenced both by extrinsic factors (e.g., water temperature and water salinity) and intrinsic factors (e.g., metabolism, diet, and reproduction), where their dual regulation on otolith accretion and chemical signatures can outweigh or limit inferences on habitat utilisation or interpretations of environmental proxies (Izzo *et al.*, 2018; Reis-Santos *et al.*, 2018b; Sturrock *et al.*, 2014).

Otolith shape is used to identify fish species and differentiate populations through morphometric variations (Smoliński *et al.,* 2020; Campana and Casselman 1993). Minimal variations in otolith shape among populations can infer connectivity, although much like otolith chemistry, extrinsic (e.g., water depth and water temperature) and intrinsic factors (e.g., ontogeny, sex, diet, or genetics) can contribute to shape variability (Vaux *et al.,* 2019; Cardinale *et al.,* 2004). For example, ontogeny can be positively correlated with otolith size and weight, and negatively with roundness and circularity due to crenulation (Barnuevo *et al.,* 2022; Hüssy 2008); whereas water depth can influence otolith elongation and overall size (Assis *et al.,* 2020; Bardarson *et al.,* 2017).

Population genetics is a powerful alternative to otolith-based techniques, providing information beyond individuals' life histories (Reis-Santos *et al.*, 2018a; Tanner *et al.*, 2016). Genetic markers identify population connectivity via gene flow, functioning on a generational to evolutionary timescale rather than an ecological timescale where homogeneity can be maintained even with limited fish movement (Leis *et al.*, 2011). Genetic markers such as Single Nucleotide Polymorphisms (SNPs) are abundant and widely distributed across genomes (1000s to 100,000s of markers), and are effective at illustrating population structure and connectivity for species with high levels of potential movement and dispersal, including many marine fishes (Bernatchez *et al.*, 2017; Anderson and Garza 2006) and freshwater species (Biesack *et al.*, 2020; Attard *et al.*, 2017).

Combining natural markers provides an opportunity to mitigate marker-specific limitations, enhance analyses, and strengthen population connectivity estimates by integrating information from different sources. Previous studies have coupled otolith-based techniques (Martino *et al.*, 2017; Mirasole *et al.*, 2017; Ferguson *et al.*, 2011) as well as a suite of molecular approaches with nonmolecular markers (Reis-Santos *et al.*, 2018; Izzo *et al.*, 2017; Abaunza *et al.*, 2008). However, there is limited understanding of the integrative power of multi-marker approaches for assessing population connectivity.

Black bream *Acanthopagrus butcheri* (Munro 1949) are endemic to southern Australia, including Tasmania, where they support important commercial and recreational fisheries. Although completing much of their life cycle within estuaries (i.e., estuarine-dependent), *A. butcheri* inhabit rivers and coastal waters due to their tolerance of dramatic shifts in water salinity and temperature (Doubleday *et al.*, 2015; Partridge and Jenkins 2002).

Current understanding of *A. butcheri* movements is largely based on individual marker approaches, including otolith chemistry (Williams *et al.*, 2018; Elsdon and Gillanders 2005b, 2006), genetics (i.e., microsatellites and allozymes) (Burridge *et al.*, 2004; Chaplin *et al.*, 1998), and artificial markers (i.e., acoustic telemetry) (Tracey *et al.*, 2020; Williams *et al.*, 2017; Sakabe and Lyle 2010); and in most cases, is spatially restricted to single-estuary studies. Movement patterns determined from otolith chemistry are known to vary within and among populations, with individuals showing resident and/or migratory behaviour (i.e., partial migration) likely influenced by extrinsic factors (e.g., freshwater flow, tidal cycles) (Tracey *et al.*, 2020; Gillanders *et al.*, 2015; Sakabe and Lyle 2010). Furthermore, molecular approaches have shown genetic structuring among estuaries, with limited fish movement occurring between adjacent sites supporting the species' estuarine dependency (Burridge *et al.*, 2004; Chaplin *et al.*, 1998). This context of habitat-use and movement behaviour provides an opportunity to investigate the scales of marker variability, as well as the effectiveness of a multi-marker approach to characterise population structure and connectivity.

Currently, assessments of status and management for A. butcheri across southern Australia are undertaken for specific fisheries (SAFS 2020) due to a poorly understood biological stock structure. In South Australia (SA), two commercial fisheries target A. butcheri; the State-wide Marine Scalefish Fishery, currently classified as 'sustainable' (Smart et al., 2022); and the Lakes and Coorong Fishery, currently classified as 'depleted' (Earl 2023). Both fisheries are assessed independently as little is known of the true spatial utilisation of biological stocks that support these fisheries (i.e., they may be exploiting the same biological stock). This limits the capacity to effectively evaluate whether the current management strategies, including spatial and temporal restrictions on both recreational and commercial fishing, are adequate and reflect the true spatial scale of the exploited stock(s) (Earl 2023; Smart et al., 2022; Elsdon and Gillanders 2006). The misalignment of population dynamics and static management boundaries are recognised in other fish species and question the effectiveness of current management strategies (Reiss et al., 2009; Coetzee et al., 2008). Implementing a multimarker approach may inform on population connectivity and therefore the appropriate management scale, enable improved stock assessment, help reduce estimation biases, and support development of strategies to better align management units with biological stocks (Berger et al., 2021; Kerr et al., 2017; Reiss et al., 2009).

This study assessed the population structure and connectivity of *A. butcheri* from across south-eastern Australia using a multi-marker approach. We utilised a range of natural markers, including genetics, otolith shape, otolith isotopic composition, and otolith elemental composition to (1) determine the discriminatory power of each marker independently and through integration; and (2) determine the degree of connectivity among *A. butcheri* populations. We hypothesised that a combination of markers functioning on ecological and evolutionary timescales will increase the power and accuracy of site-level discrimination and connectivity estimates, relative to using each marker independently. Understanding the spatial extent and connectivity of *A. butcheri* populations, and the optimum marker for accurately discriminating among them, will enable biological stock delineation and help define the appropriate scales for fisheries management.

3.3 Materials and methods

Field sampling and sample preparation

Acanthopagrus butcheri were sampled in 12 locations across South Australia and western Victoria between April 2020 and October 2021 (Fig. 3.1). Sampling was opportunistic, with samples collected where possible through collaborative efforts with recreational anglers using a rod and line, along with targeted seine netting (The University of Adelaide animal ethics, approval S-2020-069) and commercial fishery catches (i.e., Lakes and Coorong commercial fishers). Each fish was measured for total length (TL, mm) and weighed (g). Small cubes (~2 mm³) of muscle tissue were extracted from below the dorsal fin and preserved in 100% ethanol for molecular analysis. Sex was not determined due to the species rudimentary hermaphroditism potentially influencing any interpretations of gender (Gray 2022). The sagittal otoliths were extracted, cleaned in ultrapure water (Milli-Q), dried, and stored in micro-centrifuge tubes.

High contrast images of whole sagittal otoliths were taken for shape analysis under a dissection microscope with reflected light; otoliths were oriented sulcus-side up, horizontally with rostrum to the left. The left otolith from each fish was prioritised where no damage or vateritic material was found, as this can alter otolith morphometrics (Vignon 2020). Otolith edge material was chipped off from the outside edge (i.e., representing the location of capture) at the tip of the rostrum (~0.8 µg), powdered and stored in glass vials ready for isotope analysis. The other otolith of the pair was embedded in epoxy resin (*Struers Epofix*), spiked with ~30 ppm indium and sectioned using a low-speed saw for chemical analysis. Twin blades were used to obtain 250-300 µm thin sections, which were then polished using a sequence of lapping film sandpaper sheets (30, 9 and 3 µm grades). The otolith sections were then mounted onto microscope slides using ~200 ppm indium-spiked crystal bond for elemental analysis and ageing. Indium spiking was used to ensure only otolith material was analysed.



Fig. 3.1 Map of South Australia and Victoria showing all 12 *A. butcheri* capture locations grouped by geographic regions, including Spencer Gulf (SG), Gulf St Vincent (GSV), Kangaroo Island (KI), and the eastern sites (CRV). Map includes inset of Australia.

Otolith ageing

Annual growth of otolith increments has been validated for *A. butcheri* (Sarre and Potter 2000). Fish were aged by counting opaque bands under a dissecting microscope with reflected light. Three independent readings by one reader were made for each otolith to determine the final age of the fish. Adjustments to age were made based on the distance of the last band from the otolith edge and the month of capture. *Acanthopagrus butcheri* become sexually mature between 2 and 4 years of age (Cheshire *et al.,* 2013; Norriss 2002), therefore, fish <2 years old (i.e., juveniles) were removed from subsequent analyses to reduce any potential ontogenetic influence on otolith shape and chemistry. Three fish from the Middle River were identified as juveniles and removed from the study.

Otolith chemistry

Otolith sections were analysed for elemental composition using Laser-Ablation Inductively-Coupled-Plasma Mass-Spectrometry (LA-ICP-MS) (*Agilent* 7900x with attached *RESOlution* LR 193nm Excimer laser system) for two minor elements (Sr and Ca) and eight trace elements (Li, Mg, Mn, Cu, Zn, In, Ba and Pb). Spot analysis (60 µm in diameter) was performed, targeting the otolith edge reflecting the location of capture, although this was likely to represent different periods of time (e.g., number of days) for different aged fish. Two replicate National Institute of Standards and Technology (NIST612) standards and two replicate United States Geological Service synthetic calcium carbonate (MACS-3) standards were run at the beginning and end of each session, along with two NIST612 ablations every 15 samples. NIST612 was used to correct for any drift in instrumentation over time and MACS-3 was used to measure external precision. The coefficient of variation ranged between 0.4 and 1.9% for NIST612, and between 2.2 and 4.1% for MACS-3 standards depending on the element.

Raw mass-spectrometer signals were transformed from counts per sec to ppm based on subtraction of background counts and analysis of standards in *lolite* (Paton *et al.*, 2011), followed by conversion to element:Ca ratios (μ mol mol⁻¹) based on a calcium concentration of 38.8% in otoliths (Yoshinaga *et al.*, 2000). Potential outliers were investigated by fitting linear models and assessing diagnostic plots for influential cases (*stats* package in *R*; R Core Team 2021). Diagnostic plots were also used to check for normality and balance of the data for each element:Ca. Data were transformed into natural logarithms for independent element:Ca visualisation and assessment.

Isotopic readings were collected using an Isotope Ratio Mass Spectrometer (IRMS) (*Nu Instruments* NuCarb) system calibrated for carbon (δ^{13} C) and oxygen (δ^{18} O). Raw signals were collected as isotope ratios (variation of isotope ratio in the carbonate, w.r.t. Pee Dee Belemnite standard [PDB]), and drift during analysis was monitored for precision. Both otolith element and isotope data were visualised as boxplots for each capture location.

Otolith shape

Using the *R* package '*Rshape*' (Wickham 2007), otolith outlines were extracted from each whole otolith image, along with multiple morphometric parameters (i.e., perimeter, area, length, and width). Outlines were then transformed into shape coefficients using discrete wavelet analysis. Wavelet transformation was chosen over elliptical Fourier analysis as it is more suited for approximating sharp edges (Libungan *et al.,* 2015b; Graps 1995). Each shape coefficient was adjusted for allometric relationships with fish length, and the quality of wavelet reconstruction was estimated by comparing the deviation from the otolith outline. An individual from Tumby Bay was removed due to an otolith shape abnormality making it unreadable by *ShapeR*. Average otolith outline for each

capture location was plotted, along with a corresponding boxplot showing the variability in otolith shape both within and among locations.

Molecular analysis

DNA extraction and sequencing were completed by Diversity Arrays Technology (DArT, Canberra), where SNP genetic markers were generated using restriction site-associated DNA sequencing (RAD-seq, medium-density, 1.2 million reads). Data were received from DArT as a 1-row binary score for each locus. The raw SNP data underwent a filtering pipeline similar to Junge *et al.*, (2019). Four filters were applied, namely (1) removal of loci with <95% reproducibility, (2) removal of loci with >20% missing data, (3) removal of ambiguous secondary loci (*e.g.*, retaining one of two separately recorded SNP loci for the same fragment), and (4) removal of monomorphic loci (*DArTr* package in *R*; Gruber *et al.*, 2018). Quality control of data, including identification and removal of closely related fish is described in the supplementary material. Independent assessment of this marker prior to integration with otolith-based techniques included calculating pairwise fixation index values (FST) as a genetic distance matrix to assess variance among locations (*StAMPP* package in *R*; Pembleton *et al.*, 2013); with FST values increasing from 0 to 1 indicating greater genetic differentiation (Frankham *et al.*, 2012). Filtered loci binary scores were stored as a Genlight object in *R* and therefore could not undergo the same analyses as the otolith-based datasets, therefore, the data were transformed into Principal Component (PC) scores.

Data integration and analysis

To enable natural marker integration and address an assumption of increased resolution of our results merely via additional variables, an independent Principal Component Analysis (PCA) was completed for each marker by transforming all data types into PC scores, including otolith element signatures (element:Ca ratios), otolith isotope signatures (isotope ratios) and otolith shape signatures (wavelet coefficients). Selecting the minimum required number of PC scores for each data set was completed using scree plots and dependent on the cumulative percentage of total variation (often between 70-90%, Jolliffe 2002). A cut-off of 70% cumulative total variation was chosen for all four markers.

To assess the discriminatory power of each natural marker, and the likelihood that individuals could be classified back to their location of capture, we ran permutational multivariate analysis of variance (PERMANOVA) and Canonical Analysis of Principle coordinates (CAP) (*PRIMER*; Anderson 2001). Both tests were applied to each marker data set independently and to combinations of all four data sets before data reduction via PCAs (i.e., raw data). Running PERMANOVA of raw element and isotope data required z-score normalisation (mean = 0, SD = 1), which was performed in *PRIMER* to account for differences in scale of element:Ca and isotope:Ca variables. PERMANOVA and CAP were

then applied to combinations of all four markers as PC scores to assess the optimum marker integration, and whether increasing the number of markers would provide greater discriminatory power.

3.4 Results

Otolith chemistry

A total of 154 *A. butcheri* were analysed across the 12 locations (Table 3.S1). Elemental data reflected variability among capture locations (multi-element PERMANOVA, MS = 52.18, F_{11,142} = 9.228, p = 0.001), with univariate PERMANOVAs indicating significant differences across locations for all element:Ca signatures (Table 3.S2). For example, Li:Ca concentrations were lower in locations further south (i.e., Eleanor River, Chapman River, Glenelg River, and Hopkins River), while locations further east (i.e., Port River and western VIC) appeared to show higher Zn:Ca levels (Fig. 3.S1). Ba:Ca concentrations were lower for locations within the SA gulfs (e.g., Tumby Bay, Port River, and West Lakes), whereas Pb:Ca concentrations were higher in estuaries closer to and within Gulf St Vincent. CAP correctly classified 59.1% of individuals to their capture location (Fig. 3.2a, Table 3.1). No individuals were successfully classified back to Middle River (0%), while locations with the highest correct classifications (≥70%) included Western River (100%), Port River (85.7%), and Robe (70%) (Table 3.S3).

Multivariate tests using isotope signatures revealed significant differences among locations (MS = 20.13, $F_{11,142}$ = 33.82, p = 0.001), with univariate PERMANOVAs indicating significant variation for both isotope signals (Table 3.S2). δ^{13} C concentrations reflected a strong geographic and latitudinal influence, with more enriched values in estuaries sampled further north and more depleted signals across Kangaroo Island and Victoria (Fig. 3.S1). Variability of δ^{18} O was comparatively weaker across locations, with depleted values again found in Victoria (Glenelg River and Hopkins River) (Fig. 3.S1). The multi-isotope signature correctly classified 57.8% of individuals to their capture location (Fig. 3.2b, Table 3.1). Locations with \geq 70% correct classification were Glenelg River (82.4%), Tumby Bay (80%), Middle River (75%), Port River (71.4%), all of which are scattered across the sampling distribution (Table 3.S4).

Both otolith element and isotope markers were most successful in discriminating among populations at greater distances (i.e., not adjacent estuaries), with the highest correct classifications scattered across the sampling distribution and located in Gulf St Vincent, Kangaroo Island, Spencer Gulf, or Coorong, Robe, and western Victoria.



Fig. 3.2 Canonical Analysis of Principal coordinates (CAP) plots for each investigated natural marker (a) otolith elemental chemistry, (b) otolith isotopic chemistry, (c) otolith shape, and (d) genetics. Data points represent individual *A. butcheri* grouped by capture location. Legend shows locations grouped by geographic regions Spencer Gulf (SG), Gulf St Vincent (GSV), Kangaroo Island (KI), and the eastern sites (CRV).

Table 3.1 Comparisons of integrated natural markers and individuals (N out of 154, and %) correctly classified to their capture locations based on a Canonical Analysis of Principal Coordinates (CAP). Markers utilised in each combination (rows) include otolith elemental chemistry (E), otolith isotopic chemistry (I), otolith shape (S) or genetics (G). Marker combinations based on PC scores (post PCA transformation and information reduction) are indicated with an asterisk (*). Combinations or single use of raw data are indicated as (*R*). Raw data include element (element:Ca ratio, µmol mol⁻¹), isotope (isotope:Ca ratio, w.r.t. Pee Dee Belemnite standard), shape (wavelet coefficient), and genetics (all PC scores prior to information reduction via scree plot).

	ma	rker		correct classification (x/154)	correct classification (%)
Е	I	S	G		
*		*	*	146	94.8
*	*	*	*	145	94.2
	*	*	*	145	94.2
		*	*	145	94.2
*	*		*	140	90.9
*			*	140	90.9
			R	137	89.0
	*		*	137	89.0
R	R			117	76.0
R	R	R		95	61.7
*	*			94	61.0
*	*	*		93	60.4
R				91	59.1
	R			89	57.8
R		R		89	57.8
*		*		85	55.2
	R	R		75	48.7
		R		64	41.6
	*	*		50	32.5

Otolith shape

Wavelet shape reconstruction of whole *A. butcheri* otoliths generated 63 shape coefficients that revealed morphometric variations among capture locations (Fig. 3.S2). Variability in mean otolith outlines was highest across the pseudo-excisura (0-60), antirostrum (120-180), and pseudo-rostrum (300-360) regions (Fig. 3.S2). PERMANOVA showed significant differences among capture locations (MS = 3.138, $F_{11,142}$ = 4.939, p = 0.001), although an overall weaker classification accuracy (41.6%) for the 12 locations than otolith chemistry (Fig. 3.2c, Table 3.1). Locations with the highest correct classification included Harriet River and Western River (\geq 70%), while no *A. butcheri* were correctly classified back to Eleanor River (Table 3.S5).

Molecular analysis

All individuals were retained during the data filtering pipeline, which removed 24,806 loci from the sequenced SNP data received from DArT (Table 3.S6). FST values showed consistent genetic differentiation across capture locations both within Kangaroo Island and between Kangaroo Island and mainland Australia (FST >0.10, Table 3.S7). Fish from mainland locations between Gulf St Vincent and Victoria generally showed genetic similarities. Fish from Tumby Bay had FST values >0.05 with fish from the Coorong, with FST increasing with distance to the east. Higher genetic differentiation (FST >0.15) was found between *A. butcheri* from Tumby Bay, Western River and Chapman River. The PCA generated 153 scores, which through PERMANOVA showed significant differences among locations (MS = 1277, $F_{11,142}$ = 2.923, p = 0.001), and via CAP correctly classified 89% of individuals to their capture location (Table 3.1). The CAP plot identified four main clusters, grouping locations in (1) northern Kangaroo Island, (2) southern Kangaroo Island, (3) between Spencer Gulf and Gulf St Vincent, and (4) eastern capture locations (Coorong, Robe, Glenelg River, and Hopkins River) (Fig. 3.2d). The majority of locations had 100% correct classification, except Hopkins River (95%), Coorong (85.7%), Tumby Bay (70%), Eleanor River (42.9%), and Port River (0%) (Table 3.S8).

Integrated markers

The minimum number of PC scores for each marker (i.e., cumulative variance >70%) included four element PCs, one isotope PC, 19 shape PCs and 87 genetic PCs (Fig. 3.S3). The strongest combination of natural markers based on CAP was element, shape, and genetics (94.8% correct classified) (Fig. 3.3), followed by an integration of all four; isotope, shape, and genetics; and shape coupled with genetics (all 94.2% correct classified) (Table 3.1). Genetics provided the clearest separation of locations both independently (i.e., all 153 PC scores) and through marker integration, especially boosted by otolith chemical markers, while combinations of only otolith-based techniques provided weak to moderate classification in separating all 12 estuaries (32.5-76.0% correct classified). Overall, integrations were generally more effective in discriminating among populations, relative to the independent use of each natural marker. Most integrated markers were shown to complement each other, except for isotope and shape markers, which had the lowest discrimination (32.5% correct classified). The PERMANOVA using the most powerful integration (i.e., element, shape, and genetics) showed significant differences among locations (MS = 1414, $F_{11,142}$ = 4.382, p = 0.001), with CAP showing all sites with classification accuracies >90%, except Eleanor River and Tumby Bay (both 71.4%) (Table 3.S9).



Fig. 3.3 Canonical Analysis of Principal coordinates (CAP) plot for three integrated markers; otolith element, otolith shape, and genetic. Legend shows capture locations grouped by geographic regions Spencer Gulf (SG), Gulf St Vincent (GSV), Kangaroo Island (KI), and the eastern sites (CRV).



cioseu winch may leau to mgner balca signais in fish otolithis round here relative to estuaries along

mainland Australia which typically remain open (Rumbelow *et al.*, 2010). Water pollution has also been linked to otolith Pb:Ca and Zn:Ca (Søndergaard *et al.*, 2015; McKinley *et al.*, 2012), both of which were found in higher concentrations across metropolitan locations relative to rural or more isolated Kangaroo Island estuaries. A latitudinal gradient was found across the sampling range for both δ^{13} C and Li:Ca signatures, with higher concentrations further north likely influenced by higher water temperatures, as previously seen in *A. Butcheri* and other fish species (Elsdon and Gillanders 2002; Stanley *et al.*, 2015). Finding higher discrimination at greater distances for both multi-element and multi-isotope signatures supports their environmental influence, with adjacent estuaries likely more similar in environmental conditions relative to those at greater distances (e.g., at 100s kms).

The last annual otolith increment was targeted to analyse both chemical markers, although the variation in time (e.g., number of days) this area represents can vary among different aged fish (e.g., a decrease in increment width as fish grow older; Chapter 4; Morrongiello *et al.*, 2021; Martino *et al.*, 2019), thereby possibly reducing the accuracy of our results when interpreting such signals as a reflection of the location of capture. Such a limitation further stresses the potential benefit of integrating additional tagging techniques functioning at different spatiotemporal resolutions.

Otolith shape was a weak marker for *A. butcheri* at this spatial scale and to separate 12 estuaries. Areas of variability along the otolith outline were consistent with previous findings for other species, including the antirostrum in jack mackerel (*Trachurus picturatus*, Moreira *et al.*, 2019), the pseudo-rostrum in Atlantic herring (*Clupea harengus*, Libungan *et al.*, 2015a), and a combination of both in Atlantic cod (*Gadus morhua*, Stransky *et al.*, 2008). Age can influence otolith shape analysis as ontogenetic otolith shape formation is allometric and cannot be easily standardised – a known limitation of shape metrics (Wei and Zhu 2022; Vignon *et al.*, 2012; Campana and Casselman 1993). Whilst we did not include juveniles in this study and most fish were 4-6 years old, locations with larger fish (i.e., Coorong) and the smallerfish (i.e., Chapman River and Western River) showed some of the highest correct classification using otolith shape. *Acanthopagrus butcheri* is known to vary in size and growth rate across its distribution, including the average length-at-maturity from Western Australia to New South Wales (Cheshire *et al.*, 2013; Norriss *et al.*, 2002; Sarre and Potter 1999), with intrinsic factors likely playing a role in the variability of this natural marker.

Genetic markers provided the strongest discrimination among capture locations, with genetic differentiation primarily among Kangaroo Island locations and between Kangaroo Island and the mainland. Depth, currents and other barriers of dispersal and movement are likely limiting gene flow across the Kangaroo Island locations, as well as between Kangaroo Island and the mainland (i.e., Investigator Strait and Backstairs Passage) (Volk *et al.*, 2021; Hammer *et al.*, 2021). This degree of connectivity is likely associated with the frequently closed estuarine systems along Kangaroo Island

increasing genetic isolation between adjacent estuaries (Rumbelow *et al.*, 2010), while the more permanently open and connected systems along the mainland are more likely to promote gene flow within Spencer Gulf, Gulf St Vincent, and eastern locations (Coorong, Robe, Glenelg River and Hopkins River). The limited gene flow coupled with the species estuarine dependency made this the strongest marker (89% correct classification), and more accurate than the otolith chemical markers (76%) (Table 3.1).

Overall, we found that the integration of natural fish markers provided a clear and more powerful discrimination among *A. butcheri* populations than their independent application. The combination of genetics with any otolith-based technique(s) provided the highest resolution in population structuring. While a combination of only otolith-based markers provided good discriminations, they were weaker relative to the molecular approach. A study by Izzo *et al.* (2017) identified similar patterns, with genetics providing a consistently clear structuring of populations, while integrated otolith-based techniques although successful were relatively weaker. Our integration of only otolith-based techniques mitigates any assumption that merely increasing the number of variables would increase the accuracy of our results, in addition to the optimum marker combination only consisting of three out of the four markers assessed (otolith element, otolith shape, and genetics).

Multi-marker signatures among locations greatly varied with each marker combination, emphasising the importance of appropriate marker selection. The complementary integration of markers can be attributed to the diverse and marker-specific drivers of this variability. For example, otolith markers were most likely influenced by combinations of environmental factors (e.g., water chemistry, temperature, salinity) and associated intrinsic factors (ontogeny, metabolism), and genetics by gene flow linked to dispersal and movements among nearby estuaries (i.e., similarities more common between adjacent estuaries). Similar advantages of harnessing information from complementary markers has been found in previous multi-marker studies, including from observations of genetic differentiation driven by oceanographic characteristics and latitudinal patterns, or variation in otolith chemistry or migratory movements driven by location and fish growth, respectively (Reis-Santos *et al.*, 2018a; Abaunza *et al.*, 2008).

We identified minimal dispersal of *A. butcheri* along the sampling range, with markers indicating differences among populations suggesting some form of isolation. Our findings support the species' estuarine dependency with the occasional movement of individuals between adjacent estuaries across either mainland capture locations or Kangaroo Island locations, associated with the species known partial migration behaviour (Gillanders *et al.*, 2015). Population structuring of *A. butcheri* has been previously identified in south-eastern Australia, as well as the species' estuarine

dependency and limited movement occurring primarily between adjacent estuaries (Burridge *et al.*, 2004; Chaplin *et al.*, 1998). In terms of implications for management, our results suggest limited fish movement between the two major fisheries in South Australia (i.e., the State-wide Marine Scalefish Fishery and the Lakes and Coorong Fishery), although some connectivity between the Coorong and the adjacent Robe population was evident. Further investigation into the degree of connectivity present here should be considered to support reassessing *A. butcheri* management within this region, particularly given the current 'depleted' status of the Coorong population. Overall, the limited connectivity of *A. butcheri* populations across South Australia and western Victoria highlights the need for location-specific management for *A. butcheri* across South Australia and Victoria be reviewed to strengthen the link between management units and the boundaries of biological stocks (Berger *et al.*, 2021; Reiss *et al.*, 2009).

The inferences made from each marker, including the best combination of markers, are a reflection of *A. butcheri* and likely context specific. Nonetheless, the key drivers influencing marker variability provide valuable information that can be harnessed for application on similar species, spatial scales, or studies with low sampling effort. Species inhabiting similar and more homogenised environments (e.g., deep water fish) may benefit from utilising genetic markers, whereas species with high somatic variability across their distribution could implement otolith morphometrics. The generational scale genetics provides goes beyond the ecological "noise" often found in otolith-based markers, thereby providing unique tracking information that has worked effectively at this scale for *A. butcheri*, and more so when combined with other natural markers. Given the costs of obtaining information from multiple natural markers as the ones used in this study, it is important to first identify the markers that best suit the target species and the spatiotemporal range of interest. This is the most appropriate and cost-effective approach to provide a more accurate estimate of population structure and connectivity, considering our results show that merely adding markers is not always advantageous. This study shows the benefits of utilising common natural markers found in fish and their effectiveness through integration.

References

- Abaunza, P, Murta, AG, Campbell, N, Cimmaruta, R, Comesaña, AS, Dahle, G, García Santamaría, MT, Gordo, LS, Iversen, SA, MacKenzie, K, Magoulas, A, Mattiucci, S, Molloy, J, Nascetti, G, Pinto, AL, Quinta, R, Ramos, P, Sanjuan, A, Santos, AT, Stransky, C, Zimmermann, C (2008) Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. *Fisheries Research* 89, 196-209. doi:10.1016/j.fishres.2007.09.022
- Allemand, D, Mayer-Gostan, N, De Pontual, H, Boeuf, G, Payan, P (2007) Fish otolith calcification in relation to endolymph chemistry. In 'Handbook of Biomineralization.' Vol. 1 pp. 291-308.
- Amoutchi, AI, Kersten, P, Vogt, A, Kohlmann, K, Kouamelan, EP, Mehner, T (2023) Population genetics of the African snakehead fish *Parachanna obscura* along West Africa's water networks: Implications for sustainable management and conservation. *Ecology and Evolution* **13**, e9724. doi:10.1002/ece3.9724
- Anderson, EC, Garza, JC (2006) The power of single-nucleotide polymorphisms for large-scale parentage inference. *Genetics* **172**, 2567-82. doi:10.1534/genetics.105.048074
- Anderson, MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 626-639. doi:DOI 10.1139/cjfas-58-3-626
- Assis, IO, da Silva, VEL, Souto-Vieira, D, Lozano, AP, Volpedo, AV, Fabre, NN (2020) Ecomorphological patterns in otoliths of tropical fishes: assessing trophic groups and depth strata preference by shape. *Environmental Biology of Fishes* **103**, 349-361. doi:10.1007/s10641-020-00961-0
- Attard, CRM, Brauer, CJ, Sandoval-Castillo, J, Faulks, LK, Unmack, PJ, Gilligan, DM, Beheregaray, LB (2018) Ecological disturbance influences adaptive divergence despite high gene flow in golden perch (*Macquaria ambigua*): Implications for management and resilience to climate change. *Molecular Ecology* **27**, 196-215. doi:10.1111/mec.14438
- Ávila-Herrera, CL, Pacheco-Almanzar, E, Guevara-Chumacero, LM, Velázquez-Aragón, JA, Serrato-Díaz, A, Ramírez-Saad, H, Ibáñez, AL (2021) Past and recent connectivity of white mullet between the Gulf of Mexico and the Mexican Pacific inferred through sequences of the gene cytochrome *c* oxidase I and microsatellites. *Marine Biology* **168**, doi:10.1007/s00227-021-03907-x
- Bardarson, H, McAdam, BJ, Thorsteinsson, V, Hjorleifsson, E, Marteinsdottir, G (2017) Otolith shape differences between ecotypes of Icelandic cod (*Gadus morhua*) with known migratory behaviour inferred from data storage tags. *Canadian Journal of Fisheries and Aquatic Sciences* **74**, 2122-2130. doi:10.1139/cjfas-2016-0307
- Barnuevo, KDE, Morales, CJC, Calizo, JKS, Delloro, ES, Añasco, CP, Babaran, RP, Lumayno, SDP (2022) Distinct Stocks of the Redtail Scad *Decapterus kurroides* Bleeker, 1855 (Perciformes: Carangidae) from the Northern Sulu and Southern Sibuyan Seas, Philippines Revealed from Otolith Morphometry and Shape Analysis. *Fishes* 8, doi:10.3390/fishes8010012
- Bath, GE, Thorrold, SR, Jones, CM, Campana, SE, McLaren, JW, Lam, JWH (2000) Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* **64**, 1705-1714. doi:10.1016/s0016-7037(99)00419-6
- Berger, AM, Deroba, JJ, Bosley, KM, Goethel, DR, Langseth, BJ, Schueller, AM, Hanselman, DH, Bartolino, V (2021) Incoherent dimensionality in fisheries management: consequences of misaligned stock assessment and population boundaries. *ICES Journal of Marine Science* 78, 155-171. doi:10.1093/icesjms/fsaa203
- Bernatchez, L, Wellenreuther, M, Araneda, C, Ashton, DT, Barth, JMI, Beacham, TD, Maes, GE, Martinsohn, JT, Miller, KM, Naish, KA, Ovenden, JR, Primmer, CR, Young Suk, H, Therkildsen, NO, Withler, RE (2017) Harnessing the Power of Genomics to Secure the Future of Seafood. *Trends in Ecology & Evolution* 32, 665-680. doi:10.1016/j.tree.2017.06.010
- Biesack, EE, Dang, BT, Ackiss, AS, Bird, CE, Chheng, P, Phounvisouk, L, Truong, OT, Carpenter, KE (2020) Evidence for population genetic structure in two exploited Mekong River fishes across a natural riverine barrier. *Journal of Fish Biology* **97**, 696-707. doi:10.1111/jfb.14424

- Burridge, CP, Hurt, AC, Farrington, LW, Coutin, PC, Austin, CM (2004) Stepping stone gene flow in an estuarine-dwelling sparid from south-east Australia. *Journal of Fish Biology* **64**, 805-819. doi:10.1111/j.1095-8649.2004.0347.x
- Burridge, CP, Versace, VL (2006) Population genetic structuring in *Acanthopagrus butcheri* (*Pisces: Sparidae*): does low gene flow among estuaries apply to both sexes? *Marine Biotechnology* **9**, 33-44. doi:10.1007/s10126-006-6023-7
- Campana, SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* **188**, 263-297. doi:DOI 10.3354/meps188263
- Campana, SE, Casselman, JM (1993) Stock discrimination using otolith shape-analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 1062-1083. doi:DOI 10.1139/f93-123
- Cardinale, M, Doering-Arjes, P, Kastowsky, M, Mosegaard, H (2004) Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 158-167. doi:10.1139/F03-151
- Castillo, GC, Sandford, ME, Hung, TC, Tigan, G, Lindberg, JC, Yang, WR, Van Nieuwenhuyse, EE (2018) Using Natural Marks to Identify Individual Cultured Adult Delta Smelt. *North American Journal of Fisheries Management* **38**, 698-705. doi:10.1002/nafm.10066
- Chaplin JA, BG, Gill HS, McCullock R, Potter IC (1998) Are assemblages of black bream (*Acanthopagrus butcheri*) in different estuaries genetically distinct? *International Journal of Salt Lake Research* **6**, 303-321.
- Cheshire, KJM, Ye, Q, Fredberg, J, Earl, J, 2013. Aspects of reproductive biology of five key fish species in the Murray Mouth and Coorong. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Coetzee, JC, van der Lingen, CD, Hutchings, L, Fairweather, TP (2008) Has the fishery contributed to a major shift in the distribution of South African sardine? *ICES Journal of Marine Science* **65**, 1676-1688. doi:10.1093/icesjms/fsn184
- Doubleday, ZA, Izzo, C, Haddy, JA, Lyle, JM, Ye, Q, Gillanders, BM (2015) Long-term patterns in estuarine fish growth across two climatically divergent regions. *Oecologia* **179**, 1079-90. doi:10.1007/s00442-015-3411-6
- Dwivedi, AK (2022) Detection of body shape variations in vulnerable snow trout, *Schizothorax richardsonii* (Gray, 1832) from rivers of the Indian Himalayan Region. *Limnologica* **97**, doi:10.1016/j.limno.2022.126025
- Earl, J, 2018. Fishery statistics and performance indicators for the South Australian Lakes and Coorong Fishery. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Earl, J, 2020. Assessment of the South Australian Lakes and Coorong Fishery in 2018/19. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Elsdon, TS, Gillanders, BM (2002) Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 1796-1808. doi:10.1139/F02-154
- Elsdon, TS, Gillanders, BM (2005) Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. *Marine Ecology Progress Series* **285**, 233-243. doi:DOI 10.3354/meps285233
- Elsdon, TS, Gillanders, BM (2005) Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 1143-1152. doi:10.1139/f05-029
- Elsdon, TS, Gillanders, BM (2006) Identifying migratory contingents of fish by combining otolith Sr:Ca with temporal collections of ambient Sr:Ca concentrations. *Journal of Fish Biology* **69**, 643-657. doi:10.1111/j.1095-8649.2006.01136.x
- Ferguson, GJ, Ward, TM, Gillanders, BM (2011) Otolith shape and elemental composition: Complementary tools for stock discrimination of mulloway (*Argyrosomus japonicus*) in southern Australia. *Fisheries Research* **110**, 75-83. doi:10.1016/j.fishres.2011.03.014

- Frankham, R, Ballou, JD, Briscoe, DA, McInnes, KH (2012) 'Introduction to Conservation Genetics.' (Cambridge University Press:
- Gillanders, BM, Izzo, C, Doubleday, ZA, Ye, Q (2015) Partial migration: growth varies between resident and migratory fish. *Biology Letters* **11**, doi:10.1098/rsbl.2014.0850
- Gray, CA (2022) Variation in growth, length and age characteristics of estuarine *Acanthopagrus* (Sparidae) populations in New South Wales, Australia. *Regional Studies in Marine Science* **55**, doi:10.1016/j.rsma.2022.102481
- Gruber, B, Unmack, PJ, Berry, OF, Georges, A (2018) dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources* **18**, 691-699. doi:10.1111/1755-0998.12745
- Hamer, PA, Jenkins, GP, Coutin, P (2006) Barium variation in *Pagrus auratus* (Sparidae) otoliths: A potential indicator of migration between an embayment and ocean waters in south-eastern Australia. *Estuarine Coastal and Shelf Science* **68**, 686-702. doi:10.1016/j.ecss.2006.03.017
- Hammer, MP, Adams, M, Unmack, PJ, Hassell, KL, Bertozzi, T (2021) Surprising Pseudogobius: Molecular systematics of benthic gobies reveals new insights into estuarine biodiversity (Teleostei: Gobiiformes). *Molecular Phylogenetics and Evolution* **160**, 107140. doi:10.1016/j.ympev.2021.107140
- Hüssy, K (2008) Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects. Journal of Experimental Marine Biology and Ecology **364**, 35-41. doi:10.1016/j.jembe.2008.06.026
- Izzo, C, Reis-Santos, P, Gillanders, BM (2018) Otolith chemistry does not just reflect environmental conditions: A meta-analytic evaluation. *Fish and Fisheries* **19**, 441-454. doi:10.1111/faf.12264
- Izzo, C, Ward, TM, Ivey, AR, Suthers, IM, Stewart, J, Sexton, SC, Gillanders, BM (2017) Integrated approach to determining stock structure: implications for fisheries management of sardine, *Sardinops sagax*, in Australian waters. *Reviews in Fish Biology and Fisheries* **27**, 267-284. doi:10.1007/s11160-017-9468-z
- Jolliffe, IT (2002) 'Principal Component Analysis.' (Springer: New York)
- Junge, C, Donnellan, SC, Huveneers, C, Bradshaw, CJA, Simon, A, Drew, M, Duffy, C, Johnson, G, Cliff, G, Braccini, M, Cutmore, SC, Butcher, P, McAuley, R, Peddemors, V, Rogers, P, Gillanders, BM (2019) Comparative population genomics confirms little population structure in two commercially targeted carcharhinid sharks. *Marine Biology* **166**, doi:10.1007/s00227-018-3454-4
- Kerr, LA, Hintzen, NT, Cadrin, SX, Clausen, LW, Dickey-Collas, M, Goethel, DR, Hatfield, EMC, Kritzer, JP, Nash, RDM, Hidalgo, M (2017) Lessons learned from practical approaches to reconcile mismatches between biological population structure and stock units of marine fish. *ICES Journal of Marine Science* 74, 1708-1722. doi:10.1093/icesjms/fsw188
- Khan, U, Bal, H, Battal, ZS, Seyhan, K (2022) Using otolith and body shape to discriminate between stocks of European anchovy (Engraulidae: *Engraulis encrasicolus*) from the Aegean, Marmara and Black Seas. *Journal of Fish Biology* **101**, 1452-1465. doi:10.1111/jfb.15216
- Leis JM, HL, Patterson HM (2011) Estimating connectivity in marine fish populations: What works best? Oceanography and Marine biology **49**, 193-234.
- Libungan, LA, Oskarsson, GJ, Slotte, A, Jacobsen, JA, Palsson, S (2015) Otolith shape: a population marker for Atlantic herring *Clupea harengus*. *Journal of Fish Biology* **86**, 1377-95. doi:10.1111/jfb.12647
- Libungan, LA, Palsson, S (2015) ShapeR: an R package to study otolith shape variation among fish populations. *PLoS ONE* **10**, e0121102. doi:10.1371/journal.pone.0121102
- Luo, YT, Fang, DA, Zhou, YF, Xu, DP, Peng, YX, Zhang, MY, Mao, CC, Tang, XM, Xu, J, You, Y (2022) Genetic diversity, habitat relevance and conservation strategies of the silver carp in the Yangtze River by simple sequence repeat. *Frontiers in Ecology and Evolution* **10**, doi:10.3389/fevo.2022.850183
- Magson, K, Monacella, E, Scott, C, Buffat, N, Arunrugstichai, S, Chuangcharoendee, M, Pierce, SJ, Holmberg, J, Araujo, G (2022) Citizen science reveals the population structure and seasonal presence of whale sharks in the Gulf of Thailand. *Journal of Fish Biology* **101**, 540-549. doi:10.1111/jfb.15121
- Martinho, F, Pina, B, Nunes, M, Vasconcelos, RP, Fonseca, VF, Crespo, D, Primo, AL, Vaz, A, Pardal, MA, Gillanders, BM, Tanner, SE, Reis-Santos, P (2020) Water and otolith chemistry: Implications for discerning estuarine nursery habitat use of a juvenile flatfish. *Frontiers in Marine Science* **7**, doi:10.3389/fmars.2020.00347

- Martino, J, Doubleday, ZA, Woodcock, SH, Gillanders, BM (2017) Elevated carbon dioxide and temperature affects otolith development, but not chemistry, in a diadromous fish. *Journal of Experimental Marine Biology and Ecology* **495**, 57-64. doi:10.1016/j.jembe.2017.06.003
- Martino, JC, Fowler, AJ, Doubleday, ZA, Grammer, GL, Gillanders, BM (2019) Using otolith chronologies to understand long-term trends and extrinsic drivers of growth in fisheries. *Ecosphere* **10**, e02553. doi:10.1002/ecs2.2553
- McKinley, AC, Taylor, MD, Johnston, EL (2012) Relationships between body burdens of trace metals (As, Cu, Fe, Hg, Mn, Se, and Zn) and the relative body size of small tooth flounder (*Pseudorhombus jenynsii*). *Science of The Total Environment* **423**, 84-94. doi:10.1016/j.scitotenv.2012.02.007
- Mirasole, A, Gillanders, BM, Reis-Santos, P, Grassa, F, Capasso, G, Scopelliti, G, Mazzola, A, Vizzini, S (2017) The influence of high *p*CO2 on otolith shape, chemical and carbon isotope composition of six coastal fish species in a Mediterranean shallow CO2 vent. *Marine Biology* **164**, 1-15. doi:10.1007/s00227-017-3221-y
- Mohan, JA, Dewar, H, Snodgrass, OE, Miller, NR, Tanaka, Y, Ohshimo, S, Rooker, JR, Francis, M, Wells, RJD (2022) Otolith geochemistry reflects life histories of Pacific bluefin tuna. *PLoS ONE* **17**, e0275899. doi:10.1371/journal.pone.0275899
- Morales-Nin, B, Perez-Mayol, S, MacKenzie, K, Catalan, IA, Palmer, M, Kersaudy, T, Mahe, K (2022) European hake (*Merluccius merluccius*) stock structure in the Mediterranean as assessed by otolith shape and microchemistry. *Fisheries Research* **254**, doi:10.1016/j.fishres.2022.106419
- Moreira, C, Froufe, E, Vaz-Pires, P, Correia, AT (2019) Otolith shape analysis as a tool to infer the population structure of the blue jack mackerel, *Trachurus picturatus*, in the NE Atlantic. *Fisheries Research* **209**, 40-48. doi:https://doi.org/10.1016/j.fishres.2018.09.010
- Morrison, AL, Swierczek, Z, Gulson, BL (2016) Visualisation and quantification of heavy metal accessibility in smelter slags: The influence of morphology on availability. *Environmental Pollution* **210**, 271-81. doi:10.1016/j.envpol.2015.11.030
- Morrongiello, JR, Horn, PL, C, OM, Sutton, PJH (2021) Synergistic effects of harvest and climate drive synchronous somatic growth within key New Zealand fisheries. *Global Change Biology* **27**, 1470-1484. doi:10.1111/gcb.15490

Munro, ISR (1949) 'Revision of Australian Silver Breams: Mylio and Rhabdosargus.' (Queensland Museum:

- Norriss, JV, Tregonning, JE, Lenanton, RCJ, Sarre, GA, 2002. Biological synopsis of the black bream, *Acanthopagrus butcheri* (Munro) (Teleostei: Sparidae) in Western Australia with reference to information from other southern states. Department of Fisheries, Perth, Western Australia.
- Panella, G (1971) Fish otoliths: daily growth layers and periodical patterns. *Science* **173**, 1124-7. doi:10.1126/science.173.4002.1124
- Partridge, GJ, Jenkins, GI (2002) The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). *Aquaculture* **210**, 219-230. doi:10.1016/S0044-8486(01)00817-1
- Paton, C, Hellstrom, J, Paul, B, Woodhead, J, Hergt, J (2011) Iolite: Freeware for the visualisation and processing of mass spectrometric data. *Journal of Analytical Atomic Spectrometry* **26**, 2508-2518. doi:10.1039/c1ja10172b
- Pazmiño, DA, Maes, GE, Simpfendorfer, CA, Salinas-de-León, P, van Herwerden, L (2017) Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile Galapagos shark (*Carcharhinus galapagensis*). *Conservation Genetics* **18**, 1151-1163. doi:10.1007/s10592-017-0967-1
- Pembleton, LW, Cogan, NO, Forster, JW (2013) StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology Resources* **13**, 946-52. doi:10.1111/1755-0998.12129
- R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
- Reis-Santos, P, Gillanders, BM, Sturrock, AM, Izzo, C, Oxman, DS, Lueders-Dumont, JA, Hüssy, K, Tanner, SE,
 Rogers, T, Doubleday, ZA, Andrews, AH, Trueman, C, Brophy, D, Thiem, JD, Baumgartner, LJ, Willmes,
 M, Chung, M-T, Charapata, P, Johnson, RC, Trumble, S, Heimbrand, Y, Limburg, KE, Walther, BD (2022)

Reading the biomineralized book of life: expanding otolith biogeochemical research and applications for fisheries and ecosystem-based management. *Reviews in Fish Biology and Fisheries* **33**, 411-449. doi:10.1007/s11160-022-09720-z

- Reis-Santos, P, Tanner, SE, Aboim, MA, Vasconcelos, RP, Laroche, J, Charrier, G, Perez, M, Presa, P, Gillanders, BM, Cabral, HN (2018) Reconciling differences in natural tags to infer demographic and genetic connectivity in marine fish populations. *Scientific Reports* **8**, 10343. doi:10.1038/s41598-018-28701-6
- Reis-Santos, P, Vasconcelos, RP, Tanner, SE, Fonseca, VF, Cabral, HN, Gillanders, BM (2018) Extrinsic and intrinsic factors shape the ability of using otolith chemistry to characterize estuarine environmental histories. *Marine Environmental Research* **140**, 332-341. doi:10.1016/j.marenvres.2018.06.002
- Reiss, H, Hoarau, G, Dickey-Collas, M, Wolff, WJ (2009) Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries* **10**, 361-395. doi:10.1111/j.1467-2979.2008.00324.x
- Rogers, TA, Fowler, AJ, Steer, MA, Gillanders, BM (2019) Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry. *Marine and Freshwater Research* **70**, -. doi:10.1071/mf18280
- Rowell, K, Dettman, DL, Dietz, R (2010) Nitrogen isotopes in otoliths reconstruct ancient trophic position. *Environmental Biology of Fishes* **89**, 415-425. doi:10.1007/s10641-010-9687-9
- Rumbelow, K, Speziali, A, Bloomfield, A, 2010. Working Towards a Statewide Inventory of Estuaries: Advancing the
- Inventory of Estuaries in Five NRM Regions of South Australia. Adelaide.
- SAFS (2020) 'Black bream' Available at https://fish.gov.au/report/366-Black-Bream-2020 [Accessed 16/01/24]
- Sakabe, R, Lyle, JM (2010) The influence of tidal cycles and freshwater inflow on the distribution and movement of an estuarine resident fish *Acanthopagrus butcheri*. *Journal of Fish Biology* **77**, 643-60. doi:10.1111/j.1095-8649.2010.02703.x
- Sarakinis, KG, Taylor, MD, Johnson, DD, Gillanders, BM (2022) Determining population structure and connectivity through otolith chemistry of stout whiting, *Sillago robusta*. *Fisheries Management and Ecology* **29**, 760-773. doi:10.1111/fme.12577
- Sarre, GA, Potter, IC (1999) Comparisons between the reproductive biology of black bream *Acanthopagrus butcheri* (Teleostei: Sparidae) in four estuaries with widely differing characteristics. *International Journal of Salt Lake Research* **8**, 179-210.
- Smoliński, S, Schade, FM, Berg, F (2020) Assessing the performance of statistical classifiers to discriminate fish stocks using fourier analysis of otolith shape. *Canadian Journal of Fisheries and Aquatic Sciences* 77, 674-683. doi:10.1139/cjfas-2019-0251
- Søndergaard, J, Halden, N, Bach, L, Gustavson, K, Sonne, C, Mosbech, A (2015) Otolith Chemistry of Common Sculpins (*Myoxocephalus scorpius*) in a Mining Polluted Greenlandic Fiord (Black Angel Lead-Zinc Mine, West Greenland). *Water, Air, & Soil Pollution* **226**, 336. doi:10.1007/s11270-015-2605-1
- Stanley, RRE, Bradbury, IR, DiBacco, C, Snelgrove, PVR, Thorrold, SR, Killen, SS (2015) Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). *ICES Journal of Marine Science* **72**, 2350-2363. doi:10.1093/icesjms/fsv070
- Steer, MA, Fowler, AJ, McGarvery, R, Feenstra, J, Smart, J, Rogers, PJ, Earl, J, Beckmann, C, Drew, M, Matthews, D, 2018. Assessment of the South Australian Marine Scalefish Fishery in 2017. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Steer, MA, Rogers, PJ, Bailleul, F, Earl, J, 2020. Assessment of the South Australiann Marine Scalefish Fishery in 2018. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Stransky, C, Baumann, H, Fevolden, S-E, Harbitz, A, Høie, H, Nedreaas, KH, Salberg, A-B, Skarstein, TH (2008)
 Separation of Norwegian coastal cod and Northeast Arctic cod by outer otolith shape analysis.
 Fisheries Research 90, 26-35. doi:10.1016/j.fishres.2007.09.009
- Sturrock, AM, Hunter, E, Milton, JA, Johnson, RC, Waring, CP, Trueman, CN, Eimf (2015) Quantifying physiological influences on otolith microchemistry. *Methods in Ecology and Evolution* **6**, 806-816. doi:10.1111/2041-210x.12381

- Sturrock, AM, Trueman, CN, Milton, JA, Waring, CP, Cooper, MJ, Hunter, E (2014) Physiological influences can outweigh environmental signals in otolith microchemistry research. *Marine Ecology Progress Series* 500, 245-264. doi:10.3354/meps10699
- Tanner, SE, Reis-Santos, P, Cabral, HN (2016) Otolith chemistry in stock delineation: A brief overview, current challenges and future prospects. *Fisheries Research* **173**, 206-213. doi:10.1016/j.fishres.2015.07.019
- Taylor, MD, Becker, A, Quinn, J, Lowry, MB, Fielder, S, Knibb, W (2020) Stock structure of dusky flathead (*Platycephalus fuscus*) to inform stocking management. *Marine and Freshwater Research* 71, 1378-1383. doi:10.1071/Mf19364
- Toews, DP, Brelsford, A (2012) The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* **21**, 3907-30. doi:10.1111/j.1365-294X.2012.05664.x
- Tracey, SR, Hartmann, K, McAllister, J, Lyle, JM (2020) Home range, site fidelity and synchronous migrations of three co-occurring, morphologically distinct estuarine fish species. *Science of The Total Environment* **713**, 136629. doi:10.1016/j.scitotenv.2020.136629
- Vaux, F, Rasmuson, LK, Kautzi, LA, Rankin, PS, Blume, MTO, Lawrence, KA, Bohn, S, O'Malley, KG (2019) Sex matters: Otolith shape and genomic variation in deacon rockfish (*Sebastes diaconus*). *Ecology and Evolution* 9, 13153-13173. doi:10.1002/ece3.5763
- Vignon, M (2012) Ontogenetic trajectories of otolith shape during shift in habitat use: Interaction between otolith growth and environment. *Journal of Experimental Marine Biology and Ecology* **420-421**, 26-32. doi:10.1016/j.jembe.2012.03.021
- Vignon, M (2020) When the presence of a vateritic otolith has morphological effect on its aragonitic partner: trans-lateral compensation induces bias in microecological patterns in one-side-only vateritic otolith. *Canadian Journal of Fisheries and Aquatic Sciences* **77**, 285-294. doi:10.1139/cjfas-2019-0066
- Volk, DR, Konvalina, JD, Floeter, SR, Ferreira, CEL, Hoffman, EA (2021) Going against the flow: Barriers to gene flow impact patterns of connectivity in cryptic coral reef gobies throughout the western Atlantic. *Journal of Biogeography* 48, 427-439. doi:10.1111/jbi.14010
- Wei, X, Zhu, G (2022) Shape and ontogenetic changes in otolith of the ocellated icefish (*Chionodraco rastrospinosus*) from the Bransfield Strait, Antarctic. *Zoology* **153**, 126025. doi:10.1016/j.zool.2022.126025
- Wickham H (2007). Reshaping data with the reshape package. *Journal of Statistical Software*, **21**, <u>https://www.jstatsoft.org/v21/i12/</u>.
- Williams, J, Hindell, JS, Jenkins, GP, Tracey, S, Hartmann, K, Swearer, SE (2017) The influence of freshwater flows on two estuarine resident fish species show differential sensitivity to the impacts of drought, flood and climate change. *Environmental Biology of Fishes* **100**, 1121-1137. doi:10.1007/s10641-017-0632-z
- Williams, J, Jenkins, GP, Hindell, JS, Swearer, SE (2018) Fine-scale variability in elemental composition of estuarine water and otoliths: Developing environmental markers for determining larval fish dispersal histories within estuaries. *Limnology and Oceanography* **63**, 262-277. doi:10.1002/lno.10627
- Yoshinaga, J, Nakama, A, Morita, M, Edmonds, JS (2000) Fish otolith reference material for quality assurance of chemical analyses. *Marine Chemistry* **69**, 91-97. doi:10.1016/S0304-4203(99)00098-5

Supplementary Material

Table 3.S1. Summary of each *A. butcheri* capture location. Table includes collection date, sample sizes, methods of capture, and fish meta data (weight, length and age) with standard deviation. Body weight data were not collected from Victoria as only fish frames were received.

state	location		collection date	sample size	method of capture	body weight (g)		total length (mm)		age	
	1	Western River	Apr, Dec 2020	10	net	51.8	±13.3	154.4	±12.4	3.0	±0.7
South Australia	2	Middle River	Apr, Dec 2020	8	net	65.8	±23.1	158.4	±18.4	2.9	±1.0
	3	Harriet River	May, Dec 2020	11	mix	134.9	±53.4	202.6	±25.7	3.7	±1.4
	4	Eleanor River	Dec-20	7	rod	130.0	±64.2	199.0	±32.7	3.9	±1.1
	5	Chapman River	Aug, Dec 2020	6	mix	67.7	±20.6	169.3	±17.8	6.7	±2.3
	6	Tumby Bay	Jan-21	10	rod	471.5	±123.7	308.9	±22.9	6.9	±2.6
	7	Port River	Nov-21	7	net	791.7	±91.1	359.3	±13.7	5.3	±2.2
	8	West Lakes	Jul, Nov 2021	24	net	196.2	±127.1	227.6	±47.3	3.8	±1.0
	9	Coorong	Jun 2020, Jan 2021	14	mix	717.7	±134.7	355.3	±38.5	5.2	±2.4
	10	Robe	Dec 2020; Jan, May 2021	20	rod	511.9	±101.6	322.6	±20.6	9.8	±3.8
Vietoria	11	Glenelg River	Mar-Apr 2021	17	rod	-	-	313.6	±21.5	9.4	±3.0
Victoria	12	Hopkins River	Jun, Oct 2021	20	rod	-	-	348.0	±33.0	12.5	±4.2

Table 3.S2 Permutational multivariate analysis of variance results for otolith element:Ca and isotope ratios of *A. butcheri*. Values include Mean Squares (MS), pseudo-F, and P value, Degrees of freedom = 11, 142.

element:Ca	MS	pseudo-F	P(perm)
Li	0.594	35.016	0.001
Mg	0.594	35.016	0.001
Р	0.194	4.172	0.001
Mn	0.765	8.015	0.001
Cu	0.228	2.112	0.029
Zn	0.672	13.357	0.001
Sr	0.158	9.095	0.001
Ва	6.334	52.610	0.001
Pb	0.001	22.152	0.001
δ ¹³ C	144.940	53.828	0.001
δ ¹⁸ Ο	17.668	23.042	0.001



Fig. 3.S1 Boxplots of otolith (a-i) elemental and (j-k) isotopic composition. Elemental signatures plotted as log-transformed element:Ca ratio [log(μ mol/mol)] include (a) Li:Ca, (b) Mg:Ca, (c) P:Ca, (d) Mn:Ca, (e) Cu:Ca, (f) Zn:Ca, (g) Sr:Ca, (h) Ba:Ca, and (i) Pb:Ca. Isotopic signatures are plotted as isotope ratios (w.r.t. Pee Dee Belemnite standard [PDB]) and include (j) carbon [δ^{13} C] and (k) oxygen [δ^{18} O].

Table 3.S3 Summary table of leave-one-out allocation (cross-validation) results of canonical analysis of principle coordinates (CAP) for *A. butcheri* otolith multi-element signatures. Values represent individuals assigned to each collection site and (%) correctly classified.

	Western River	Middle River	Harriet River	Eleanor River	Chapman River	Tumby Bay	Port River	West Lakes	Coorong	Robe	Glenelg River	Hopkins River	total (<i>n</i>)	correct (%)
Western River	10	0	0	0	0	0	0	0	0	0	0	0	10	100.0
Middle River	4	0	3	0	0	1	0	0	0	0	0	0	8	0.0
Harriet River	1	0	5	1	0	2	0	0	0	2	0	0	11	45.5
Eleanor River	0	0	1	3	1	0	0	0	2	0	0	0	7	42.9
Chapman River	0	0	0	2	1	0	0	0	0	3	0	0	6	16.7
Tumby Bay	2	0	1	0	0	6	0	0	1	0	0	0	10	60.0
Port River	1	0	0	0	0	0	6	0	0	0	0	0	7	85.7
West Lakes	3	0	0	0	0	1	2	15	0	3	0	0	24	62.5
Coorong	1	0	1	0	0	3	0	0	9	0	0	0	14	64.3
Robe	0	0	1	4	0	1	0	0	0	14	0	0	20	70.0
Glenelg River	0	0	0	3	1	0	0	0	0	0	11	2	17	64.7
Hopkins River	0	0	2	1	0	1	0	0	0	1	4	11	20	55.0
Table 3.S4 Summary table of leave-one-out allocation (cross-validation) results of canonical analysis of principle coordinates (CAP) for *A. butcheri* otolith isotopic signatures. Values represent individuals assigned to each collection site and (%) correctly classified.

	Western River	Middle River	Harriet River	Eleanor River	Chapman River	Tumby Bay	Port River	West Lakes	Coorong	Robe	Glenelg River	Hopkins River	total (<i>n</i>)	correct (%)
Western River	5	0	1	2	0	0	1	0	0	0	0	1	10	50.0
Middle River	1	6	0	1	0	0	0	0	0	0	0	0	8	75.0
Harriet River	4	0	4	1	1	0	1	0	0	0	0	0	11	36.4
Eleanor River	0	1	2	3	0	0	0	0	0	0	0	1	7	42.9
Chapman River	0	2	0	0	4	0	0	0	0	0	0	0	6	66.7
Tumby Bay	0	0	0	0	0	8	0	2	0	0	0	0	10	80.0
Port River	0	0	1	0	0	0	5	1	0	0	0	0	7	71.4
West Lakes	0	0	0	0	0	6	2	16	0	0	0	0	24	66.7
Coorong	0	0	0	0	0	0	1	5	8	0	0	0	14	57.1
Robe	0	0	0	0	0	1	1	3	2	5	0	8	20	25.0
Glenelg River	0	0	0	0	0	0	0	0	0	0	14	3	17	82.4
Hopkins River	0	0	0	0	0	0	1	1	0	1	6	11	20	55.0



Fig. 3.S2 Discrete wavelet analysis of *A. butcheri* otolith shape. Plots include (a) mean otolith shape based on Wavelet reconstruction for all 12 capture locations, with axes numbers representing angles in degrees (°) based on polar coordinates; and (b) means ± standard deviation of the Wavelet coefficients for all *A. butcheri* otoliths (primarily y axis), along with the proportion of variance among capture locations (intraclass correlations [ICC], black line); the x axis also shows angle in degrees (°) based on polar coordinates. Areas of interest across otolith outline labelled (grey).

Table 3.S5 Summary table of leave-one-out allocation (cross-validation) results of canonical analysis of principle coordinates (CAP) for *A. butcheri* otolith shape signatures. Values represent individuals assigned to each collection site and (%) correctly classified.

	Western River	Middle River	Harriet River	Eleanor River	Chapman River	Tumby Bay	Port River	West Lakes	Coorong	Robe	Glenelg River	Hopkins River	total (n)	correct (%)
Western River	7	1	0	2	0	0	0	0	0	0	0	0	10	70.0
Middle River	3	2	0	1	0	0	0	2	0	0	0	0	8	25.0
Harriet River	0	0	8	2	0	0	0	0	0	0	0	1	11	72.7
Eleanor River	1	0	4	0	0	0	0	0	0	2	0	0	7	0.0
Chapman River	0	0	1	0	4	0	0	0	0	0	0	1	6	66.7
Tumby Bay	1	0	0	2	0	3	4	0	0	0	0	0	10	30.0
Port River	0	0	0	0	0	4	1	0	0	0	1	1	7	14.3
West Lakes	2	1	3	0	1	1	0	11	0	4	1	0	24	45.8
Coorong	0	0	0	1	0	0	3	0	8	0	1	1	14	57.1
Robe	0	0	1	0	2	1	4	3	0	5	1	3	20	25.0
Glenelg River	0	0	0	0	0	0	1	1	0	2	8	5	17	47.1
Hopkins River	1	0	0	1	0	0	0	0	1	5	5	7	20	35.0

Table 3.S6 Summary of number of loci retained after each genetic filter for the entire *A. butcheri* sampling distribution.

step	action	loci retained
0	Raw data	33,493
1	Remove loci <95% reproducibility	22,215
2	Keep SNPs with data missing <20% overall individuals	22,215
3	Remove ambiguous SNPs - i.e., same position with more than two alleles	16,603
4	Remove monomorphic loci, including those with all NAs	8,687

Table 3.S7 A pairwise comparison of each capture location based on genetic distance (FST). Comparisons are colour coded by significance values, where values greater than 0.15 can be considered as indicative of differentiation between populations (Frankham *et al.*, 2002).

	Western River	Middle River	Harriet River	Eleanor River	Chapman River	Tumby Bay	Port River	West Lakes	Coorong	Robe	Glenelg River
Western River	0										
Middle River	0.056	0									
Harriet River	0.135	0.107	0								
Eleanor River	0.138	0.108	0.007	0							
Chapman River	0.155	0.127	0.078	0.079	0						
Tumby Bay	0.157	0.129	0.125	0.131	0.153	0					
Port River	0.138	0.109	0.104	0.112	0.137	0.034	0				
West Lakes	0.123	0.098	0.094	0.099	0.120	0.030	0.001	0			
Coorong	0.132	0.102	0.099	0.101	0.124	0.054	0.028	0.027	0		
Robe	0.124	0.098	0.097	0.102	0.120	0.052	0.026	0.024	0.008	0	
Glenelg River	0.131	0.105	0.100	0.101	0.123	0.068	0.043	0.042	0.034	0.031	0
Hopkins River	0.130	0.105	0.100	0.104	0.125	0.071	0.046	0.046	0.040	0.038	0.013

FST

0

0.10

0.15

Table 3.S8 Summary table of leave-one-out allocation (cross-validation) results of canonical analysis of principle coordinates (CAP) for *A. butcheri* genetic signatures. Values represent individuals assigned to each collection site and (%) correctly classified.

	Western River	Middle River	Harriet River	Eleanor River	Chapman River	Tumby Bay	Port River	West Lakes	Coorong	Robe	Glenelg River	Hopkins River	total (n)	correct (%)
Western River	10	0	0	0	0	0	0	0	0	0	0	0	10	100.0
Middle River	0	8	0	0	0	0	0	0	0	0	0	0	8	100.0
Harriet River	0	0	11	0	0	0	0	0	0	0	0	0	11	100.0
Eleanor River	0	0	4	3	0	0	0	0	0	0	0	0	7	42.9
Chapman River	0	0	0	0	6	0	0	0	0	0	0	0	6	100.0
Tumby Bay	0	0	0	0	0	7	0	3	0	0	0	0	10	70.0
Port River	0	0	0	0	0	0	0	7	0	0	0	0	7	0.0
West Lakes	0	0	0	0	0	0	0	24	0	0	0	0	24	100.0
Coorong	0	0	0	0	0	0	0	0	12	2	0	0	14	85.7
Robe	0	0	0	0	0	0	0	0	0	20	0	0	20	100.0
Glenelg River	0	0	0	0	0	0	0	0	0	0	17	0	17	100.0
Hopkins River	0	0	0	0	0	0	0	0	0	0	1	19	20	95.0





Table 3.59 Summary table of leave-one-out allocation (cross-validation) results of canonical analysis of principle coordinates (CAP) for *A. butcheri* multivariate signatures generated from a combination of otolith element, otolith shape, and genetic markers. Values represent individuals assigned to each collection site and (%) correctly classified.

	Western River	Middle River	Harriet River	Eleanor River	Chapman River	Tumby Bay	Port River	West Lakes	Coorong	Robe	Glenelg River	Hopkins River	total (n)	correct (%)
Western River	10	0	0	0	0	0	0	0	0	0	0	0	10	100.0
Middle River	0	8	0	0	0	0	0	0	0	0	0	0	8	100.0
Harriet River	0	0	10	1	0	0	0	0	0	0	0	0	11	90.9
Eleanor River	0	0	2	5	0	0	0	0	0	0	0	0	7	71.4
Chapman River	0	0	0	0	6	0	0	0	0	0	0	0	6	100.0
Tumby Bay	0	0	0	0	0	9	0	1	0	0	0	0	10	90.0
Port River	0	0	0	0	0	0	5	2	0	0	0	0	7	71.4
West Lakes	0	0	0	0	0	0	0	24	0	0	0	0	24	100.0
Coorong	0	0	0	0	0	0	0	0	13	0	1	0	14	92.9
Robe	0	0	0	0	0	0	0	0	0	20	0	0	20	100.0
Glenelg River	0	0	0	0	0	0	0	0	0	0	17	0	17	100.0
Hopkins River	0	0	0	0	0	0	0	0	0	0	1	19	20	95.0

Relatedness

There is the possibility that fish caught within a site on the same day or from the same net haul are closely related. As relatedness can influence population structure inference, random sampling was validated using an Identity-By-Descent (IBD) analysis (*SNPRelate* package in *R*; Zheng *et al.* 2012). Kinship coefficient values were generated using the KING [robust] method of moment for each possible pairing of *A. butcheri*, both within and among capture locations; with a kinship coefficient \ge 0.25 representing a sibling-sibling or parent-offspring relationship (Sun and Dimitromanolakis 2014). All analyses were performed in *R* (R Core team 2016).

Two pairs of individuals had a kinship coefficient \geq 0.25. Therefore, an individual from each pair was removed from the study.

Pairwise comparison of *A. butcheri* samples with kinship coefficient values \geq 0.18. Table includes both samples for each pairing, the proportion of SNPs with zero identity-by-state (IBSO) and kinship coefficient value (kinship). Samples removed indicated with an asterisk (*).

pair	sample ID 1	sample ID 2	IBSO	kinship
1	ABHa3FT8-08*	ABHa3FT8-04	0.0001	0.4659
2	ABCh2FT8-01*	ABCh1FT8-01	0.0003	0.4593
3	ABCoFT1-15	ABCoFT1-17	0.0087	0.2385
4	ABCoFT1-17	ABCoFT2-02	0.0063	0.2320
5	ABTbFT1-08	ABTbFT1-09	0.0103	0.1812

- Frankham, R, Ballou, JD, Briscoe, DA, McInnes, KH (2012) 'Introduction to Conservation Genetics.' (Cambridge University Press: Cambridge).
- Sun, L, Dimitromanolakis, A (2014) PREST-plus identifies pedigree errors and cryptic relatedness in the GAW18 sample using genome-wide SNP data. *BMC Proc* **8**, S23.
- Zheng, X, Levine, D, Shen, J, Gogarten, SM, Laurie, C, Weir, BS (2012) A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* 28, 3326-8.

CHAPTER 4

ENSO effects on growth of an estuarine fish

Title of Paper	ENSO effects on growth of an estua	arine fish		
Publication Status	Published	C Acce	pted for P	ublication
	Submitted for Publication	X Unpu manu	ublished ar uscript styl	nd Unsubmitted work written in le
Publication Details				
Principal Author				
Name of Principal Author (Candidate)	Koster Georgien Sarakinis			
Contribution to the Paper	Conceptualization (Equal); Data cu Project administration (Lead); Visu review & editing (Equal).	rration (Lead); alization (Equa	Formal ana al); Writing	alysis (Equal); Investigation (Equal); – original draft (Lead); Writing –
Overall percentage (%)	70%			
Certification:	This paper reports on original res Research candidature and is not si party that would constrain its inclus	earch I conduc Ibject to any ob sion in this thes	cted during oligations o sis. I am the	the period of my Higher Degree by r contractual agreements with a thirc e primary author of this paper.
Signature	puling		Date	21/02/2024
Co-Author Contributions				
By signing the Statement of Authorship, i. the candidate's stated contri	each author certifies that: bution to the publication is accurate i	as detailed ab	ove):	
ii. permission is granted for the	e candidate in include the publication	in the thesis; a	ind	
iii. the sum of all co-author con	tributions is equal to 100% less the c	andidate's stat	ed contribu	ition.
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4.1 Abstract

Understanding how animal growth varies in response to rapidly changing environmental and climatic conditions can aid in predicting future growth responses that may have broader fitness implications and allow for optimised and sustainable management solutions. We assessed the growth response of black bream Acanthopagrus butcheri to varying environmental conditions using otolith growth chronologies from nine estuaries along south-eastern Australia between 1981 and 2016, comprising 31,006 otolith increment measurements from 4,792 fish. We used a mixed modelling approach, assessing both local-scale effects (water flow and temperature) and regional-scale effects (El Niño Southern Oscillation, ENSO), as well as investigating possible spatial synchrony among A. butcheri populations. Regional-scale, annual-lagged ENSO effect (Southern Oscillation Index) was the most important factor explaining variations in yearly growth. Annual-lagged, local-scale effects elicited mixed growth responses among estuaries, likely also reflecting system-specific differences in environmental conditions. Cases of spatial synchrony were identified across both a latitudinal and longitudinal gradient. The majority of synchrony occurred in the years following a shift between El Niño and La Niña conditions, further emphasising the importance of regional scale climatic effects on this species' growth. Our findings emphasise how large-scale climate factors play a major role in population dynamics and productivity at large spatial scales, despite a diversity of estuarine conditions and surrounding environments.

Keywords: otolith; growth; chronology; synchrony; Acanthopagrus butcheri; ENSO

4.2 Introduction

Rapid environmental changes to aquatic ecosystems driven by extreme weather events, climate change, and overexploitation have led to shifts in species' distributions and increasing cases of local extinction (Nikolaou and Katsanevakis 2023; Pecl *et al.* 2014; Booth *et al.*, 2011). These climatic and anthropogenic stressors are predicted to further increase and alter ecosystems and their biodiversity, including impacting population resilience to disturbance and causing the exceedance of physiological tolerances (Martino *et al.*, 2019; Frank *et al.*, 2016; Ong *et al.*, 2016). For example, changes in fish growth driven by environmental (extrinsic) effects can result in increased mortality rates, altered population age and size structures, and ultimately increased risk of population collapse (Kraak *et al.*, 2019; Carozza *et al.*, 2018; Pinsky and Byler 2015). Therefore, understanding growth responses to rapidly changing environmental and climatic conditions can help determine future population health and resilience.

The El Niño Southern Oscillation (ENSO) is a major interannual climatic phenomenon that occurs in the tropical Pacific. Oscillating between a warm phase (El Niño) and a cool phase (La Niña), ENSO is one of the most powerful drivers of year-to-year variations in global climate and has a major role in shaping biological responses across marine and terrestrial ecosystems worldwide (Lovelock *et al.*, 2017; Jiménez-Muñoz *et al.*, 2016; Meuser *et al.*, 2013). This phenomenon has been shown to have a variety of impacts on fish growth, including cases of declining growth during strong El Niño events and positive growth responses during La Niña events (Reis-Santos *et al.*, 2021; Ong *et al.*, 2016). At the same time, changes in fish growth have also been associated with global ocean warming (i.e., increase) (Nicolle *et al.*, 2022; Martino *et al.*, 2019), or local-scale drivers such as rainfall, water flow, sea surface temperature or fishing pressure (Morrongiello *et al.*, 2021; Doubleday *et al.*, 2015; Pinsky and Byler 2015).

The spatial scale at which the environment drives variations in biological responses may lead to synchronous responses in fish growth across populations of widely distributed species (Felts *et al.*, 2020; Liebhold *et al.*, 2004). These temporally coincident changes in growth can impact long-term population and species resilience as they become more vulnerable to stochastic events (Campana *et al.*, 2023; Tanner *et al.*, 2020). For instance, if unexpected changes in the environment slow growth or increase mortality across multiple populations simultaneously, their ability to recover might be significantly impeded, potentially leading to extirpation. Regional fluctuations in environmental conditions can induce spatial synchrony of fish growth, including global temperatures, climate effects, and hydrological variables (Feiner *et al.*, 2019; Dembkowski *et al.*, 2016; Stocks *et al*, 2014; Liebhold *et al.*, 2004). Other drivers of spatial synchrony among fish populations include dispersal between adjacent populations (Hopson and Fox 2019; Koening 2001), broad-scale species

exploitation (Frank *et al.,* 2016), and interactions with other spatially synchronous species (Olin *et al.,* 2020; Liebhold *et al.,* 2004). Overall, understanding the mechanisms driving spatial synchrony and identifying potential correlations between environmental drivers and fish growth dynamics helps us predict how fish will respond to environmental shifts and if these synchronised changes can leave populations more susceptible to external threats or capitalising on optimum conditions to boost productivity (Campana *et al.,* 2023; Tanner *et al.,* 2020; Stocks *et al.,* 2014).

Determining growth variability in aquatic animals can be achieved by analysing naturally occurring hard structures (e.g., bones, scales, shells) and the rate of material formation (Dong *et al.*, 2018; Morrongiello and Thresher 2015). A structure commonly used to investigate fish growth are otoliths (ear stones), which accrete material throughout an individual's life, forming both daily and annual increments that reflect an individual's life history (Campana 2001; Pannella 1971). In addition to intrinsic drivers of growth, such as fish age (Morrongiello *et al.*, 2015; Handeland *et al.*, 2008), extrinsic variables can influence this biomineralisation process, making these structures a valuable tool for generating long-term ecological datasets (Morrongiello *et al.* 2012; Black *et al.* 2008). Therefore, harnessing the information recorded by otoliths can assist in determining past, present, and predicted future growth responses to changing environmental conditions (Rountrey *et al.* 2014; Stock *et al.* 2011; Rypel 2009).

Black bream Acanthopagrus butcheri (Munro 1949) is a generalist species distributed across dramatically different bioregions in southern Australia, from Western Australia (WA) to New South Wales (NSW). They inhabit estuaries of varying sizes influenced by catchments of highly variable rainfall, runoff, and regional-scale events (e.g., droughts and floods) (Jaffrés et al., 2022; Wetz and Yoskowitz 2013; Norriss *et al.*, 2002), with sub-tropical regions prone to flooding and dry Mediterranean type climate regions often affected by droughts. Acanthopagrus butcheri rely on estuaries to complete much of their life cycle (i.e., estuarine-dependent) but can move to coastal waters and lower areas of rivers (Doubleday et al., 2015; Partridge and Jenkins 2002). Although able to tolerate fluctuating environmental conditions (e.g., water salinity and water temperature) (Sakabe et al., 2010), an ecological risk assessment investigating species' sensitivity to climate change (e.g., abundance, distribution, and phenology) identified A. butcheri as 'highly sensitive' to key climate change drivers (i.e., freshwater flow, temperature, and salinity), predicting the species is likely to undergo a range contraction under climate change (Pecl et al. 2014). Furthermore, a study by Cottingham et al., (2018) found that decreased freshwater discharge into Western Australian estuaries resulted in reduced A. butcheri growth. Nonetheless, our understanding of the long-term effects of both local-scale and regional-scale environmental variables on this species growth is

limited, as well as if these influences are driving environmentally induced synchronised growth along a broad longitudinal and latitudinal gradient.

This study assessed the growth response of A. butcheri to past and present environmental conditions across south-eastern Australian estuaries. By generating growth chronologies and using a multi-decadal, mixed modelling approach, we (1) investigated the inter-annual growth of A. butcheri and the impacts of intrinsic factors (e.g., fish age) and extrinsic factors (e.g., water flow, temperature, and ENSO) at both a local- and regional-scale; and (2) investigated potential spatial synchrony in A. butcheri growth. We hypothesise that local-scale drivers will have a greater impact on fish growth than regional-scale effects, given the species' known estuarine dependency and limited movement across its distribution (Chapter 2). Furthermore, the variable local-scale environmental conditions across the species distribution (e.g., estuary size, temperature, and water flow) likely trigger more dominant and estuary-specific growth responses than regional-scale effects. With increasing temperatures, we predict an increase in productivity and subsequent fish growth, while an increase in water flow will not only increase nutrients entering the systems from further upstream but also food availability, therefore increasing fish growth. We also predict variable effects on fish growth with increasing ENSO conditions dependent on estuary-specific characteristics. For example, fish in smaller closed systems are likely to be more sensitive to environmental change given the stability of such estuaries compared to larger open systems, resulting in a negative growth response to increasing La Niña conditions (e.g., flooding) and El Niño conditions (e.g., drought). Overall, we hypothesise there to be limited spatial synchrony due to the importance of estuary-specific environmental variability, with any cases of synchrony driven by ENSO-driven weather events (i.e., drought or flooding) and occurring between estuaries geographically close together. This information is key to understanding the species resilience to environmental change.

4.3 Materials and Methods

Data collection

Acanthopagrus butcheri otolith samples were collected from nine estuaries across south-eastern Australia from 2001 to 2016 (Fig. 4.1) and accessed from historical collections provided by Australian government agencies and Universities in South Australia, Tasmania, and New South Wales. Additionally, information from *A. butcheri* otoliths collected from estuaries in Victoria was obtained as pre-measured growth data from the Victorian Fisheries Authority (VFA). Whole otoliths were embedded in epoxy resin and sectioned transversely (~250–300 µm thin) using a low-speed saw. Sections were polished using different grades of lapping paper and then photographed using a compound microscope under reflected light. Otolith aging was undertaken along the ventral sulcus

side and confirmed along a secondary visible axis by counting the number of opaque bands (Fig. 4.2). Each sample was aged twice by a single reader, with an additional age estimate made if values did not match and repeated if required until a mode age was obtained. The distance between each growth increment, from otolith core to otolith edge, was measured using the software *ImageJ* (Schneider *et al.,* 2012), with each increment assigned a corresponding fish age and year of formation, as well as Age-At-Capture (AAC) (Table 4.1). Potential outliers in increment size were identified by plotting increment lengths by fish age, with one individual removed from subsequent analyses.



Fig. 4.1 Map of south-eastern Australia, with an inset of Australia showing all nine *A. butcheri* sampling estuaries. Samples were collected from South Australia, Victoria, New South Wales, and Tasmania.

Parameter	Description
Fixed effects	
Age	Age when otolith increment formed (yr)
Age At Capture (AAC)	Year when sample was captured
Year	Formation of annual otolith increment (October to September)
Water Flow	Mean monthly water flow either at estuary or upstream (mL/day)
Air Temperature	Mean daily air temperature used as proxy for water temperature (C)
Sea-Surface Temperature (SST)	Mean monthly values
Southern Oscillation Index (SOI)	Mean monthly values
Random effects	
Fish ID	Individual fish ID
Year	Year when otolith increment formed
Age	Age when otolith increment formed (yr)
Estuary	Estuary where individual was captured

Table 4.1 Description of parameters used in analysis for both Fixed and Random effects.

Environmental data were collected from online databases as monthly mean values between 1980 and 2016. Southern Oscillation Index (SOI) values were obtained from the National Oceanic and Atmospheric Administration (NOAA) and incorporated as a regional-scale predictor variable (National Oceanic and Atmospheric Administration, 2024). Local-scale environmental data were collected from the Bureau of Meteorology (BOM) and included coastal air temperature (temperature, C) from each estuary, Sea-Surface Temperature (SST, C), and river flow (ML/day) (Bureau of Meteorology, 2024a). We used local air temperature and adjacent coastal SST as predictor variables as estuarine water temperature data were largely unavailable across the sampling distribution and over time. There was no significant correlation between air temperature and adjacent coastal SST ($R^2 = 0.624$, P = 0.189). Fish sampled from Gippsland Lakes could not be further categorised to smaller estuaries within the system that they may have been sampled from or regularly returned to (e.g., Williams et al., 2017). Therefore, fish from Gippsland Lakes required integration of flow data from multiple rivers (e.g., Mitchell River, Tambo River, Nicholson River, and La Trobe River) as these all drain into the terminal lakes system. We aggregated monthly values across these rivers to provide a composite Gippsland Lakes signal. Unfortunately, no flow data have been recorded for Little Swanport, nor anywhere within the corresponding catchment range. Therefore, extrinsic modelling was not performed for A. butcheri captured in Little Swanport. Monthly environmental data were then averaged from October to September of the following year to match the growth year of A. butcheri (date of birth, October 1st) (Elsdon and Gillanders 2006). Estuary size, geomorphology and latitude vary greatly across the estuaries sampled, therefore all local-scale environmental data were scaled using z-score normalisation (mean = 0, SD = 1), to account for differences in range and magnitude of environmental variables and facilitate model convergence.



Fig. 4.2 Microscope image of an *A. butcheri* otolith section under reflected light. Annual growth increments (opaque bands, white circles) indicate age of fish (six years).

Data analysis

To partition intrinsic and extrinsic variation of annual otolith growth we developed a series of increasingly complex mixed effects models (Morrongiello & Thresher 2015). Otolith increment widths and Age data were log-transformed to meet assumptions of normality and homoscedasticity. Increment width was the response variable in all models, with Age and AAC treated as intrinsic fixed effects (R package *Ime4*; Bates et al., 2015). An Estuary interaction was added to Age, to account for potential variation in age-specific growth among estuaries (i.e., overall fish size, size at sexual maturity) and in habitat availability during both juvenile and adult life histories among estuaries. Similarly, an *Estuary* interaction was added to AAC to address the potential variation of selectivity among estuaries (i.e., exploitation is likely to vary) whereby fast growing fish that reach a minimum size threshold might have higher mortality in heavier fished populations. Random effects included a random Age slope within each individual (Age | FishID) to allow for individual-level deviations in the age growth relationship from estuary averages and account for repeated measurements made within the same individual. We also fitted a random year-specific effect within each estuary (1|Estuary:Year) to account for estuary-specific deviations in environmental conditions that could affect growth but that are not covered by fixed effect environmental variables (see below). The random intercept for Estuary: Year also allows for separate estimates per estuary for each year and thus the production of annual growth biochronologies.

The random effects component of the maximal model was optimized using restricted maximum likelihood (REML) and candidate models compared using Akaike's information criterion corrected for small sample sizes (AICc) (*stats* package in *R*; R Core Team 2023). The optimal random effects model was then used to select the appropriate fixed effect structure (model fitted with

maximum likelihood, ML) based on the lowest delta AICc (ΔAICc) value (R package *MuMIn*; Burnham *et al.*, 2003). To interpret the predicted intrinsic growth effects, estimated marginal means were calculated using Best Linear Unbiased Predictors (BLUPs), which were extracted from the best-fitted model and plotted by estuary as growth chronologies (R package *ggeffects*; Lüdecke 2018), with positive values in BLUPs reflecting good (above average) growth, whereby negative values reflecting years with lower, below average growth. The explained variance in otolith growth for each model was evaluated using marginal and conditional R-squared values for the fixed effects and combined fixed and random effects, respectively.

We then used combinations of local-scale and regional-scale predictor variables to assess the influence of extrinsic effects on growth of back bream across the different estuaries. As fish can have a delayed growth response to extrinsic effects (e.g., Morrongiello *et al.*, 2021), an annual lag response was added for each environmental variable by assigning the previous year's data to investigate whether past environmental conditions affected fish growth. Both an *Estuary* and *Age* interaction was applied to each predictor variable to account for known variability in environmental signals among estuaries, and the varying impacts these would have on each *estuary* and fish maturity stage. A quadratic term for each predictor variable coupled with an *Estuary* interaction was also added to evaluate potential non-linear relationships with fish growth. Estimated marginal means were calculated and plotted using predictor variables from both the best-fitted regional-scale and best-fitted local-scale models.

Spatial synchrony

We tested for spatial synchrony of *A. butcheri* growth among estuaries using pairwise 11-year centrealigned running correlations (Morrongiello *et al.*, 2021). Spatial synchrony required temporal detrending of each growth chronology using an auto-regressive model, which utilised BLUP estimates to generate residuals. Pairwise testing for spatial synchrony was completed between all combinations of estuaries, assessing each 11-year window rather than the entire year range. As significant correlations can arise by chance, and here we performed multiple correlations (i.e., the multiple comparison problem), a permutation analysis was used to identify the critical threshold of significance. The running correlation coefficient was then calculated for each growth year, with values passing the critical threshold deemed growth years of significant spatial synchrony between estuaries (*stats* package in *R*; R Core Team 2023).

4.4 Results

A total of 4,792 *A. butcheri* otoliths were analysed comprising 31,006 annual growth increment measurements spanning 1981-2016 (Table 4.2, Fig. 4.S1). Annual increment width decreased with fish age (Fig. 4.S2). In terms of environmental data, fluctuations in SOI over time reflected shifts between La Niña conditions (positive SOI values) and the El Niño conditions (negative SOI values), including during the late 1980s, late 1990s and mid to late 2000s (Fig. 4.S3a). Estuaries varied in local-scale environmental conditions, including SST (Fig. 4.S3b) showing similar patterns over time with higher temperatures in the northernmost estuaries (i.e., Lake Coila and Lake Brou, NSW) and cooler temperatures in the southernmost estuaries but overall showed a similar latitudinal gradient as SST (Fig. 4.S3c). Water flow varied the most among estuaries, as would be expected given the variation in estuary size and type, with large systems permanently open via dredging showing higher freshwater flow, such as the Gippsland Lakes (1x10⁴ km² of catchment) and the Coorong (1x10⁶ km² of catchment), compared to smaller systems that are intermittently closed, including Lake Coila and Lake Tyers (10s km² of catchment) (Fig. 4.S3d, Table 4.2).

Table 4.2 *Acanthopagrus butcheri* sampling estuaries with corresponding Australian state, estuary type, sample size, total number of otolith increment measurements, and year range. For estuary type, O indicates open whereas IC indicates intermittently closed.

state	estuary	estuary type	sample size	no. of increment measurements	year range
South Australia	Coorong	0	193	1,244	1983-2016
Victoria	Hopkins River	IC	753	5,062	1983-2013
	Gippsland Lakes	0	1,554	9,309	1981-2016
	Lake Tyers	IC	1,006	7,470	1982-2015
	Mallacoota	IC	678	4,117	1984-2013
New South Wales	Lake Brou	IC	102	602	1986-2005
	Lake Coila	IC	105	485	1990-2004
Tasmania	Swan River	0	201	1527	1988-2006
	Little Swanport	0	200	1190	1988-2006
TOTAL			4792	31006	1981-2016

Intrinsic effects

The best supported random effects structure for all estuaries based on the lowest Δ AlCc values consisted of a random slope for age within individuals, and a random intercept term that varied by both estuary and year (1|Estuary:Year), indicating differences in age-dependent growth among

individuals and differences in estuary-dependent growth across years. The best supported intrinsic model included an estuary interaction for both age and AAC (Table 4.S1) with the overall best model being: Age x Estuary + AAC x Estuary + (Age|FishID) + (1|Estuary:Year) (Table 4.S2). R-squared values indicated that both the intrinsic fixed and random effects explained a large portion of the growth variability (marginal = 0.627, conditional = 0.823). Predicted growth declined with increasing age (Fig. 4.3a), with a positive relationship between predicted growth and AAC for all *A. butcheri* populations except for Lake Tyers and Mallacoota (Fig. 4.3b). *Acanthopagrus butcheri* growth was highest (fastest) in Lake Tyers (Victoria) and lowest (slowest) in Swan River and Little Swanport (Tasmania). Fish growth in Lake Tyers was higher compared to the geographically adjacent and larger Gippsland Lakes (Fig. 4.3a). Similarly, fish in Lake Coila and Lake Brou estuaries, both small systems, had higher overall growth compared to Coorong, a large system whose *A. butcheri* are commercially targeted.

Growth chronologies generated from the best fitted intrinsic model revealed variations in BLUPs over time among estuaries, with minimal error in predicted growth (Fig. 4.S4). Considerable interannual fluctuations in annual growth appeared after 1990-1995, with estuaries showing below average growth during this period (i.e., negative BLUPs). Some clear patterns across estuaries include a decline in growth of *A. butcheri* after 1990 in Hopkins River, Gippsland Lakes and Lake Tyers (adjacent estuaries, Victoria), and Swan River (Tasmania). Almost all estuaries show a spike in growth (i.e., above average; positive BLUPs) around the year 2000, except for *A. butcheri* collected from Lake Brou (New South Wales) and Mallacoota (Victoria). The spike in growth from fish in the Coorong appeared to be delayed and occurred around 2005. Signals showed higher error at the start of each chronology where there are lower numbers of increments underpinning estimates.



Fig. 4.3 Conditional effects plots generated using the best-fitted intrinsic model. Predicted *A. butcheri* otolith growth plotted in response to (a) Age and (b) Age-At-Capture (AAC). Colours indicate each estuary.

Extrinsic effects

Overall, the best supported extrinsic predictor model explaining *A. butcheri* growth variability comprised the regional-scale annual-lagged SOI term interacting with both *Estuary* and *Age* as well as a quadratic term for SOI (Table 4.3, Table 4.S3, Fig. 4.S5). Predicted *A. butcheri* growth in all estuaries was in general highest at neutral SOI conditions, with growth generally declining as conditions moved into El Niño (low SOI) and La Niña (High SOI) conditions. But there were cases of fish growth with increasing La Niña conditions (between 0-1 SOI), before a continued increase in conditions either triggered a negative response (e.g., *A. butcheri* from Hopkins River, Gippsland Lakes, and Lake Tyers) or reached a plateau in growth (e.g., *A. butcheri* from Swan River) (Fig. 4.4a). Growth rates showed change in response to SOI as fish grew, with error in predicted growth being greater at younger ages in response to changes in both regional and local scale extrinsic effects (Fig. 4.S5). Interestingly, *A. butcheri* showed a strong negative growth response during early life in Lake Coila (i.e., the northernmost estuary) with decreasing SOI (Fig. 4.S5a), which was also reflected by the strong positive correlation fish from this estuary showed with SOI (i.e., continued growth with increasing La Niña conditions) (Fig. 4.4a). R-squared calculations indicated that both fixed and

random effects explained a substantial amount of the variability in *A. butcheri* growth for the best fitted regional-scale model (marginal = 0.637, conditional = 0.816). Table 4.3 Results of the best-fitted extrinsic models based on delta Akaike information criterion (Δ AICc) values. Table includes temporal and spatial scales, model parameters, and degrees of freedom (*df*). Note, this model did not include data from Little Swanport as we lacked flow data for this estuary.

model	temporal scale	spatial scale	parameters	df	ΔAICc
1	annual-lagged	regional	intrinsic effects + SOI _{lag} x Estuary x Age + SOI ² _{lag}	46	0.000
2	annual	regional	intrinsic effects + SOI _{ann} x Estuary x Age + SOI ² _{ann}	46	42.100
3	annual-lagged	local	intrinsic effects + Flow _{lag} x Estuary x Age + Temperature _{lag} x Estuary x Age + Temperature ² _{lag} + SST _{lag} x Estuary x Age + SST ² _{lag} x Estuary x Age	85	78.000
4	annual	local	intrinsic effects + Flow _{ann} x Estuary x Age + Temperature _{ann} x Estuary x Age + SST _{ann} x Estuary x Age + SST ² _{ann} x Estuary	85	93.100



Fig. 4.4 Conditional effects plots generated using the best-fitted regional-scale model (a) and localscale model (b-d). Predicted *A. butcheri* otolith growth plotted in response to annual-lagged (i.e., past year [PY]) (a) Southern Oscillation Index (SOI), (b) air temperature, (c) Sea-Surface Temperature (SST), and (d) water flow. Extrinsic variables have been plotted as centre-scaled values. Colours indicate each estuary (excluding Little Swanport).

For comparison, the best supported local-scale model (ΔAICc=78) included annual-lagged air temperature, SST, and water flow predictor variables, all with an *Estuary* and *Age* interaction reflecting estuary-specific and age-dependent extrinsic effects (Table 4.S4). Predicted growth in all estuaries except Gippsland Lakes, Lake Coila, and Swan River increased with increasing air temperature (Fig. 4.4b), with a shift from negative growth during early life history to positive growth as fish aged in Mallacoota (Fig. 4.S5b). Predicted fish growth also varied in response to increasing SST, with fish from Gippsland Lakes, Lake Tyers, Lake Coila, and Swan River having a positive relationship, while *A. butcheri* from Coorong, Hopkins River, Mallacoota, and Lake Brou had a negative growth response (Fig. 4.4c). However, *A. butcheri* from Lake Tyers showed a negative growth response to increasing SST during early life before shifting to positive, with the opposite shift from positive to negative growth present in Mallacoota (Fig. 4.S5c). Predicted growth varied in response to increasing water flow, with a negative relationship in both large open systems (Coorong and Gippsland Lakes) and smaller, intermittently closed systems (Lake Brou and Lake Coila) (Fig. 4.4d). A positive growth relationship with increasing flow was found for *A. butcheri* collected in Lake Tyers and Swan River, while fish from negative to positive growth with increasing water flow. A shift from negative to positive growth with increasing water flow as fish aged was present in Hopkins River, Mallacoota (both in Victoria), and Lake Tyers (New South Wales) (Fig. 4.S5d). Overall, explained variance for fixed and random effects in the local-scale model were 0.654 (marginal) and 0.817 (conditional), respectively.

Spatial synchrony

Overall, we found limited evidence for spatial synchrony in growth, with only 21 of the 109 possible 11-year centre-aligned sliding windows showing significant spatial synchrony in growth, all of which were positive. Nine pairwise tests (i.e., between estuaries) showed significance at some point in time, including in the 1990s and late 2000s (Fig. 4.5, Fig. 4.6). The longest cases of significant spatial synchrony were found between estuaries that were considerably separated by latitude, including A. butcheri from Lake Coila and Little Swanport (~1000 km apart, 1991-2004, Fig. 4.5a) but also A. butcheri from Gippsland Lakes and Swan River (100s km apart, 1989-2001, Fig. 4.5b). Other cases of synchrony occurred between longitudinally separated estuaries across a large distance (e.g., Coorong and Lake Coila, ~1600 km apart, Fig. 4.6a), and geographically close (e.g., Gippsland Lakes and Lake Tyers, ~10 km apart, Fig. 4.6c). Overall, cases of significant spatial synchrony occurred between A. butcheri from multiple estuaries but generally only for a single 11-year window or two. Namely, between Coorong and Lake Coila (1993-2003, Fig. 4.6a), Gippsland Lakes and Hopkins River (1985-1995, Fig. 4.6b), Gippsland Lakes and Lake Tyers (1990-2001, Fig. 4.6c), Swan River and Lake Tyers (1991-2002, Fig. 4.6d), Lake Coila and Swan River (1991-2001, Fig. 4.6e), Hopkins River and Lake Tyers (2001-2011, Fig. 4.6f), and between A. butcheri from Gippsland Lakes and Mallacoota (2002-2012, Fig. 4.6g). Negative spatial synchrony was apparent for Gippsland Lakes and Hopkins River (Fig. 4.6b), and between spatially adjacent estuaries Gippsland Lakes and Lake Tyers (Fig. 4.6c), although these were not significant as neither case passed the critical [negative] threshold. As the best fitted extrinsic growth model consisted of an annual-lagged ENSO effect, SOI values were mapped onto growth chronologies, which highlighted some cases of spatial synchrony occurring in the years

following a shift between negative SOI values (El Niño conditions) and positive SOI values (La Niña conditions) (Fig. 4.5, Fig. 4.6). Namely, between Gippsland Lakes and Lake Tyers, Hopkins River and Lake Tyers, as well as Gippsland Lakes and Mallacoota (Fig. 4.6).



Fig. 4.5 Two cases of prolonged significant spatial synchrony in *A. butcheri* growth variability between estuaries (a-b). Spatial synchrony tests comprised pairwise comparisons between estuary-specific growth chronologies (Best Linear Unbiased Predictors [BLUP]) by growth year (left) and Running Correlation Coefficient (RRC) values generated for each corresponding growth year assessed (right). Coloured vertical bands along growth chronologies indicate years of La Niña conditions (light blue) and El Niño conditions (light red). Horizontal red lines indicate critical RCC thresholds indicating significant spatial synchrony (unique for each test).



Fig. 4.6 Seven cases of significant spatial synchrony in *A. butcheri* growth variability between estuaries (a-g). Spatial synchrony tests comprised pairwise comparisons between estuary-specific growth chronologies (Best Linear Unbiased Predictors [BLUP]) by growth year (left) and Running Correlation Coefficient (RCC) values generated for each corresponding growth year assessed (right). Coloured vertical bands along growth chronologies indicate years of La Niña conditions (light blue) and El Niño conditions (light red). Horizontal red lines indicate critical RCC thresholds indicating significant spatial synchrony (unique for each test).

4.5 Discussion

Annual-lagged, regional-scale ENSO effects best explained A. butcheri growth variability. Fish generally grew fastest in neutral SOI conditions and had decreased growth in El Niño and La Niña periods, which are characterised by drought and floods, respectively (Bureau of Meteorology, 2024b). The higher error in early predicted growth in response to changes in both regional and local scale extrinsic effects during early life history relative to adult years reflects the species sensitivity and potential vulnerability to environmental change during this life history stage. We also detected some instances of synchrony across estuaries and over time. The presence of synchronised growth among these environmentally heterogeneous estuaries highlights the potential impact and vulnerability of the species with a higher risk of population collapse and altered population structure from stochastic events (i.e., drought or flooding). We detected estuary-specific differences in the fish Age and AAC terms which supports the species' known variability in both average size and age at sexual maturity across its distribution range (Norriss et al., 2002; Sarre and Potter 1999). Such variability has also been shown at smaller spatial scales, including across Western Australia and New South Wales (Gray 2022; Sarre and Potter 2000). Furthermore, the unique temporal patterns in growth observed among estuaries (i.e., location-specific growth patterns) is indicative of known isolated populations and the limited connectivity previously identified across the species' distribution range using various genetic approaches (Chapter 2; Burridge and Versace 2006; Farrington et al., 2000; Chaplin et al., 1998), and otolith-based techniques (Chapter 3).

ENSO effects on annual growth

Annual-lagged, regional-scale SOI was the most important factor explaining variations in yearly *A*. *butcheri* growth across south-eastern Australia. A decline in predicted growth reflects the potential long-term impacts of increasing climatic events, specifically intensifying El Niño (i.e., hot and dry) and La Niña conditions (i.e., cold and wet) (National Oceanic and Atmospheric Administration, 2024). Similar responses have been found in other species, including a negative growth response to

intensifying SOI (i.e., increasing or decreasing) in snapper (e.g., Chrysophrys auratus, Martino et al., 2019), a positive response with increasing SOI in barramundi (Lates calcarifer, Leahy and Robins 2021) and rock blackfish (Girella elevata, Stocks et al., 2014), and a decline in growth with decreasing SOI in dusky grouper (Epinephelus marginatus, Reis-Santos et al., 2021). The annual-lagged, regionalscale effect complements the best fitted local-scale extrinsic model (annual-lagged), with climatic events triggering regional-scale droughts and/or flooding, thereby altering estuary-specific conditions and A. butcheri having a delayed growth response. ENSO events have been previously shown to alter and even synchronise salinity patterns among estuaries (Tolan 2007; Schmidt and Luther 2002), impact seagrasses and mangrove ecosystems through rising sea levels and temperature (Holbrook et al., 2021), as well as impact phytoplankton communities by influencing a range of environmental conditions (e.g., precipitation, turbidity, and water temperature) (López Abbate et al., 2017 Sathicq et al., 2015). Reductions in habitat availability from increasing ENSO events such as droughts or flooding also influence abundance and spatial distribution of estuarine species during early life stages (Filho et al., 2022; Belarmino et al., 2021), which could possibly have a greater impact on younger A. butcheri growth. This is shown not only by the higher sensitivity of younger fish (i.e., higher error in predicted growth) to changes in both regional and local scale extrinsic effects but also in A. butcheri from Lake Coila, the northernmost estuary, where younger fish show a strong negative growth response to increasing El Niño conditions compared to the remaining sampling range, which would be expected from a population inhabiting waters that border the species thermal tolerance.

Local-scale effects on annual growth

Acanthopagrus butcheri showed a mixed response to the local-scale variables. Freshwater flow produced little effect on *A. butcheri* from Hopkins River and Mallacoota but in larger systems with larger catchments and intensified flushing events (i.e., Coorong and Gippsland Lakes), as well as intermittently closed systems with large estuaries (i.e., Lake Brou and Lake Coila), growth showed a negative response to increased flow. Dramatic shifts in water conditions can alter water salinity and the halocline commonly present in estuarine environments (Jeppesen *et al.*, 2023; Filho *et al.*, 2022) with increased salinity stratification inducing *A. butcheri* spawning and increased juvenile growth (Jenkins *et al.*, 2018; Williams *et al.*, 2012; Partridge and Jenkins 2002). This influence of salinity on *A. butcheri* growth is shown in cases of negative growth responses of younger *A. butcheri* with increasing flow in Hopkins River, Mallacoota, and Lake Tyers (all intermittently closed), possibly reflecting their presence further upstream and higher sensitivity to a reduction in water salinity, whereas a shift to positive growth as fish grew older reflecting adult life within estuaries where increased flow would not greatly reduce salinity. Changes in water salinity and flow also trigger

varying growth responses within populations that exhibit partial migration or diadromy (e.g., *L. calcarifer*), comprising faster growing migrants compared to estuarine residents (Roberts *et al.*, 2019; Gillanders *et al.*, 2015). Further research into how migratory patterns vary across the distribution may shed further light on the long-term effects of such climatically induced disruptions to these estuaries.

Temperature-based effects showed varying growth trends among estuaries, specifically an inverse relationship with increasing SST and air temperature for most estuaries, which may explain why local-scale effects produced a weaker model than the regional-scale effects. The influence both SST and air temperature have on A. butcheri growth might possibly reflect the different relationship both variables have with estuary water temperature. Marine environments across south-eastern Australia are rapidly warming and have been labelled climate change hotspots (Hobday and Pecl 2014; Frusher et al., 2007; Ridgway 2007), suggesting higher latitude estuaries may be more sensitive to future fluctuations in SST. However, local air temperature is likely a better reflection of water temperature across all the different estuary types we sampled, with cases of a positive growth response of A. butcheri to air temperature matching previous trends shown by Australian estuarine and coastal species, including mulloway (Argyrosomus japonicus, Nicolle et al., 2022; Izzo et al., 2016), estuary perch (Percalates colonorum, Morrongiello et al., 2014), and eastern trumpeter (Pelates sexlineatus, O'Connor and Booth 2021). Studies have also shown positive growth responses of both A. butcheri and A. australis to estuary water temperature or air temperature (O'Connor and Booth 2021; Cottingham et al., 2016; Doubleday et al., 2015). The different influences both SST and air temperature have on estuaries is further supported by A. butcheri growth in Mallacoota, with a shift from positive to negative growth with increasing SST as fish grow older and the opposite shift with increasing air temperature.

Widespread exploitation from both commercial and recreational fishers has previously been suggested to impact growth (Morrongiello *et al.*, 2021; Frank *et al.*, 2016; Sarre and Potter 2000). Larger systems such as the Coorong or Gippsland Lakes have a commercial fishery targeting *A. butcheri*, which could impact growth patterns observed here across estuaries. Size-based fishing results in the removal of not only adults but younger individuals that are fast growing and recruit to the fishery at a younger age than slower-growing conspecifics, with overexploitation likely giving rise to slower somatically growing populations in these estuaries (i.e., the Rosa Lee Phenomenon) (Kraak *et al.*, 2019; Rouyer *et al.*, 2011; Lee 1912). The higher commercial catch of *A. butcheri* across their distribution during the 1980s, including within the Coorong (South Australia, Earl 2020) and Gippsland Lakes (Victoria, Conron *et al.*, 2016) was followed by a decline over the following decades that led to the Coorong stock being classified as 'depleted' (SAFS 2020). The faster growing

individuals were captured from smaller estuaries (e.g., Lake Tyers, Lake Brou, and Lake Coila, 10s km²). Cases of slower growing fish in smaller systems were only present in lower latitude estuaries, which is likely driven by environmental conditions rather than fishing effort, as has been previously found for *A. butcheri* growth (Izzo *et al.*, 2016; Doubleday *et al.*, 2015).

Spatial synchrony in annual growth

The presence of some spatial synchrony in growth between different estuary types and sizes further indicated local-scale, estuary-specific influences are less important than regional scale processes (Coulson et al., 2014). Such regional-scale, climate-induced synchronous growth has also been shown at similar regional-scales, including along the Western Australian coast, Bering Sea and Atlantic Ocean (1000s km) (Tanner et al., 2020; Ong et al. 2018, 2019; Matta et al., 2010). Our best-fitted regional-scale model (annual-lagged) supported such growth responses, with some cases of significant spatial synchrony occurring in the years following a large shift between ENSO conditions (i.e., El Niño and La Niña conditions), a climate-related change in growth previously shown in other south-eastern Australian fish (e.g., C. auratus and Sillaginodes punctatus, Jenkins et al., 2022). Spatial synchrony found both latitudinally and longitudinally supports our best-fitted model inference of regional-scale, climate-induced habitat alterations, which is likely promoting synchronous growth responses among A. butcheri populations. Cases of long-term significant spatial synchrony were also identified (i.e., over multiple 11-year sliding windows). Interestingly, more prolonged synchrony was only present across latitudinally separated populations, which has previously been shown in other fish species as well as in A. butcheri populations (Uvanović et al., 2023; Ong et al., 2018). As 11-year windows were used to produce robust results, this limited the number of assessable running correlations for estuaries with short year ranges (e.g., Lake Coila with 14 growth years and Swan River with 18 growth years). Therefore, more spatial synchrony may be occurring outside of these year ranges and further research incorporating contemporary samples and recent major ENSO events may shed light on prolonged patterns. Given the known variability across this species' spatial scale, both in water temperature and estuary type, our findings of prolonged spatial synchrony further support regional-scale and overall climatic context effects on A. butcheri growth, as opposed to spatial proximity of estuaries or similarities in the physical estuarine habitat (e.g., small or large catchments, open or closed systems). However, cases of spatial synchrony across south-eastern Australia (longitudinally) were not found consistently after large shifts in ENSO conditions. Inconsistent spatial synchrony coupled with cases of spatial asynchrony occurring between geographically close locations suggests local-scale effects still play a role in A. butcheri growth variability (Gray et al., 2022), with more extreme weather events triggering the synchronised responses found in this study.

Acanthopagrus butcheri showed a clear growth response to extrinsic factors across the sampling distribution, with regional-scale climatic effects inducing a significant change in *A. butcheri* growth across south-eastern Australia. El Niño Southern Oscillation events best explained a substantial amount of growth variability with an annual-lagged growth response from *A. butcheri* likely due to extreme weather events altering their estuaries and subsequently impacting fish growth in the years that followed. Furthermore, varying growth responses to local-scale extrinsic drivers reflects highly variable environmental conditions among estuaries, with higher growth sensitivity to changing environmental conditions, specifically SOI, shown by younger fish. Our findings highlight the power of otolith growth chronologies for understanding the long-term impact of climate change and extreme weather events on widely distributed fish inhabiting variable estuarine conditions.

References

- Bates, D, Mächler, M, Bolker, B, Walker, S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, doi:10.18637/jss.v067.i01
- Belarmino, E, de Nóbrega, MF, Grimm, AM, Copertino, MD, Vieira, JP, Garcia, AM (2021) Long-term trends in the abundance of an estuarine fish and relationships with El Nino climatic impacts and seagrass meadows reduction. *Estuarine Coastal and Shelf Science* **261**, doi:10.1016/j.ecss.2021.107565
- Black, BA, Boehlert, GW, Yoklavich, MM (2008) Establishing climate-growth relationships for yelloweye rockfish (*Sebastes ruberrimus*) in the northeast Pacific using a dendrochronological approach. *Fisheries Oceanography* **17**, 368-379. doi:10.1111/j.1365-2419.2008.00484.x
- Booth, DJ, Bond, N, Macreadie, P (2011) Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research* **62**, 1027-1042. doi:10.1071/Mf10270
- Bureau of Meteorology. (2023b). ENSO impacts rainfall. http://www.bom.gov.au/climate/enso/history/ln-2010-12/ENSO-rainfall.shtml
- Bureau of Meteorology. (2023a). Water data online. <u>http://www.bom.gov.au/waterdata/</u>
- Burnham, KP, Anderson, DR, Burnham, KP (2003) 'Model selection and multimodel inference: a practical information-theoretic approach.' (Springer: New York)
- Burridge, CP, Versace, VL (2006) Population genetic structuring in *Acanthopagrus butcheri* (*Pisces: Sparidae*): does low gene flow among estuaries apply to both sexes? *Marine Biotechnology* **9**, 33-44. doi:10.1007/s10126-006-6023-7
- Campana, SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59**, 197-242. doi:10.1006/jfbi.2001.1668
- Campana, SE, Smolinski, S, Black, BA, Morrongiello, JR, Alexandroff, SJ, Andersson, C, Bogstad, B, Butler, PG, Denechaud, C, Frank, DC, Geffen, AJ, Godiksen, JA, Gronkjaer, P, Hjorleifsson, E, Jonsdottir, IG, Meekan, M, Mette, M, Tanner, SE, van der Sleen, P, von Leesen, G (2023) Growth portfolios buffer climate-linked environmental change in marine systems. *Ecology* **104**, e3918. doi:10.1002/ecy.3918
- Carozza, DA, Bianchi, D, Galbraith, ED, Bates, A (2018) Metabolic impacts of climate change on marine ecosystems: Implications for fish communities and fisheries. *Global Ecology and Biogeography* **28**, 158-169. doi:10.1111/geb.12832
- Chaplin JA, BG, Gill HS, McCullock R, Potter IC (1998) Are assemblages of black bream (*Acanthopagrus butcheri*) in different estuaries genetically distinct? *International Journal of Salt Lake Research* **6**, 303-321.
- Cheshire, KJM, Ye, Q, Fredberg, J, Earl, J (2013) Aspects of reproductive biology of five key fish species in the Murray Mouth and Coorong. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Conron, S, Giri, K., Hall, K., Hamer, P., 2016. Gippsland Lakes Fisheries Assessment.
- Cottingham, A, Hall, NG, Potter, IC (2016) Factors influencing growth of *Acanthopagrus butcheri* (Sparidae) in a eutrophic estuary have changed over time. *Estuarine, Coastal and Shelf Science* **168**, 29-39. doi:10.1016/j.ecss.2015.10.031
- Cottingham, A, Peisheng H, Hipsey, MR, Hall, NG, Ashworth, A, Williams, J, Potter, IC (2018). "Growth, Condition, and Maturity Schedules of an Estuarine Fish Species Change in Estuaries Following Increased Hypoxia Due to Climate Change." *Ecology and Evolution* **8** (14): 7111– 30. doi:10.1002/ece3.4236
- Coulson, PG, Black, BA, Potter, IC, Hall, NG (2013) Sclerochronological studies reveal that patterns of otolith growth of adults of two co-occurring species of Platycephalidae are synchronised by water temperature variations. *Marine Biology* **161**, 383-393. doi:10.1007/s00227-013-2343-0
- Dembkowski, DJ, Willis, DW, Wuellner, MR (2016) Synchrony in larval yellow perch abundance: the influence of the Moran Effect during early life history. *Canadian Journal of Fisheries and Aquatic Sciences* **73**, 1567-1574. doi:10.1139/cjfas-2015-0310

- Dong, ZG, Chen, YH, Ge, HX, Li, XY, Wu, HL, Wang, CH, Hu, Z, Wu, YJ, Fu, GH, Lu, JK, Che, H (2018) Response of growth and development of the Pacific oyster (*Crassostrea gigas*) to thermal discharge from a nuclear power plant. *BMC Ecology* **18**, 31. doi:10.1186/s12898-018-0191-y
- Doubleday, ZA, Izzo, C, Haddy, JA, Lyle, JM, Ye, Q, Gillanders, BM (2015) Long-term patterns in estuarine fish growth across two climatically divergent regions. *Oecologia* **179**, 1079-90. doi:10.1007/s00442-015-3411-6
- Earl, J, 2020. Assessment of the South Australian Lakes and Coorong Fishery in 2018/19. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Elsdon, TS, Gillanders, BM (2006) Identifying migratory contingents of fish by combining otolith Sr:Ca with temporal collections of ambient Sr:Ca concentrations. *Journal of Fish Biology* **69**, 643-657. doi:10.1111/j.1095-8649.2006.01136.x
- Farrington, LW, Austin, CM, Coutin, PC (2000) Allozyme variation and stock structure in the black bream, *Acanthopagrus butcheri* (Munro) (Sparidae) in southern Australia: implications for fisheries management, aquaculture and taxonomic relationship with (Gunther). *Fisheries Management and Ecology* 7, 265-279. doi:DOI 10.1046/j.1365-2400.2000.00178.x
- Feiner, ZS, Coulter, DP, Linn, MD, Höök, TO (2019) A question of scale: Weak evidence for broad regional synchrony in fish year-class strength within or among species in inland lakes. *Fisheries Research* 214, 45-55. doi:10.1016/j.fishres.2019.01.025
- Felts, EA, Fincel, MJ, Graeb, BDS (2020) Effects of reservoir elevation and spatial synchrony on walleye recruitment in Lake Oahe, Missouri River. North American Journal of Fisheries Management 40, 1133-1145. doi:10.1002/nafm.10477
- Frank, KT, Petrie, B, Leggett, WC, Boyce, DG (2016) Large scale, synchronous variability of marine fish populations driven by commercial exploitation. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 8248-53. doi:10.1073/pnas.1602325113
- Frusher, SD, Hobday, AJ, Jennings, SM, Creighton, C, D'Silva, D, Haward, M, Holbrook, NJ, Nursey-Bray, M, Pecl, GT, van Putten, EI (2013) The short history of research in a marine climate change hotspot: from anecdote to adaptation in south-east Australia. *Reviews in Fish Biology and Fisheries* doi:10.1007/s11160-013-9325-7
- Gillanders, BM, Izzo, C, Doubleday, ZA, Ye, Q (2015) Partial migration: growth varies between resident and migratory fish. *Biology Letters* **11**, doi:10.1098/rsbl.2014.0850
- Gray, CA (2022) Variation in growth, length and age characteristics of estuarine *Acanthopagrus* (Sparidae) populations in New South Wales, Australia. *Regional Studies in Marine Science* **55**, doi:10.1016/j.rsma.2022.102481
- Handeland, SO, Imsland, AK, Stefansson, SO (2008) The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture* **283**, 36-42. doi:10.1016/j.aquaculture.2008.06.042
- Hobday, AJ, Pecl, GT (2013) Identification of global marine hotspots: sentinels for change and vanguards for adaptation action. *Reviews in Fish Biology and Fisheries* **24**, 415-425. doi:10.1007/s11160-013-9326-6
- Holbrook, NJ, Claar, DC, Hobday, AJ, McInnes, KL, Oliver, ECJ, Gupta, AS, Widlansky, MJ, Zhang, X (2020) ENSO-driven ocean extremes and their ecosystem impacts. In 'El Niño Southern Oscillation in a Changing Climate.' pp. 409-428.
- Hopson, J, Fox, JW (2019) Occasional long distance dispersal increases spatial synchrony of population cycles. *Jounral of Animal Ecology* **88**, 154-163. doi:10.1111/1365-2656.12905
- Izzo, C, Doubleday, ZA, Grammer, GL, Barnes, TC, Delean, S, Ferguson, GJ, Ye, QF, Gillanders, BM (2016) Multispecies response to rapid environmental change in a large estuary system: A biochronological approach. *Ecological Indicators* **69**, 739-748. doi:10.1016/j.ecolind.2016.05.019
- Izzo, C, Doubleday, ZA, Grammer, GL, Gilmore, KL, Alleway, HK, Barnes, TC, Disspain, MCF, Giraldo, AJ, Mazloumi, N, Gillanders, BM (2016) Fish as proxies of ecological and environmental change. *Reviews in Fish Biology and Fisheries* 26, 265-286. doi:10.1007/s11160-016-9424-3

- Jaffrés, JBD, Cuff, B, Cuff, C, Knott, M, Rasmussen, C (2022) Hydrological characteristics of Australia: national catchment classification and regional relationships. *Journal of Hydrology* **612**, doi:10.1016/j.jhydrol.2022.127969
- Jenkins, GP, Coleman, RA, Barrow, JS, Morrongiello, JR (2022). Environmental drivers of fish population dynamics in an estuarine ecosystem of south-eastern Australia. *Fisheries Management and Ecology*, *29*(5), 693-707. doi:0.1111/fme.12559
- Jenkins, GP, Kent, JA, Woodland, RJ, Warry, F, Swearer, SE, Cook, PLM (2018) Delayed timing of successful spawning of an estuarine dependent fish, black bream *Acanthopagrus butcheri*. *Journal of Fish Biology* **93**, 931-941. doi:10.1111/jfb.13806
- Jiménez-Muñoz, JC, Mattar, C, Barichivich, J, Santamaria-Artigas, A, Takahashi, K, Malhi, Y, Sobrino, JA, Schrier, G (2016) Record-breaking warming and extreme drought in the Amazon rainforest during the course of El Nino 2015-2016. *Scientific Reports* **6**, 33130. doi:10.1038/srep33130
- Koenig, WD (2001) Spatial autocorrelation and local disappearances in wintering North American birds. *Ecology* **82**, 2636-2644. doi:Doi 10.1890/0012-9658(2001)082[2636:Saaldi]2.0.Co;2
- Kraak, SBM, Haase, S, Minto, C, Santos, J (2019) The Rosa Lee phenomenon and its consequences for fisheries advice on changes in fishing mortality or gear selectivity. *ICES Journal of Marine Science* 76, 2179-2192. doi:10.1093/icesjms/fsz107
- Leahy, SM, Robins, JB (2021) River flows affect the growth of a tropical finfish in the wet-dry rivers of northern Australia, with implications for water resource development. *Hydrobiologia* **848**, 4311-4333. doi:10.1007/s10750-021-04641-7
- Leal Filho, W, Nagy, GJ, Martinho, F, Saroar, M, Erache, MG, Primo, AL, Pardal, MA, Li, C (2022) Influences of climate change and variability on estuarine ecosystems: an impact study in selected European, South American and Asian countries. *International Journal of Environmental Research and Public Health* **19**, doi:10.3390/ijerph19010585
- Lee, RM (1912) An investigation into the methods of growth determination in fishes by means of scales. *ICES* Journal of Marine Science **s1**, 3-34. doi:10.1093/icesjms/s1.63.3
- Liebhold, A, Koenig, WD, Bjornstad, ON (2004) Spatial synchrony in population dynamics. *Annual Review of Ecology Evolution and Systematics* **35**, 467-490. doi:10.1146/annurev.ecolsys.34.011802.132516
- Lopez Abbate, MC, Molinero, JC, Guinder, VA, Perillo, GME, Freije, RH, Sommer, U, Spetter, CV, Marcovecchio, JE (2017) Time-varying environmental control of phytoplankton in a changing estuarine system. *Science of The Total Environment* **609**, 1390-1400. doi:10.1016/j.scitotenv.2017.08.002
- Lovelock, CE, Feller, IC, Reef, R, Hickey, S, Ball, MC (2017) Mangrove dieback during fluctuating sea levels. *Scientific Reports* **7**, 1680. doi:10.1038/s41598-017-01927-6
- Lüdecke, D (2018) ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software* **3**, doi:10.21105/joss.00772
- Martino, JC, Fowler, AJ, Doubleday, ZA, Grammer, GL, Gillanders, BM (2019) Using otolith chronologies to understand long-term trends and extrinsic drivers of growth in fisheries. *Ecosphere* **10**, e02553. doi:10.1002/ecs2.2553
- Matta, ME, Black, BA, Wilderbuer, TK (2010) Climate-driven synchrony in otolith growth-increment chronologies for three Bering Sea flatfish species. *Marine Ecology Progress Series* **413**, 137-145. doi:10.3354/meps08689
- Meuser, E, Mooers, AØ, Cleary, DFR (2013) El Niño and biodiversity. In 'Encyclopedia of Biodiversity.' (Ed. SA Levin.) pp. 155-163. (Academic Press: Waltham)
- Morrongiello, JR, Horn, PL, C, OM, Sutton, PJH (2021) Synergistic effects of harvest and climate drive synchronous somatic growth within key New Zealand fisheries. *Global Change Biology* **27**, 1470-1484. doi:10.1111/gcb.15490
- Morrongiello, JR, Thresher, RE (2015) A statistical framework to explore ontogenetic growth variation among individuals and populations: a marine fish example. *Ecological Monographs* **85**, 93-115. doi:10.1890/13-2355.1
- Morrongiello, JR, Thresher, RE, Smith, DC (2012) Aquatic biochronologies and climate change. *Nature Climate Change* **2**, 849-857. doi:10.1038/Nclimate1616
Munro, ISR (1949) 'Revision of Australian Silver Breams: Mylio and Rhabdosargus.' (Queensland Museum:

- Nicolle, P, Hughes, J, Fowler, A, Schilling, HT (2022) Long-term increase in growth of an estuarine predator, mulloway *Argyrosomus japonicus*, predicted to continue under future warming scenarios. *Marine Ecology Progress Series* **688**, 1-17. doi:10.3354/meps14048
- National Oceanic and Atmospheric Administration. (2024). Southern oscillation index. https://www.ncei.noaa.gov/access/monitoring/enso/soi
- Nikolaou, A, Katsanevakis, S (2023) Marine extinctions and their drivers. *Regional Environmental Change* 23, doi:10.1007/s10113-023-02081-8
- Norriss, JV, Tregonning, JE, Lenanton, RCJ, Sarre, GA, 2002. Biological synopsis of the black bream, *Acanthopagrus butcheri* (Munro) (Teleostei: Sparidae) in Western Australia with reference to information from other southern states. Department of Fisheries, Perth, Western Australia.
- O'Connor, C, Booth, DJ (2021) Response of estuarine fishes to elevated temperatures within temperate Australia: Implications of climate change on fish growth and foraging performance. *Journal of Experimental Marine Biology and Ecology* **544**, doi:10.1016/j.jembe.2021.151626
- Olin, AB, Banas, NS, Wright, PJ, Heath, MR, Nager, RG (2020) Spatial synchrony of breeding success in the blacklegged kittiwake *Rissa tridactyla* reflects the spatial dynamics of its sandeel prey. *Marine Ecology Progress Series* **638**, 177-190. doi:10.3354/meps13252
- Ong, JJ, Rountrey, AN, Zinke, J, Meeuwig, JJ, Grierson, PF, O'Donnell, AJ, Newman, SJ, Lough, JM, Trougan, M, Meekan, MG (2016) Evidence for climate-driven synchrony of marine and terrestrial ecosystems in northwest Australia. *Global Change Biology* **22**, 2776-86. doi:10.1111/gcb.13239
- Ong, JJL, Rountrey, AN, Black, BA, Nguyen, HM, Coulson, PG, Newman, SJ, Wakefield, CB, Meeuwig, JJ, Meekan, MG (2018) A boundary current drives synchronous growth of marine fishes across tropical and temperate latitudes. *Global Change Biology* **24**, 1894-1903. doi:10.1111/gcb.14083
- Panella, G (1971) Fish otoliths: daily growth layers and periodical patterns. *Science* **173**, 1124-7. doi:10.1126/science.173.4002.1124
- Partridge, GJ, Jenkins, GI (2002) The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). *Aquaculture* **210**, 219-230. doi:10.1016/S0044-8486(01)00817-1
- Pecl, GT, Ward, TM, Doubleday, ZA, Clarke, S, Day, J, Dixon, C, Frusher, S, Gibbs, P, Hobday, AJ, Hutchinson, N, Jennings, S, Jones, K, Li, XX, Spooner, D, Stoklosa, R (2014) Rapid assessment of fisheries species sensitivity to climate change. *Climatic Change* 127, 505-520. doi:10.1007/s10584-014-1284-z
- Pinsky, ML, Byler, D (2015) Fishing, fast growth and climate variability increase the risk of collapse. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20151053. doi:10.1098/rspb.2015.1053
- R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
- Reis-Santos, P, Condini, MV, Albuquerque, CQ, Saint'Pierre, TD, Garcia, AM, Gillanders, BM, Tanner, SE (2021) El Nino - Southern Oscillation drives variations in growth and otolith chemistry in a top predatory fish. *Ecological Indicators* **121**, doi:10.1016/j.ecolind.2020.106989
- Reis-Santos, P, Gillanders, BM, Sturrock, AM, Izzo, C, Oxman, DS, Lueders-Dumont, JA, Hüssy, K, Tanner, SE, Rogers, T, Doubleday, ZA, Andrews, AH, Trueman, C, Brophy, D, Thiem, JD, Baumgartner, LJ, Willmes, M, Chung, M-T, Charapata, P, Johnson, RC, Trumble, S, Heimbrand, Y, Limburg, KE, Walther, BD (2022)
 Reading the biomineralized book of life: expanding otolith biogeochemical research and applications for fisheries and ecosystem-based management. *Reviews in Fish Biology and Fisheries* 33, 411-449. doi:10.1007/s11160-022-09720-z
- Ridgway, KR (2007) Long-term trend and decadal variability of the southward penetration of the east australian current. *Geophysical Research Letters* **34**, doi:10.1029/2007gl030393
- Rountrey, AN, Coulson, PG, Meeuwig, JJ, Meekan, M (2014) Water temperature and fish growth: otoliths predict growth patterns of a marine fish in a changing climate. *Global Change Biology* **20**, 2450-8. doi:10.1111/gcb.12617
- Rouyer, T, Fromentin, JM, Hidalgo, M, Stenseth, NC (2014) Combined effects of exploitation and temperature on fish stocks in the Northeast Atlantic. *ICES Journal of Marine Science* **71**, 1554-1562. doi:10.1093/icesjms/fsu042

- Rypel, AL (2009) Climate-growth relationships for largemouth bass (*Micropterus salmoides*) across three southeastern USA states. *Ecology of Freshwater Fish* **18**, 620-628. doi:10.1111/j.1600-0633.2009.00379.x
- SAFS (2020), 'Black bream' Available at https://fish.gov.au/report/366-Black-Bream-2020 [Accessed 16/01/24]
- Sakabe, R, Lyle, JM (2010) The influence of tidal cycles and freshwater inflow on the distribution and movement of an estuarine resident fish *Acanthopagrus butcheri*. *Journal of Fish Biology* **77**, 643-60. doi:10.1111/j.1095-8649.2010.02703.x
- Sarre, GA, Potter, IC (1999) Comparisons between the reproductive biology of black bream *Acanthopagrus butcheri* (Teleostei: Sparidae) in four estuaries with widely differing characteristics. *International Journal of Salt Lake Research* **8**, 179-210.
- Sarre, GA, Potter, IC (2000) Variation in age compositions and growth rates of *Acanthopagrus butcheri* (Sparidae) among estuaries:: some possible contributing factors. *Fishery Bulletin* **98**, 785-799.
- Sathicq, MB, Bauer, DE, Gomez, N (2015) Influence of el nino southern oscillation phenomenon on coastal phytoplankton in a mixohaline ecosystem on the southeastern of South America: Rio de la Plata estuary. *Marine Pollution Bulletin* **98**, 26-33. doi:10.1016/j.marpolbul.2015.07.017
- Schmidt, N, Luther, NE (2002) ENSO impacts on salinity in Tampa Bay, Florida. *Estuaries* **25**, 976-984. doi:Doi 10.1007/Bf02691345
- Schneider, CA, Rasband, WS, Eliceiri, KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671-5. doi:10.1038/nmeth.2089
- Stocks, J, Stewart, J, Gray, CA, West, RJ (2011) Using otolith increment widths to infer spatial, temporal and gender variation in the growth of sand whiting. *Fisheries Management and Ecology* **18**, 121-131. doi:10.1111/j.1365-2400.2010.00761.x
- Stocks, JR, Gray, CA, Taylor, MD (2014) Synchrony and variation across latitudinal gradients: The role of climate and oceanographic processes in the growth of a herbivorous fish. *Journal of Sea Research* **90**, 23-32. doi:10.1016/j.seares.2014.03.002
- Tanner, SE, Giacomello, E, Menezes, GM, Mirasole, A, Neves, J, Sequeira, V, Vasconcelos, RP, Vieira, AR, Morrongiello, JR (2020) Marine regime shifts impact synchrony of deep-sea fish growth in the northeast Atlantic. *Oikos* 129, 1781-1794. doi:10.1111/oik.07332
- Tolan, JM (2007) El Nino-Southern Oscillation impacts translated to the watershed scale: Estuarine salinity patterns along the Texas Gulf Coast, 1982 to 2004. *Estuarine Coastal and Shelf Science* **72**, 247-260. doi:10.1016/j.ecss.2006.10.018
- Tracey, SR, Hartmann, K, McAllister, J, Lyle, JM (2020) Home range, site fidelity and synchronous migrations of three co-occurring, morphologically distinct estuarine fish species. *Science of The Total Environment* **713**, 136629. doi:10.1016/j.scitotenv.2020.136629
- Uvanović, H, Peharda, M, Pavin, N, Thébault, J, Mazzoldi, C, Mihanović, H, Župan, I (2023) Developing a sclerochronology network in the Adriatic Sea: Growth synchrony among populations of *Callista chione*. *Regional Studies in Marine Science* **64**, doi:10.1016/j.rsma.2023.103009
- Wetz, MS, Yoskowitz, DW (2013) An 'extreme' future for estuaries? Effects of extreme climatic events on estuarine water quality and ecology. *Marine Pollution Bulletin* **69**, 7-18. doi:10.1016/j.marpolbul.2013.01.020
- Williams, J, Hindell, JS, Jenkins, GP, Tracey, S, Hartmann, K, Swearer, SE (2017) The influence of freshwater flows on two estuarine resident fish species show differential sensitivity to the impacts of drought, flood and climate change. *Environmental Biology of Fishes* **100**, 1121-1137. doi:10.1007/s10641-017-0632-z
- Williams, J, Hindell, JS, Swearer, SE, Jenkins, GP (2012) Influence of freshwater flows on the distribution of eggs and larvae of black bream *Acanthopagrus butcheri* within a drought-affected estuary. *Journal of Fish Biology* **80**, 2281-301. doi:10.1111/j.1095-8649.2012.03283.x

Supplementary Material



Fig. 4.S1 *Acanthopagrus butcheri* samples mapped across the year range investigated. Horizontal bands indicate each fish from the year of birth (left) to the year of capture (right), with colours indicating each estuary.



Fig. 4.S2 Box and whisker plot of otolith annual increment measurements by age of formation (2-25) for all *A. butcheri* across all nine estuaries.



Fig. 4.S3 Environmental data through time coloured by estuary. Environmental data include (a) Southern Oscillation Indices (SOI), (b) air temperature, (c) Sea-Surface Temperature (SST), and (d) water flow (log-transformed). Due to close spatial proximity of estuaries, data from Lake Tyers and Gippsland Lakes were identical for both air temperature and SST and have been mapped onto plots b and c as a single signal (red). Environmental data for Little Swanport has been excluded as the estuary was removed from all extrinsic-based tests.



Fig. 4.S4 Growth chronologies for each *A. butcheri* estuary by growth year generated using the bestfitted intrinsic model (Best Linear Unbiased Predictors [BLUP]). Colours indicate each estuary, including (a) Coorong, (b), Hopkins River, (c) Gippsland Lakes, (d) Lake Tyers, (e) Mallacoota, (f) Lake Brou, (g) Lake Coila, (h) Swan River, and (i) Little Swanport.



Fig. 4.S5 Conditional effects plots generated using the best-fitted regional-scale model (a) and localscale model (b-d). Predicted *A. butcheri* otolith growth plotted in response to annual-lagged (i.e., past year [PY]) (a) Southern Oscillation Index (SOI), (b) air temperature, (c) Sea-Surface Temperature (SST), and (d) water flow. Extrinsic variables have been plotted as centre-scaled values and further grouped by age (Age = X). Colours indicate each estuary (excluding Little Swanport).

Table 4.S1 Fixed effects parameter estimates and random effects variance of the best-fitted intrinsic model for explaining annual *A. butcheri* growth variability. Table includes Standard Deviation (SD) and Standard Error (SE) values.

Random effects	Variance	SD	Correlation
FishID	0.050	0.223	-
Age	0.013	0.116	-0.810
Estuary:Year	0.010	0.098	-
Residual	0.055	0.232	-
Fixed effects	Estimate		SE
Intercept	5.753		0.043
Age	-0.888		0.029
Hopkins River	-0.001		0.053
Gippsland Lakes	0.021		0.049
Lake Tyers	0.072		0.051
Mallacoota	0.187		0.051
Lake Brou	-0.144		0.074
Lake Coila	-0.369		0.096
Swan River	-0.224		0.062
Little Swanport	-0.147		0.069
AAC	0.010		0.005
Age:Hopkins River	0.017		0.032
Age:Gippsland Lakes	0.108		0.031
Age:Lake Tyers	0.174		0.031
Age:Mallacoota	0.040		0.032
Age:Lake Brou	0.157		0.051
Age:Lake Coila	0.168		0.080
Age:Swan River	0.022		0.040
Age:Little Swanport	-0.066		0.046
Hopkins River:AAC	-0.004		0.006
Gippsland Lakes:AAC	-0.008		0.006
Lake Tyers:AAC	-0.018		0.006
Mallacoota:AAC	-0.013		0.006
Lake Brou:AAC	-0.002		0.009
Lake Coila:AAC	0.014		0.016
Swan River:AAC	0.003		0.007
Little Swanport:AAC	0.012		0.009

Table 4.S2 Results of the best-fitted intrinsic models based on delta Akaike information criterion values (Δ AICc). Table also includes degrees of freedom values (*df*). Little Swanport was included in the following intrinsic models but excluding from the extrinsic models due to a lack of environmental data.

Intrinsic model	df	ΔAICc
Age x Estuary + AAC x Estuary + Age FishID + 1 Estuary: Year	32	0.000
Age x Estuary + AAC x Estuary + 1 FishID + 1 Estuary:Year	30	270.400

Table 4.S3 Fixed effects parameter estimates and random effects variance of the best-fitted regionalscale extrinsic model for explaining annual *A. butcheri* growth variability. Table includes Standard Deviation (SD) and Standard Error (SE) values.

Random effects	Variance	SD	Correlation
FishID	0.045	0.211	
Age	0.012	0.11	-0.82
Estuary:Year	0.008	0.092	
Residual	0.055	0.234	
Fixed effects	Estimate		SE
Intercept	5.752		0.044
Age	-0.867		0.032
Hopkins River	0.01		0.053
Gippsland Lakes	0.043		0.05
Lake Tyers	0.141		0.052
Mallacoota	0.204		0.053
Lake Brou	-0.124		0.073
Lake Coila	-0.443		0.097
Swan River	-0.219		0.062
AAC	0.007		0.006
SOI	0.089		0.039
SOI ²	-0.014		0.008
Age:Hopkins River	-0.011		0.035
Age:Gippsland Lakes	0.072		0.034
Age:Lake Tyers	0.108		0.034
Age:Mallacoota	0.016		0.035
Age:Lake Brou	0.172		0.056
Age:Lake Coila	0.214		0.082
Age:Swan River	0.014		0.043
Hopkins River:AAC	0		0.007
Gippsland Lakes:AAC	-0.004		0.006
Lake Tyers:AAC	-0.017		0.006
Mallacoota:AAC	-0.011		0.007
Lake Brou:AAC	-0.006		0.01
Lake Coila:AAC	0.014		0.016
Swan River:AAC	0.005		0.008

Hopkins River:SOI	-0.061	0.047
Gippsland Lakes:SOI	-0.095	0.044
Lake Tyers:SOI	-0.166	0.046
Mallacoota:SOI	-0.083	0.046
Lake Brou:SOI	-0.151	0.069
Lake Coila:SOI	0.328	0.102
Swan River:SOI	0.009	0.054
Age:SOI	-0.061	0.022
Age:Hopkins River:SOI	0.071	0.025
Age:Gippsland Lakes:SOI	0.091	0.023
Age:Lake Tyers:SOI	0.139	0.024
Age:Mallacoota:SOI	0.071	0.024
Age:Lake Brou:SOI	0.102	0.038
Age:Lake Coila:SOI	-0.135	0.066
Age:Swan River:SOI	0.042	0.027

Table 4.S4 Fixed effects parameter estimates and random effects variance of the best-fitted localscale extrinsic model for explaining annual *A. butcheri* growth variability. Table includes Standard Deviation (SD) and Standard Error (SE) values.

Random effects	Variance	SD	Correlation
FishID	0.041	0.202	-
Age	0.011	0.105	-0.800
Estuary:Year	0.008	0.089	-
Residual	0.055	0.234	-
Fixed effects	Estimate		SE
Intercept	5.765		0.045
Age	-0.963		0.037
Hopkins River	-0.013		0.055
Gippsland Lakes	0.034		0.052
Lake Tyers	0.060		0.054
Mallacoota	0.157		0.053
Lake Brou	-0.138		0.077
Lake Coila	-0.409		0.100
Swan River	-0.210		0.070
AAC	0.024		0.008
Flow	-0.010		0.038
Temperature	0.109		0.063
Temperature ²	-0.002		0.016
SST	-0.053		0.058
Age:Hopkins River	0.083		0.040
Age:Gippsland Lakes	0.176		0.038
Age:Lake Tyers	0.242		0.039
Age:Mallacoota	0.122		0.039
Age:Lake Brou	0.212		0.074
Age:Lake Coila	0.175		0.127
Age:Swan River	0.133		0.048
Hopkins River:AAC	-0.018		0.008

Gippsland Lakes:AAC	-0.019	0.008
Lake Tyers:AAC	-0.034	0.008
Mallacoota:AAC	-0.028	0.008
Lake Brou:AAC	-0.012	0.014
Lake Coila:AAC	0.026	0.026
Swan River:AAC	-0.017	0.009
Hopkins River:Flow	-0.046	0.042
Gippsland Lakes:Flow	-0.063	0.043
Lake Tyers:Flow	-0.056	0.044
Mallacoota:Flow	-0.030	0.045
Lake Brou:Flow	0.001	0.107
Lake Coila:Flow	0.018	0.072
Swan River:Flow	0.028	0.060
Age:Flow	-0.034	0.026
Hopkins River:Temperature	-0.019	0.073
Gippsland Lakes:Temperature	-0.092	0.068
Lake Tyers:Temperature	-0.081	0.072
Mallacoota:Temperature	-0.219	0.072
Lake Brou:Temperature	-0.083	0.103
Lake Coila:Temperature	-0.240	0.133
Swan River: Temperature	-0.176	0.094
Age:Temperature	0.009	0.027
Hopkins River:Temperature ²	0.021	0.021
Gippsland Lakes: Temperature ²	-0.013	0.017
Lake Tyers: Temperature ²	0.017	0.018
Mallacoota: Temperature ²	0.007	0.017
Lake Brou: Temperature ²	-0.003	0.025
Lake Coila: Temperature ²	-0.017	0.023
Swan River: Temperature ²	-0.011	0.033
Hopkins River:SST	0.031	0.068
Gippsland Lakes:SST	0.050	0.064
Lake Tvers:SST	0.005	0.067
Mallacoota:SST	0.139	0.065
Lake Brou:SST	0.023	0.109
Lake Coila:SST	0.175	0.157
Swan River:SST	0.191	0.087
Age:SST	-0.014	0.030
Age:Hopkins River:Flow	0.069	0.027
Age:Gippsland Lakes:Flow	0.065	0.027
Age:Lake Tvers:Flow	0.109	0.027
Age:Mallacoota:Flow	0.063	0.027
Age:Lake Brou:Flow	0.020	0.060
Age:Lake Coila:Flow	-0.013	0.060
Age:Swan River:Flow	0.041	0.030
Age:Hopkins		
River:Temperature	-0.046	0.031
Age:Gippsland		
Lakes:Temperature	-0.025	0.028
Age:Lake Tyers:Temperature	-0.021	0.029
Age:Mallacoota:Temperature	0.071	0.030
Age:Lake Brou:Temperature	0.008	0.051
- •		

Age:Lake Coila:Temperature	0.061	0.072
Age:Swan River:Temperature	0.018	0.044
Age:Hopkins River:SST	0.030	0.033
Age:Gippsland Lakes:SST	0.046	0.031
Age:Lake Tyers:SST	0.063	0.032
Age:Mallacoota:SST	-0.041	0.033
Age:Lake Brou:SST	0.010	0.057
Age:Lake Coila:SST	-0.020	0.093
Age:Swan River:SST	-0.031	0.045

CHAPTER 5

Otolith chemistry reveals varying movement

patterns across estuaries

Title of Paper	Fish otolith chemistry reveals varying movement	patterns ac	ross estuaries
Publication Status	Published Acce	pted for Pt	ublication
	Submitted for Publication	ublished an uscript style	nd Unsubmitted work written in e
Publication Details			
Principal Author			
Name of Principal Author (Candidate)	Koster Georgien Sarakinis		
Contribution to the Paper	Conceptualization (Equal); Data curation (Lead); Methodology (Equal); Project administration (Lea Writing – original draft (Lead); Writing – review &	Formal ana d); Softwar editing (Eq	lysis (Lead); Investigation (Lead); e (Equal); Visualization (Lead); ual).
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conduc Research candidature and is not subject to any of party that would constrain its inclusion in this thes	eted during oligations of sis. I am the	the period of my Higher Degree by r contractual agreements with a third e primary author of this paper.
Signature	Juntin	Date	3/02/2024
Co-Author Contribution	s / J		1
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Signature	145	Date	21/02/2024
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Signature	f.C.	Date	14 / 02 / 2024
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Contribution to the Paper	Conceptualization (Equal); Funding acqui editing (Equal).	sition (Lead); Sup	ervision (Lead); Writing – review &
Signature	BM Gillanders	Date	19 Feb 2024

5.1 Abstract

Understanding animal movement across a species distribution can reveal the appropriate spatial scales to manage wild populations. Harnessing natural tagging techniques provides information on animal movement spanning an individual's entire life history. We used otolith chemistry to investigate potential movement patterns of black bream Acanthopagrus butcheri in southern Australian estuaries. Specifically, we harnessed otolith Ba:Ca and Sr:Ca signals spanning each individual fish's life history (i.e., otolith profiles) and performed K-means cluster analysis to group fish based on similarities in otolith chemistry. Results showed a mixture of stable and fluctuating signals, reflecting differences in the environments experienced across individual life histories and the presence of resident and migratory fish. Ba:Ca was best in representing fish movement, with varying signals found within and among estuaries. More stable and lower magnitude changes in Ba:Ca signals, reflecting resident behaviour, were found more often in intermittently closed systems with large estuaries, while fish with fluctuating Ba:Ca signals reflecting shifts across large environmental gradients, including movement outside of estuaries (i.e., into rivers or coastal waters), were found more often in open systems and intermittently closed systems with small estuaries. Sr:Ca signals were almost identical across the entire sampling distribution, suggesting a more ontogenetic influence. The variability in movement patterns appeared to be estuary-specific, with some populations utilising surrounding water bodies (i.e., rivers and coastal waters) more than others. Our findings stress the importance of considering the diversity in movement patterns for this estuarine species, with management extending across the salinity gradient for select populations with increased migratory behaviour. We highlight the power of otolith chemistry in characterising fish movement and its application in investigating population dynamics of estuarine fish.

Keywords: movement patterns; chemical profile; *Acanthopagrus butcheri*; otolith chemistry; estuary; Australia

5.2 Introduction

Understanding animal movement patterns within and among populations can uncover valuable information regarding habitat use, and the spatial scales required for appropriate management. Fish movement patterns can be assessed through both applied and natural tagging techniques. Artificial tags, including acoustic telemetry (Williams *et al.*, 2017; Gannon *et al.*, 2015; Calò *et al.*, 2013) and archival tags (Goetz *et al.*, 2018; Block *et al.*, 2001) are powerful techniques able to provide both movement and environmental information, although are temporally limited to the date of tag deployment and can reduce fish growth and survival (Jepsen *et al.*, 2015). Alternatively, natural tagging can access information encoded from birth to capture, and in aquatic ecology are commonly the hard parts of animals, including vertebrae in elasmobranchs (Frazier *et al.*, 2023; McMillan *et al.*, 2018), shells in bivalves (Dong *et al.*, 2018; Nielsen *et al.*, 2008), and otoliths (i.e., ear stones) in fish (Reis-Santos *et al.*, 2023; Campana 1999). Natural tags provide a suite of valuable information. For example, fish otoliths provide information relating to a fish's life history, such as growth rate, individual movement, habitat use, and natal origin (Rogers *et al.*, 2019; Morrongiello and Thresher 2015; Elsdon *et al.*, 2008).

Otoliths are polycrystalline, calcium carbonate structures formed in the inner ear of teleost fish that continuously accrete material over time to form both daily and annual increments reflecting fish age and growth (Reis-Santos et al., 2023; Campana 2001). This accretion process incorporates minor and trace elements from the ambient water, either binding to calcium ions within the calcium carbonate matrix or becoming trapped in the interstitial spaces (Doubleday et al., 2014). Because otoliths are metabolically inert, their chemical composition reflects the environments fish inhabit during the time of otolith accretion, and analysis across the growth axis reflect an individual's life history from otolith core (location of birth) to otolith edge (location of capture) (Sarakinis et al., 2022; Izzo et al., 2017). Element incorporation in otoliths can act as environmental indicators due to their correlation with ambient water chemistry (Reis-Santos et al., 2018, 2013). Most notably, otolith Sr and Ba have shown a clear positive and negative correlation with water salinity, respectively (Izzo et al., 2017; Elsdon and Gillanders 2005; Tabouret et al., 2010). This relationship has been used to assess the movement of species across water salinity gradients throughout their life history (i.e., across riverine, estuarine, and marine waters). For example, studies have demonstrated movement and habitat use in barramundi (e.g., Lates calcarifer, Nazir et al., 2023; Walther et al., 2011), Murray Cod (e.g., Maccullochella peelii, Thiem et al., 2021;) and chinook salmon (e.g., Oncorhynchus tshawytscha, Brennan et al., 2019). Additional environmental indicators include stable Oxygen isotopes previously linked to water temperature (Stanley et al., 2015; Elsdon and Gillanders 2002), heavy metals such as Pd, Cu, and Zn linked to water pollution (Sinnatamby et al., 2019; McKinley et

al., 2012), and Mn linked to hypoxia (Limburg *et al.*, 2011). However, element incorporation can also be physiologically influenced; for example, by somatic growth, ontogeny or reproduction, which can dampen the environmental signals encoded by otoliths (Grammer *et al.*, 2017; Sturrock *et al.*, 2015). Nonetheless, otolith chemistry has long demonstrated its power to reconstruct environmental histories and movement patterns, particularly across freshwater, estuarine and marine environments. Ultimately, harnessing trace elements as natural markers to investigate habitat utilisation and individual fish movement can allow us to determine the movement patterns of populations, and whether this differs across a species distribution.

The black bream Acanthopagrus butcheri is a generalist species endemic to southern Australia, spanning from Western Australia to New South Wales (Norriss et al., 2002). Able to tolerate dramatic shifts in water temperature and salinity, A. butcheri inhabit rivers, estuaries, and coastal waters, although require estuaries to complete their life cycle (i.e., estuarine-dependent) (Doubleday et al., 2015; Partridge and Jenkins 2002). Spawning typically occurs during austral spring and summer in the upper reaches of streams feeding estuaries (Jenkins et al., 2018; Williams et al., 2012; Sakabe et al., 2011), where eggs hatch and larvae recruit within estuaries and become sexually mature between two and four years of age (Cheshire et al., 2013; Roberts et al., 2010; Norriss 2002). Previous research has shown limited connectivity of A. butcheri populations across southern Australian estuaries, with both otolith-based techniques and genetic approaches finding high population structuring across its distribution range (Chapter 1; Chapter 2; Burridge and Versace 2006; Chaplin et al., 1998). Although such studies have established that limited fish movement is occurring among estuaries, our understanding of movement within estuaries and adjacent habitats (i.e., rivers and coastal waters) is limited. Otolith chemistry has revealed life history variations in A. butcheri, where populations are comprised of coexisting migratory and resident life cycles (i.e., partial migration) (Tracey et al., 2020; Gillanders et al., 2015). Although populations exhibit partial migration, our understanding of the ratio of resident and migrant life histories within estuaries and across this species' distribution is limited. Furthermore, little is known of potential shifts in these ratios with increasing climatic effects. An ecological risk assessment of species' sensitivity to climate change using various attributes (i.e., abundance, distribution, and phenology) predicted A. butcheri to undergo a range contraction (Pecl et al., 2014), which could decrease population size and promote shifts in migratory behaviours.

The main aim of this study was to investigate *A. butcheri* movement patterns both within and among southern Australian estuaries of varying sizes and types (i.e., open and closed systems). Specifically, we analysed otolith Ba and Sr signals spanning each fish's life history (i.e., otolith profile) to (1) investigate variations in *A. butcheri* movement patterns within estuaries using cluster analysis

and (2) characterise the ratio of movement patterns among estuaries. We hypothesise that due to the species' ability to partially migrate, populations will consist of both stable chemical signals reflecting resident behaviour, and fluctuating signals reflecting migratory behaviour. We also hypothesise that due to the large distribution range of *A. butcheri* across estuaries of highly variable environmental conditions (e.g., freshwater flow, estuary type and size), coupled with evidence of limited fish movement among these estuaries, the ratio of movement patterns within estuaries may differ across the sampling distribution. Understanding *A. butcheri* movement can determine dependencies on adjacent environments (i.e., rivers and coastal waters) and how this might vary among populations across the distribution, and the appropriate management required.

5.3 Materials and Methods

Sampling and preparation

Acanthopagrus butcheri were sampled from eight estuaries across southern Australia variable in both size and type (i.e., open or intermittently closed), including from Western Australia, South Australia, Victoria, and Tasmania (Fig. 5.1). Methods of capture included targeted seine netting (The University of Adelaide animal ethics, approval S-2020-069), as well as rod and line via collaborative efforts with recreational anglers and commercial fishery catches (i.e., Lakes and Coorong commercial fishers). The sagittal otoliths were extracted and cleaned in ultrapure water (Milli-Q), with the left otolith of the pair embedded in epoxy resin (*Struers Epofix*) spiked with ~30 ppm indium to ensure only otolith material was analysed. Epoxy blocks were sectioned using a low-speed saw and twin blades, making a transverse section through the otolith core to obtain 250-300 μ m thin sections, which were then polished using a sequence of lapping film sandpaper sheets (30, 9 and 3 μ m grades). The otolith sections were mounted onto microscope slides using ~200 ppm indium-spiked crystal bond for elemental analysis and aging.





Chemical analysis

Otolith sections were analysed using Laser-Ablation Inductively-Coupled Plasma Mass-Spectrometry (LA-ICP-MS) (*Agilent* 7900x with attached *RESOlution* LR 193nm Excimer laser system) for Sr, Ba and Ca. Indium was also analysed but only as a marker for contamination from the epoxy and crystal bond. Laser ablations consisted of 30 µm transects running along the sulcal groove of each otolith at ~3.3 µm/sec with a pulse rate of 10 Hz from the otolith core to the otolith edge, reflecting each individual's entire life history. Two replicate National Institute of Standards and Technology (NIST612) standards and two replicate United States Geological Service synthetic calcium carbonate (MACS-3) standards were also run at the beginning and end of each session, along with two NIST612 ablations around every 50 minutes of analysis. NIST612 was used to correct for drift in instrumentation over time and MACS-3 was used as a long-term external precision measurement. The coefficient of variation ranged between 0.34 and 0.46% for NIST612, and between 2.90 and 4.69% for MACS-3 standards depending on the element.

Raw mass-spectrometer signals were transformed from counts per sec to parts per million (ppm) based on subtraction of background counts and analysis of standards in *lolite* (Paton *et al.,* 2011), followed by conversion to element:Ca ratios (µmol mol⁻¹) based on a calcium concentration of 38.8% in otoliths (Yoshinaga *et al.,* 2000). Two fish samples contained spikes in In throughout their otolith profile and were removed from subsequent analyses.

Ageing and measurements

High contrast images of otolith sections were taken for aging and transect measurements under a dissection microscope with reflected light. Annual growth of otolith increments has been validated for A. butcheri (Sarre and Potter 2000; Morison et al., 1998), therefore, fish were aged by counting opaque bands, with annual growth increments forming in October (i.e., 1 October to 30 September) (Elsdon and Gillanders 2006). Three independent readings by one reader were made for each otolith, with an additional reading completed until a mode age was determined for each fish. All juvenile A. *butcheri* (samples ≤2 years old) were removed to reduce discrimination of otolith profiles based on life history stage and not movement pattern (i.e., grouping juveniles). The distance between each confirmed annual growth increment along the ablated transect was then measured using the software *ImageJ* (Schneider et al., 2012), with each increment assigned a corresponding fish age and year of formation. Sections of each ablated transect before the otolith core and beyond the otolith edge were also measured. To investigate life history stages independently along each otolith profile (e.g., juvenile and/or adult years), increment measurements were used in conjunction with the known lengths of each otolith profile, which were calculated using ablation time and speed. This allowed chemical signals to be segmented by annual increment length (i.e., fish age), while allowing the start and end of each transect outside the annual increments to be removed.

Cluster analysis

The variability of Ba:Ca and Sr:Ca concentrations in each otolith profile were compared among individuals within estuaries to identify potential groups of fish. Features from Ba:Ca and Sr:Ca time series were extracted, including the mean and variance, spike, linearity, curvature, entropy, and trend per element (*R* package *tsfeatures*, Hyndman *et al.*, 2023). These integrated data were then scaled using z-score normalisation (mean = 0, SD = 1) to account for different magnitudes across otolith Ba:Ca and Sr:Ca. Environmental variability across the sampling distribution is likely to influence profile types in each estuary differently, therefore, subsequent analyses were run on each estuary independently. Using both Ba:Ca and Sr:Ca data, a K-means clustering analysis was performed on individuals within each estuary in order to predict the number of potential clusters (K) (*R* package *stats*, R core team 2023), ranging from 2-5. Clusters were then visualised as scatterplots of each predicted K for each estuary. Additionally, a silhouette score was calculated for each

predicted K, which determined the similarities of each fish within a cluster compared to other clusters (*R* package *factoextra*, Kassambara and Mundt 2020). A higher silhouette score indicates a more appropriate predicted K. Using multiple lines of evidence, including silhouette score, symmetry of scatterplot clusters, absence of cluster overlap, and relative equality in cluster sizes, a predicted K was then selected for each estuary. Although silhouette scores were prioritised due to being an empirical test, scores that suggested clusters that overlapped or were unequal in size were not selected, and instead the next best was chosen.

Following clustering, chemical profiles were smoothed using a rolling mean approach (window size = 20 values) to reduce signal noise and reveal fluctuations that may reflect shifts in habitats (*R* package *zoo*, Zeileis and Grothendieck 2005). The length of each annual growth increment was then fit within a distance range of 0 to 1 μ m across all otolith profiles to plot proportionate lengths and allow for comparisons of signals among each age class to be made.

5.4 Results

Cluster analysis

A total of 239 *A. butcheri* were analysed from eight estuaries, including large open systems (e.g., 100s km², Coorong), and smaller, intermittently closed systems (e.g., <1 km², Robe) (Table 5.1). Other intermittently closed systems consisted of large bodies of water adjacent to the coast (i.e., Lake Coila, Lake Brou, West Lakes, and Bremer Bay [Wellstead Estuary]), and long rivers with small, narrow estuaries (i.e., Glenelg River and Hopkins River). Average fish age did not vary greatly across the sampling distribution, with older fish sampled from VIC estuaries (12 ±4 years old) and TAS estuaries (16 ±6 years old), and younger fish from WA estuaries (9 ±5 years old) and SA estuaries (8 ±4 years old) (Table 5.1). Little variability was identified in Sr:Ca signals, with almost all fish showing a gradual increase over time coupled with oscillations within annual increments. Ba:Ca signals showed variability both within and among estuaries, including fluctuations and spikes in concentration both during early and adult growth years, as well as profiles with almost flat signals throughout entire life histories.

Table 5.1 *Acanthopagrus butcheri s*ampling estuaries with corresponding Australian state, collection year, age data, estuary type (i.e., open or intermittently closed), samples size, and proportion of profile types.

state estuary	collection		age age SD estuary				profile type (%)			
	year	age		estuary type	i type sample size	А	В	С	other	
Western Australia	Bremer Bay	2020	8.69	±4.99	IC	25	-	-	-	100
South Australia	West Lakes	2021	7.28	±4.40	IC	47	51.1	48.9	-	-
	Coorong	2020	6.23	±3.61	0	27	40.7	59.3	-	-
	Robe	2020	9.68	±3.71	IC	18	77.8	22.2	-	-
Victoria	Glenelg River	2021	11.9	±3.26	IC	35	20	48.6	31.4	-
	Hopkins River	2021	14.7	±3.95	IC	27	25.9	33.3	40.7	-
	Lake Tyers	2021	12.3	±3.58	IC	39	59	41	-	-
Tasmania	Port Huon	2021	15.9	±6.06	0	21	52.4	47.6	-	-
TOTAL						239	40.6	48	3.9	10.5

Overall, almost all estuaries were predicted to have between two and three clusters (Table 5.2). The larger silhouette scores were primarily for K=2 (Table 5.2), with overlapping of predicted clusters in scatterplots almost consistently present past K=3, deeming further cluster predictions (i.e., K>5) unnecessary. Using multiple lines of evidence, two clusters (i.e., K=2) were predicted for intermittently closed estuaries West Lakes (Fig. 5.S1), Robe (Fig. 5.S2) (both South Australia), and Lake Tyers (Fig. 5.S3) (Victoria); as well as for large open estuaries Coorong (Fig. 5.S4) (South Australia) and Port Huon (Fig. 5.S5) (Tasmania). *Acanthopagrus butcheri* collected from Glenelg River (Fig. 5.S6) and Hopkins River (Fig. 5.S7) (both Victoria) were best separated into three clusters (K=3), while *A. butcheri* from Bremer Bay (Fig. 5.S8), which were initially separated into two clusters (i.e., the minimum number that can be predicted using this approach) best suited a single cluster based on the similarities between chemical signals of the initial two clusters predicted and the analysis appearing to separate clusters based on profile length (i.e., limited time series features to discriminate among fish) (Fig. 5.S9).

Table 5.2 Summary of silhouette scores generated for each predicted cluster for each estuary where *A. butcheri* were sampled from. Table includes the predicted cluster chosen for each estuary (bolded), with the exception of Bremer Bay which was determined to be a single cluster.

octuary		predicted number of clusters						
estuary	2	3	4	5				
Bremer Bay	0.238	0.170	0.181	0.187				
West Lakes	0.149	0.151	0.154	0.170				
Coorong	0.176	0.181	0.183	0.189				
Robe	0.216	0.169	0.194	0.208				
Glenelg River	0.235	0.182	0.154	0.156				
Hopkins River	0.151	0.174	0.156	0.178				
Lake Tyers	0.183	0.174	0.134	0.147				
Port Huon	0.168	0.159	0.171	0.163				

Chemical profiles

Mapping of otolith Sr:Ca concentration by fish age for each individual showed a gradual increase as fish aged, although cyclical fluctuations within annual growth increments were still found (Fig. 5.S10, S11). Cases where an inverse change in concentration between Sr:Ca and Ba:Ca were found included *A. butcheri* from Robe, where profile type B showed a shift from high to low Sr:Ca concentrations during the first two growth years with an opposite trend for Ba:Ca concentrations (Fig. 5.2, Fig. 5.S10). Additionally, increasing Sr:Ca occurred more frequently during the start and end of each otolith growth year, while Ba:Ca signals appeared to spike more during the middle of each growth year. However, due to the little difference in Sr:Ca otolith profiles both within and among estuaries, (Fig. 5.S10, S11), only Ba:Ca otolith profiles were plotted and interpreted for movement patterns as this element showed consistent variability both within and among estuaries.

For estuaries where K=2, profile types showed either a relatively stable Ba:Ca signal throughout an individual's life with small oscillations in some estuaries (profile type A), or a Ba:Ca signal with large spikes and magnitude variations from the baseline, more irregular in range and over time, occurring primarily during the first few years, although continuing across life history in some estuaries (profile type B) (Fig. 5.2). Cases where K=3 included an additional pattern that consisted of fluctuating Ba:Ca signals very similar to profile type B but with more irregular high range oscillations (profile type C) (Fig. 5.3). *Acanthopagrus butcheri* sampled from Bremer Bay (WA) were mapped based on their initial separation into two clusters (Fig. 5.4), however, they showed virtually identical otolith Ba:Ca signals and were grouped as a single profile type, taking into consideration that cluster analysis can only predict a minimum of two clusters. Therefore, these profiles were not treated as

either profile type A, B, or C as there was no variability within the estuary to interpret a 'stable' or fluctuating Ba:Ca signal.

Overall, profile type A was more common in West Lakes, Robe, Lake Tyers, and Port Huon, profile type B was more common in the Coorong and Glenelg River, and profile type C in Hopkins River. Most individuals analysed in this study showed the more stable and low Ba profile type A (48.9%), followed by the more variable and oscillating Ba:Ca profile type B and C (40.6%), along with individuals from Bremer Bay with a single movement pattern (10.5%) (Table 5.1).



Fig. 5.2 Otolith profiles from adult *Acanthopagrus butcheri* collected from Port Huon (n=21), West Lakes (n=47), Coorong (n=27), Robe (n=18), and Lake Tyers (n=39). Ba:Ca ratio (μ mol/mol) plotted by fish age running from otolith core to otolith edge, with grey vertical bands representing each annual growth increment and the first band representing the otolith core. All increments have been made equal in length to interpret patterns among individual fish. Colour of otolith profiles represent otolith profile type A (blue, left) and profile type B (green, right).



Fig. 5.3 Otolith profiles from adult *Acanthopagrus butcheri* collected from Glenelg River (n=35) and Hopkins River (n=27). Ba:Ca ratio (µmol/mol) plotted by fish age running from otolith core to otolith edge, with grey vertical bands representing annual growth increments and the first band representing the otolith core. All increments have been made equal in length to interpret patterns among individual fish. Colour of otolith profiles represent otolith profile type A (blue, left) and profile type B (green, middle), and profile type C (yellow, right).



Fig. 5.4 Otolith profiles from adult *Acanthopagrus butcheri* collected from Bremer Bay (*n*=25). Ba:Ca ratio (µmol/mol) plotted by fish age running from otolith core to otolith edge, with grey vertical bands representing each annual growth increment and the first band representing the otolith core. All increments have been made equal in length to interpret patterns among individual fish. Colour of otolith profiles represent a single otolith profile although separated by the initial predicted cluster, including cluster 1 (light purple) and cluster 2 (dark purple), with both clusters overlayed to show the similarities that deemed the fish to be a part of a single profile type.

5.5 Discussion

Acanthopagrus butcheri otolith profiles varied both within and among estuaries, potentially indicating the variable life histories exhibited by this estuarine species (i.e., partial migration, Tracey et al., 2020; Gillanders et al., 2015). Two main otolith profile types were identified across the sampling distribution, including more stable Ba:Ca signals likely reflecting individuals that stay in the same areas, subject to limited variations in environmental conditions, and more oscillating Ba:Ca signals with large spikes during early life stages and/or adult life stages likely reflecting movements or more drastic changes in conditions. Our interpretation of both Ba:Ca and Sr:Ca otolith profiles as potential fish movement patterns builds on previous work in A. butcheri (Elsdon and Gillanders 2005), as well as in other estuarine species such as barramundi (Lates calcarifer, Walther et al., 2011; Nazir et al., 2023), and southern flounder (Paralichthys lethostigma, Nims and Walther 2014; Farmer et al., 2013), which also consisted of stable signals (i.e., profile type A), and oscillating signals (i.e., profile type B and C). The differences in profile type ratios among estuaries is likely the result of variable estuary sizes and types, as well as estuary-specific environmental conditions influencing each A. butcheri population differently, which is supported by the presence of additional profile types predicted in only a few estuaries (i.e., Glenelg River and Hopkins River), and only a single profile type predicted in A. butcheri otoliths collected from Bremer Bay.

Stable otolith Ba:Ca signals identified among estuaries (i.e., profile type A) mirror the resident behaviour shown previously in A. butcheri life histories (Elsdon and Gillanders 2005), as well as the otolith profiles of other estuarine and freshwater species such as Australian whitebait (Lovettia sealii, Schmidt et al., 2014), and inconnu (Stenodus leucichthys, Howland et al., 2001). The high genetic structuring across A. butcheri's distribution range supports the presence of resident individuals across the sampling distribution (Chapter 2; Farrington et al., 2000; Burridge and Versace 2006; Burridge et al., 2004), with limited movement outside or among estuaries (i.e., estuaryhopping, Gillanders *et al.*, 2015). The minimal movement inferred by profile type A even during early life history may be the result of eggs hatching closer to the estuary, where larvae eventually recruit, rather than further upstream (Williams et al., 2012; Roberts et al., 2010). These individuals appear to remain in estuarine conditions throughout their entire life history. Profile type A appeared almost flat in some estuaries (i.e., Port Huon, West Lakes, and Coorong), whereas the relatively stable Ba:Ca signals in Lake Tyers, Glenelg River, and Hopkins River showed small range oscillations, as well as cases of single fluctuations per fish (i.e., Robe). In such cases, otolith profiles would never be fully stable, with small observed variations likely the result of changes in estuarine conditions (e.g., chemistry, salinity, temperature, freshwater flow) (Avigliano et al., 2017; Elsdon and Gillanders 2005;

Howland *et al.*, 2001). Overall, there are clear differences in profile types from fish collected in the same estuaries.

The large Ba:Ca spikes in fish with profile type B occurred mostly over a single growth year, with inconsistent Ba:Ca concentrations within each estuary (i.e., presence of profile type A) suggesting these individuals are moving further upstream and into freshwater environments (Reis-Santos et al., 2023; Gillanders et al., 2015; Elsdon and Gillanders 2005). Similar patterns were found among varying estuary types and size, more so with spikes in Ba:Ca readings during early life (i.e., fish ages one and two), reflecting the movement of this species in early life between rivers and estuaries (Jenkins et al., 2018; Roberts et al., 2010). Early life fluctuations in otolith Ba:Ca have been found in other estuarine species, such as catfish (Genidens barbus, Avigliano et al., 2017) and southern flounder (Paralichthys lethostigma, Nims and Walther 2014). Past research on A. butcheri from this region did not find such large spikes in otolith Ba:Ca (Elsdon and Gillanders 2005), suggesting a change in water salinity might be coupled with additional environmental influences (e.g., water temperature), which has been shown in this species (Elsdon and Gillanders 2002), and other fish (Collingsworth et al., 2010; DiMaria et al., 2010; Stanley et al., 2015). The potential dual regulation of biological and environmental factors on otolith Ba:Ca signals (e.g., diet and temperature) might also explain the variability shown here and among sampling locations, considering the vast spatial scale and environmental heterogeneity present across this sampling distribution (Walther et al., 2010). Age and size at sexual maturity does vary across this species distribution range (Norriss et al., 2002; Sarre and Potter 1999), which could explain the differences in chemical signals among estuaries (i.e., time spent in freshwater and estuarine environments during early life stages). Therefore, spikes in Ba:Ca in the first year for A. butcheri from West Lakes, Lake Tyers, and Hopkins River, may represent similar movement patterns as the Ba:Ca spikes that occur in the second year for A. butcheri collected from Coorong and Robe. These years of Ba:Ca spikes would suggest a single movement upstream and into freshwater environments for these A. butcheri. Similar single movements followed by establishment into estuaries for the remainder of an individual's life have been previously shown in Ba:Ca profiles in other species (Avigliano et al., 2017; Nims and Walther 2014), as well as match A. butcheri profiles from Port Huon, Robe, and Lakes Tyers during adult years where profile type B signals appear to match the stable signals of profile type A. Alternatively, A. butcheri that continue to move outside of their estuary during these years (i.e., additional Ba:Ca spikes) were collected from West Lakes and Coorong. A combination of larger freshwater discharge and accessible areas in these larger systems could be promoting fish movement, as has been shown in other species such as Murray Cod (Maccullochella peelii, Tonkin et al., 2022; Koehn 2009), galaxias (Galaxiidae, Amtstyaetter et al., 2021) and carp gudgeons (Hypseleotris spp., Lyon et al., 2010). Sampling was

limited to estuarine environments and *A. butcheri's* main occurrence areas, so we cannot discard the occurrence of other profiles or that migratory life histories are underrepresented in comparison to fish collected from adjacent coastal and freshwater environments. Future opportunities to collect *A. butcheri* from these environments where they are less abundant would be of great interest.

Cases where an additional profile type was found were in the Glenelg River and Hopkins River (i.e., profile type C) and consisted of more irregular patterns than profile type B and individuals spending their adult years in areas of higher freshwater conditions, relative to individuals with profile type A. However, patterns shown by profile type B and type C were similar, with type C also including individuals that likely move out of estuaries (although irregularly) based on increased concentration of otolith Ba:Ca relative to stable signals shown by individuals from the same estuaries with profile type A. Furthermore, the gradual increase in Ba:Ca overtime seen in A. butcheri from Glenelg River, and in some cases from Hopkins River, may be the result of a stronger ontogenetic influence in otolith Ba:Ca concentrations relative to the remaining sampling distribution, an effect that has influenced otolith Ba:Ca in other species (Walther et al., 2010; Comyns et al., 2008; Ruttenberg et al., 2005). Estuaries where the majority of fish showed migratory behaviour (i.e., profile type B and C) were either open systems (i.e., Coorong), or intermittently closed systems with small, narrow estuaries at their deltas (i.e., Glenelg River and Hopkins River). Estuaries with a permanent connection to rivers and coastal waters may provide more opportunity for individuals to move out of estuarine environments and across the salinity gradient and possibly among other estuaries. Genetic research suggested that A. butcheri from Coorong and Robe were a single population (i.e., gene flow between locations which is likely associated with movement) (Chapter 1), as well as cases of fish movement between Glenelg River and Hopkins River, which supports the presence of fish with more migratory behaviour in these systems (i.e., profile type B and C). Furthermore, the limited estuarine waters present near the mouth of both Hopkins River and Glenelg River, relative to the large estuaries of other intermittently closed systems (e.g., Lake Coila and Lake Brou) may promote fish movement upstream into freshwater when these systems are closed, resulting in fluctuating Ba:Ca otolith signals (i.e., profile type B and C).

Only a single otolith profile type was identified in *A. butcheri* from Bremer Bay, with fluctuations in Ba:Ca signals overtime and no cases of stable signals. The absence of a 'stable' signal, or a larger oscillating signal with Ba:Ca spikes to provide a baseline reference, suggests that signals from Bremer Bay could reflect either profile type A, B or C. For example, all *A. butcheri* might be residents, with highly variable freshwater flow in the Western Australian estuary producing fluctuating otolith Ba:Ca signals in sedentary fish similar to profile type A. Alternatively, all *A. butcheri* from Bremer Bay could move in and out of estuarine environments, similar to individuals with profile type B and C in Glenelg

River and Hopkins River, or reflect a more collective, population-wide movement pattern (Johnson *et al.,* 2016; Berdahl *et al.,* 2014). The high genetic structuring found across Western Australian *A. butcheri* populations (i.e., limited movement among estuaries) supports the likelihood that these individuals display more resident behaviour (Chaplin *et al.,* 1998), and the similarity in profile types within Bremer Bay further suggests that although the species can partially migrate, not all estuaries may exhibit such variability. Further investigation and information on environmental conditions and habitat use patterns (e.g., with artificial tagging or telemetry) will be key to help clarify the observed patterns in Bremer Bay.

Otolith Sr:Ca signals gradually increased in almost all fish samples, suggesting ontogenetic influence, a pattern seen before in sparids (e.g., *Chrysophrys auratus,* Martino *et al.*, 2021). Such physiological effects commonly influence otolith Sr:Ca concentrations in marine species (Brown and Severin 2009). Additionally, the annual cyclical pattern shown in Sr:Ca signals may suggest a combination of ontogenetics and seasonal freshwater flow into the estuaries causing dips in otolith Sr:Ca, as has been shown in *A. butcheri* otolith chemistry (Elsdon and Gillanders 2005). Cases of an inverse relationship between otolith Sr:Ca and Ba:Ca signals support the known inverse relationship these two elements have with water salinity (Izzo *et al.*, 2017; Tabouret *et al.*, 2010). Our findings here show that although there is environmental influence on otolith Sr:Ca, the similarities in Sr:Ca profiles within and among estuaries suggest a weaker representation of movement patterns relative to the Ba:Ca variability for *A. butcheri* within estuaries. As we continue to grow our understanding of the relationship between otolith elemental chemistry and the environment, there is potential that additional elements (e.g., Mn:Ca or Li:Ca) could also help reconstruct habitat use, namely regarding water salinity and hypoxia (Reis-Santos *et al.*, 2018; Williams *et al.*, 2018; Limburg *et al.*, 2011).

Our study highlights the variable otolith profiles displayed by *A. butcheri* across their distribution range, and subsequently a variety of movement patterns that characterise each population. The presence of resident and migratory behaviours both within and among estuaries, including fish with single movement events and those that consistently move throughout their life, were likely influenced by estuary-specific variables (e.g., type and size). Some populations depend more on their surrounding environments (i.e., rivers and coastal waters) than others as shown by the larger amount of migratory behaviour in open systems and small estuaries, while the majority of fish in intermittently closed systems with large estuaries tended to display resident behaviour. Ba:Ca was shown to represent unique otolith profiles and movement patterns of *A. butcheri*, more so than Sr:Ca signals. Different ratios of movement patterns among estuaries implies managing partial migrant species is complex. Management strategies should consider location and estuary type, and this work stresses the importance of location-specific management for intermittently closed systems with

increased resident behaviour (i.e., West Lakes, Robe, and Lake Tyers), and management that extends beyond estuarine environments and across the salinity gradient for fish with increased migratory behaviour in open systems (i.e., Coorong) and intermittently closed systems with small, narrow estuaries (i.e., Glenelg River and Hopkins River). The variability in otolith chemistry shown by *A. butcheri* highlights the power of this natural marker, and the potential for understanding fish movement and discriminating between population dynamics for this species elsewhere, as well as similar estuarine species.

References

- Amtstaetter, F, Yen, JDL, Hale, R, Koster, W, O'Connor, J, Stuart, I, Tonkin, Z (2021) Elevated river discharge enhances the immigration of juvenile catadromous and amphidromous fishes into temperate coastal rivers. *Journal of Fish Biology* **99**, 61-72. doi:10.1111/jfb.14699
- Avigliano, E, Leisen, M, Romero, R, Carvalho, B, Velasco, G, Vianna, M, Barra, F, Volpedo, AV (2017) Fluviomarine travelers from South America: Cyclic amphidromy and freshwater residency, typical behaviors in *Genidens barbus* inferred by otolith chemistry. *Fisheries Research* 193, 184-194. doi:10.1016/j.fishres.2017.04.011
- Berdahl, A, Westley, PAH, Levin, SA, Couzin, ID, Quinn, TP (2014) A collective navigation hypothesis for homeward migration in anadromous salmonids. *Fish and Fisheries* **17**, 525-542. doi:10.1111/faf.12084
- Block, BA, Dewar, H, Blackwell, SB, Williams, TD, Prince, ED, Farwell, CJ, Boustany, A, Teo, SLH, Seitz, A, Walli, A, Fudge, D (2001) Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. Science 293, 1310-1314. doi:DOI 10.1126/science.1061197
- Brennan, SR, Cline, TJ, Schindler, DE (2019) Quantifying habitat use of migratory fish across riverscapes using space-time isotope models. *Methods in Ecology and Evolution* **10**, 1036-1047. doi:10.1111/2041-210x.13191
- Brown, RJ, Severin, KP (2009) Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Canadian Journal of Fisheries and Aquatic Sciences* **66**, 1790+.
- Burridge, CP, Hurt, AC, Farrington, LW, Coutin, PC, Austin, CM (2004) Stepping stone gene flow in an estuarine-dwelling sparid from south-east Australia. *Journal of Fish Biology* **64**, 805-819. doi:10.1111/j.1095-8649.2004.0347.x
- Burridge, CP, Versace, VL (2006) Population genetic structuring in *Acanthopagrus butcheri* (*Pisces: Sparidae*): does low gene flow among estuaries apply to both sexes? *Marine Biotechnology* **9**, 33-44. doi:10.1007/s10126-006-6023-7
- Calò, A, Félix-Hackradt, F, Garcia, J, Hackradt, CW, Rocklin, D, Treviño Otón, J, García-Charton, J, García Charton, A (2013) A review of methods to assess connectivity and dispersal between fish populations in the Mediterranean Sea. In 'Advances in Oceonography and Limnology.' Vol. 4 pp. 150-175.
- Campana, SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* **188**, 263-297. doi:DOI 10.3354/meps188263
- Campana, SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59**, 197-242. doi:10.1006/jfbi.2001.1668
- Chaplin JA, BG, Gill HS, McCullock R, Potter IC (1998) Are assemblages of black bream (*Acanthopagrus butcheri*) in different estuaries genetically distinct? *International Journal of Salt Lake Research* **6**, 303-321.
- Cheshire, KJM, Ye, Q, Fredberg, J, Earl, J, 2013. Aspects of reproductive biology of five key fish species in the Murray Mouth and Coorong. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Collingsworth, PD, Gillanders, B, Van Tassell, JJ, Olesik, JW, Marschall, EA (2010) Effects of temperature and elemental concentration on the chemical composition of juvenile yellow perch (*Perca flavescens*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **67**, 1187-1196. doi:10.1139/f10-050
- Comyns, BH, Rakocinski, CF, Peterson, MS, Shiller, AM (2008) Otolith chemistry of juvenile spotted seatrout *Cynoscion nebulosus* reflects local natal regions of coastal Mississippi, USA. *Marine Ecology Progress Series* **371**, 243-252. doi:10.3354/meps07604
- DiMaria, RA, Miller, JA, Hurst, TP (2010) Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. *Environmental Biology of Fishes* **89**, 453-462. doi:10.1007/s10641-010-9665-2

- Dong, ZG, Chen, YH, Ge, HX, Li, XY, Wu, HL, Wang, CH, Hu, Z, Wu, YJ, Fu, GH, Lu, JK, Che, H (2018) Response of growth and development of the Pacific oyster (Crassostrea gigas) to thermal discharge from a nuclear power plant. BMC Ecology 18, 31. doi:10.1186/s12898-018-0191-y
- Doubleday, ZA, Harris, HH, Izzo, C, Gillanders, BM (2014) Strontium randomly substituting for calcium in fish otolith aragonite. Analytical Chemistry 86, 865-869. doi:10.1021/ac4034278
- Doubleday, ZA, Izzo, C, Haddy, JA, Lyle, JM, Ye, Q, Gillanders, BM (2015) Long-term patterns in estuarine fish growth across two climatically divergent regions. Oecologia 179, 1079-90. doi:10.1007/s00442-015-3411-6
- Elsdon, TS, Gillanders, BM (2002) Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. Canadian Journal of Fisheries and Aquatic Sciences 59, 1796-1808. doi:10.1139/F02-154
- Elsdon, TS, Gillanders, BM (2005) Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. Canadian Journal of Fisheries and Aquatic Sciences 62, 1143-1152. doi:10.1139/f05-029
- Elsdon, TS, Gillanders, BM (2006) Identifying migratory contingents of fish by combining otolith Sr:Ca with temporal collections of ambient Sr:Ca concentrations. Journal of Fish Biology 69, 643-657. doi:10.1111/j.1095-8649.2006.01136.x
- Elsdon, TS, Wells, BK, Campana, SE, Gillanders, BM, Jones, CM, Limburg, KE, Secor, DH, Thorrold, SR, Walther, BD (2008) Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology: An Annual Review 46, 297-330. doi:DOI 10.1201/9781420065756.ch7
- Farmer, TM, DeVries, DR, Wright, RA, Gagnon, JE (2013) Using seasonal variation in otolith microchemical composition to indicate largemouth bass and southern flounder residency patterns across an estuarine salinity gradient. Transactions of the American Fisheries Society 142, 1415-1429. doi:10.1080/00028487.2013.806348
- Farrington, LW, Austin, CM, Coutin, PC (2000) Allozyme variation and stock structure in the black bream, Acanthopagrus butcheri (Munro) (Sparidae) in southern Australia: implications for fisheries management, aquaculture and taxonomic relationship with (Gunther). Fisheries Management and *Ecology* **7**, 265-279. doi:DOI 10.1046/j.1365-2400.2000.00178.x
- Frazier, BS, Vinyard, EA, Fields, AT, Driggers, WB, Grubbs, RD, Adams, DH, Drymon, JM, Gardiner, JM, Hendon, JM, Hoffmayer, E, Hueter, RE, Wells, RJD, Wiley, TR, Portnoy, DS (2023) Age, growth and maturity of the bonnethead Sphyrna tiburo in the U.S. Gulf of Mexico. Environmental Biology of Fishes 106, 1597-1617. doi:10.1007/s10641-023-01439-5
- Gannon, R, Payne, NL, Suthers, IM, Gray, CA, van der Meulen, DE, Taylor, MD (2015) Fine-scale movements, site fidelity and habitat use of an estuarine dependent sparid. Environmental Biology of Fishes 98, 1599-1608. doi:10.1007/s10641-015-0385-5
- Gillanders, BM, Izzo, C, Doubleday, ZA, Ye, Q (2015) Partial migration: growth varies between resident and migratory fish. Biology Letters 11, doi:10.1098/rsbl.2014.0850
- Goetz, FW, Jasonowicz, AJ, Roberts, SB (2018) What goes up must come down: Diel vertical migration in the deep-water sablefish (Anoplopoma fimbria) revealed by pop-up satellite archival tags. Fisheries Oceanography 27, 127-142. doi:10.1111/fog.12239
- Grammer, GL, Morrongiello, JR, Izzo, C, Hawthorne, PJ, Middleton, JF, Gillanders, BM (2017) Coupling biogeochemical tracers with fish growth reveals physiological and environmental controls on otolith chemistry. Ecological Monographs 87, 487-507. doi:10.1002/ecm.1264
- Howland, KL, Tonn, WM, Babaluk, JA, Tallman, RF (2001) Identification of freshwater and anadromous inconnu in the Mackenzie River system by analysis of otolith strontium. Transactions of the American Fisheries Society 130, 725-741. doi:10.1577/1548-8659(2001)130<0725:lofaai>2.0.Co;2
- Hyndman, R, Kang, Y, Montero-Manso, P, O''Hara-Wild, M, Talagala, T, Wang, E, Yang, Y (2023) 'tsfeatures: time series feature extraction.' Available at

https://pkg.robjhyndman.com/tsfeatures/,https;//github.com/robjhyndman/tsfeatures.

- Izzo, C, Doubleday, ZA, Grammer, GL, Disspain, MCF, Ye, QF, Gillanders, BM (2017) Seasonally resolved environmental reconstructions using fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 74, 23-31. doi:10.1139/cjfas-2016-0055
- Jenkins, GP, Kent, JA, Woodland, RJ, Warry, F, Swearer, SE, Cook, PLM (2018) Delayed timing of successful spawning of an estuarine dependent fish, black bream *Acanthopagrus butcheri*. *Journal of Fish Biology* **93**, 931-941. doi:10.1111/jfb.13806
- Jepsen, N, Thorstad, EB, Havn, T, Lucas, MC (2015) The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. *Animal Biotelemetry* **3**, doi:10.1186/s40317-015-0086-z
- Johnson, RC, Garza, JC, MacFarlane, RB, Grimes, CB, Phillis, CC, Koch, PL, Weber, PK, Carr, MH (2016) Isotopes and genes reveal freshwater origins of Chinook salmon *Oncorhynchus tshawytscha* aggregations in California's coastal ocean. *Marine Ecology Progress Series* **548**, 181-196. doi:10.3354/meps11623
- Kassambara, A, Mundt, F (2020) 'Factoextra: extract and visualize and results of multivariate data analysis.' Available at https://CRAN.R-project.org/package=factoextra
- Limburg, KE, Olson, C, Walther, Y, Dale, D, Slomp, CP, Hoie, H (2011) Tracking Baltic hypoxia and cod migration over millennia with natural tags. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 177-182. doi:10.1073/pnas.1100684108
- Lyon, J, Stuart, I, Ramsey, D, O'Mahony, J (2010) The effect of water level on lateral movements of fish between river and off-channel habitats and implications for management. *Marine and Freshwater Research* **61**, 271-278. doi:10.1071/Mf08246
- Martino, JC, Doubleday, ZA, Fowler, AJ, Gillanders, BM (2021) Identifying physiological and environmental influences on otolith chemistry in a coastal fishery species. *Marine and Freshwater Research* **72**, doi:10.1071/mf20196_co
- McKinley, AC, Taylor, MD, Johnston, EL (2012) Relationships between body burdens of trace metals (As, Cu, Fe, Hg, Mn, Se, and Zn) and the relative body size of small tooth flounder (*Pseudorhombus jenynsii*). *Science of The Total Environment* **423**, 84-94. doi:10.1016/j.scitotenv.2012.02.007
- McMillan, MN, Huveneers, C, Semmens, JM, Gillanders, BM (2018) Natural tags reveal populations of conservation dependent school shark use different pupping areas. *Marine Ecology Progress Series* 599, 147-156. doi:10.3354/meps12626
- Morison, AK, Coutin, PC, Robertson, SG (1998) Age determination of black bream, *Acanthopagrus butcheri* (Sparidae), from the Gippsland Lakes of south-eastern Australia indicates slow growth and episodic recruitment. *Marine and Freshwater Research* **49**, doi:10.1071/mf97237
- Morrongiello, JR, Thresher, RE (2015) A statistical framework to explore ontogenetic growth variation among individuals and populations: a marine fish example. *Ecological Monographs* **85**, 93-115. doi:10.1890/13-2355.1
- Nazir, A, Chen, T-Y, Wang, P-L, Shiao, J-C (2023) Reconstructing habitat use, identifying origin and discrimination of the barramundi (wild and farmed) populations using otolith stable isotope analysis. *Estuarine, Coastal and Shelf Science* **285**, doi:10.1016/j.ecss.2023.108317
- Nielsen, JK, Helama, S, Schöne, B (2008) Shell growth history of geoduck clam (*Panopea generosa*) in parry passage, british columbia, canada: temporal variation in annuli and the pacific decadal oscillation. *Journal of Oceanography* **64**, 951-960. doi:DOI 10.1007/s10872-008-0078-1
- Nims, MK, Walther, BD (2014) Contingents of Southern Flounder from Subtropical Estuaries Revealed by Otolith Chemistry. *Transactions of the American Fisheries Society* **143**, 721-731. doi:10.1080/00028487.2014.892535
- Norriss, JV, Tregonning, JE, Lenanton, RCJ, Sarre, GA, 2002. Biological synopsis of the black bream, *Acanthopagrus butcheri* (Munro) (Teleostei: Sparidae) in Western Australia with reference to information from other southern states. Department of Fisheries, Perth, Western Australia.
- Partridge, GJ, Jenkins, GI (2002) The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). *Aquaculture* **210**, 219-230. doi:10.1016/S0044-8486(01)00817-1
- Paton, C, Hellstrom, J, Paul, B, Woodhead, J, Hergt, J (2011) Iolite: Freeware for the visualisation and processing of mass spectrometric data. *Journal of Analytical Atomic Spectrometry* **26**, 2508-2518. doi:10.1039/c1ja10172b
- Pecl, GT, Ward, TM, Doubleday, ZA, Clarke, S, Day, J, Dixon, C, Frusher, S, Gibbs, P, Hobday, AJ, Hutchinson, N, Jennings, S, Jones, K, Li, XX, Spooner, D, Stoklosa, R (2014) Rapid assessment of fisheries species sensitivity to climate change. *Climatic Change* 127, 505-520. doi:10.1007/s10584-014-1284-z
- R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
- Reis-Santos, P, Gillanders, BM, Sturrock, AM, Izzo, C, Oxman, DS, Lueders-Dumont, JA, Hüssy, K, Tanner, SE, Rogers, T, Doubleday, ZA, Andrews, AH, Trueman, C, Brophy, D, Thiem, JD, Baumgartner, LJ, Willmes, M, Chung, M-T, Charapata, P, Johnson, RC, Trumble, S, Heimbrand, Y, Limburg, KE, Walther, BD (2022) Reading the biomineralized book of life: expanding otolith biogeochemical research and applications for fisheries and ecosystem-based management. *Reviews in Fish Biology and Fisheries* 33, 411-449. doi:10.1007/s11160-022-09720-z
- Reis-Santos, P, Tanner, SE, Elsdon, TS, Cabral, HN, Gillanders, BM (2013) Effects of temperature, salinity and water composition on otolith elemental incorporation of Dicentrarchus labrax. *Journal of Experimental Marine Biology and Ecology* **446**, 245-252. doi:10.1016/j.jembe.2013.05.027
- Reis-Santos, P, Vasconcelos, RP, Tanner, SE, Fonseca, VF, Cabral, HN, Gillanders, BM (2018) Extrinsic and intrinsic factors shape the ability of using otolith chemistry to characterize estuarine environmental histories. *Marine Environmental Research* **140**, 332-341. doi:10.1016/j.marenvres.2018.06.002
- Roberts, DG, Gray, CA, West, RJ, Ayre, DJ (2010) Marine genetic swamping: hybrids replace an obligately estuarine fish. *Molecular Ecology* **19**, 508-20. doi:10.1111/j.1365-294X.2009.04501.x
- Rogers, TA, Fowler, AJ, Steer, MA, Gillanders, BM (2019) Discriminating natal source populations of a temperate marine fish using larval otolith chemistry. *Frontiers in Marine Science* **6**, doi:10.3389/fmars.2019.00711
- Rowell, K, Dettman, DL, Dietz, R (2010) Nitrogen isotopes in otoliths reconstruct ancient trophic position. *Environmental Biology of Fishes* **89**, 415-425. doi:10.1007/s10641-010-9687-9
- Ruttenberg, BI, Hamilton, SL, Hickford, MJH, Paradis, GL, Sheehy, MS, Standish, JD, Ben-Tzvi, O, Warner, RR (2005) Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. *Marine Ecology Progress Series* **297**, 273-281.
- Sakabe, R, Lyle, JM, Crawford, CM (2011) The influence of freshwater inflows on spawning success and early growth of an estuarine resident fish species, *Acanthopagrus butcheri*. *Journal of Fish Biology* **78**, 1529-44. doi:10.1111/j.1095-8649.2011.02959.x
- Sarakinis, KG, Taylor, MD, Johnson, DD, Gillanders, BM (2022) Determining population structure and connectivity through otolith chemistry of stout whiting, *Sillago robusta*. *Fisheries Management and Ecology* **29**, 760-773. doi:10.1111/fme.12577
- Sarre, GA, Potter, IC (1999) Comparisons between the reproductive biology of black bream *Acanthopagrus butcheri* (Teleostei: Sparidae) in four estuaries with widely differing characteristics. *International Journal of Salt Lake Research* **8**, 179-210.
- Sarre, GA, Potter, IC (2000) Variation in age compositions and growth rates of *Acanthopagrus butcheri* (Sparidae) among estuaries:: some possible contributing factors. *Fishery Bulletin* **98**, 785-799.
- Schmidt, DJ, Crook, DA, Macdonald, JI, Huey, JA, Zampatti, BP, Chilcott, S, Raadik, TA, Hughes, JM (2014) Migration history and stock structure of two putatively diadromous teleost fishes, as determined by genetic and otolith chemistry analyses. *Freshwater Science* **33**, 193-206. doi:10.1086/674796
- Schneider, CA, Rasband, WS, Eliceiri, KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671-5. doi:10.1038/nmeth.2089
- Sinnatamby, RN, Loewen, TN, Luo, Y, Pearson, DG, Bicalho, B, Grant-Weaver, I, Cuss, CW, Poesch, M, Shotyk, W (2019) Spatial assessment of major and trace element concentrations from Lower Athabasca Region trout-perch (*Percopsis omiscomaycus*) otoliths. *Science of The Total Environment* 655, 363-373. doi:10.1016/j.scitotenv.2018.11.168

- Stanley, RRE, Bradbury, IR, DiBacco, C, Snelgrove, PVR, Thorrold, SR, Killen, SS (2015) Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). *ICES Journal of Marine Science* **72**, 2350-2363. doi:10.1093/icesjms/fsv070
- Sturrock, AM, Hunter, E, Milton, JA, Johnson, RC, Waring, CP, Trueman, CN, Leder, E (2015) Quantifying physiological influences on otolith microchemistry. *Methods in Ecology and Evolution* **6**, 806-816. doi:10.1111/2041-210x.12381
- Sturrock, AM, Trueman, CN, Milton, JA, Waring, CP, Cooper, MJ, Hunter, E (2014) Physiological influences can outweigh environmental signals in otolith microchemistry research. *Marine Ecology Progress Series* **500**, 245-264. doi:10.3354/meps10699
- Tabouret, H, Bareille, G, Claverie, F, Pecheyran, C, Prouzet, P, Donard, OF (2010) Simultaneous use of strontium:calcium and barium:calcium ratios in otoliths as markers of habitat: application to the European eel (*Anguilla anguilla*) in the Adour basin, South West France. *Marine Environmental Research* **70**, 35-45. doi:10.1016/j.marenvres.2010.02.006
- Thiem, JD, Baumgartner, LJ, Fanson, B, Sadekov, A, Tonkin, Z, Zampatti, BP (2021) Contrasting natal origin and movement history informs recovery pathways for three lowland river species following a mass fish kill. *Marine and Freshwater Research* **73**, 237-246. doi:10.1071/mf20349
- Tonkin, Z, Moloney, P, Lyon, J, Kitchingman, A, O'Mahony, J, Raymond, S, Hackett, G, Saddlier, S, Greenfield, A, Wood, D, Hale, R (2022) Movement behavior of a threatened native fish informs flow management in a modified floodplain river system. *Ecosphere* **13**, doi:10.1002/ecs2.3916
- Tracey, SR, Hartmann, K, McAllister, J, Lyle, JM (2020) Home range, site fidelity and synchronous migrations of three co-occurring, morphologically distinct estuarine fish species. *Science of The Total Environment* **713**, 136629. doi:10.1016/j.scitotenv.2020.136629
- Walther, BD, Dempster, T, Letnic, M, McCulloch, MT (2011) Movements of diadromous fish in large unregulated tropical rivers inferred from geochemical tracers. *PLoS ONE* **6**, e18351. doi:10.1371/journal.pone.0018351
- Walther, BD, Kingsford, MJ, O'Callaghan, MD, McCulloch, MT (2010) Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. *Environmental Biology of Fishes* **89**, 441-451. doi:10.1007/s10641-010-9661-6
- Williams, J, Hindell, JS, Jenkins, GP, Tracey, S, Hartmann, K, Swearer, SE (2017) The influence of freshwater flows on two estuarine resident fish species show differential sensitivity to the impacts of drought, flood and climate change. *Environmental Biology of Fishes* **100**, 1121-1137. doi:10.1007/s10641-017-0632-z
- Williams, J, Hindell, JS, Swearer, SE, Jenkins, GP (2012) Influence of freshwater flows on the distribution of eggs and larvae of black bream *Acanthopagrus butcheri* within a drought-affected estuary. *Journal of Fish Biology* **80**, 2281-301. doi:10.1111/j.1095-8649.2012.03283.x
- Williams, J, Jenkins, GP, Hindell, JS, Swearer, SE (2018) Fine-scale variability in elemental composition of estuarine water and otoliths: Developing environmental markers for determining larval fish dispersal histories within estuaries. *Limnology and Oceanography* **63**, 262-277. doi:10.1002/lno.10627
- Yoshinaga, J, Nakama, A, Morita, M, Edmonds, JS (2000) Fish otolith reference material for quality assurance of chemical analyses. *Marine Chemistry* **69**, 91-97. doi:10.1016/S0304-4203(99)00098-5
- Zeileis, A, Grothendieck, G (2005) Infrastructure for regular and irregular time series. *Journal of Statistical* Software

Supplementary Material



Fig. 5.S1 Scatterplots of K-means clusters for *A. butcheri* collected from West Lakes. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S2 Scatterplots of K-means clusters for *A. butcheri* collected from Robe. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S3 Scatterplots of K-means clusters for *A. butcheri* collected from Lake Tyers. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S4 Scatterplots of K-means clusters for *A. butcheri* collected from Coorong. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S5 Scatterplots of K-means clusters for *A. butcheri* collected from Port Huon. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S6 Scatterplots of K-means clusters for *A. butcheri* collected from Glenelg River. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S7 Scatterplots of K-means clusters for *A. butcheri* collected from Hopkins River. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S8 Scatterplots of K-means clusters for *A. butcheri* collected from Bremer Bay. Each plot contains the same individuals, although are grouped independently by a unique number of predicted clusters (i.e., colours specific to each plot). Individuals are mapped in two dimensions, with both x axis (Dimension 1) and y axis (Dimension 2) showing the percentage of explained variance.



Fig. 5.S9 Otolith profiles for adult *Acanthopagrus butcheri* collected from Bremer Bay (n=25). Sr:Ca ratio (µmol/mol) data displayed as time series and plotted by fish age running from otolith core to otolith edge, with grey vertical bands representing each annual growth increment and the first band representing the otolith core. All increments have been made equal in length to interpret patterns among individual fish. Colour of otolith profiles represent a single otolith profile although separated by the initial predicted cluster, including cluster 1 (light purple) and cluster 2 (dark purple), with both clusters overlaid to show the similarities that deemed the fish to be a part of a single profile type.



Fig. 5.S10 Otolith profiles from adult *Acanthopagrus butcheri* collected from Port Huon (n=21), West Lakes (n=47), Coorong (n=27), Robe (n=18), and Lake Tyers (n=39). Sr:Ca ratio (µmol/mol) data displayed as time series and plotted by fish age running from otolith core to otolith edge, with grey vertical bands representing each annual growth increment and the first band representing the otolith core. All increments have been made equal in length to interpret patterns among individual fish. Colour of otolith profiles represent otolith profile type A (blue, left) and profile type B (green, right), and ordered vertically by estuary.



Fig. 5.S11 Otolith profiles from adult *Acanthopagrus butcheri* collected from Glenelg River (*n*=35) and Hopkins River (*n*=27). Sr:Ca ratio (µmol/mol) data displayed as time series and plotted by fish age running from otolith core to otolith edge, with grey vertical bands representing each annual growth increment and the first band representing the otolith core. All increments have been made equal in length to interpret patterns among individual fish. Colour of otolith profiles represent otolith profile type A (blue, left) and profile type B (green, middle), and profile type C (yellow, right), and ordered vertically by estuary.

CHAPTER 6

General discussion

In the face of overexploitation and climate change, understanding population dynamics and connectivity of commercially and recreationally important fish species and how they respond to fishing pressure and environmental change is imperative for future sustainable management of their populations. Using black bream *Acanthopagrus butcheri* as a case study, I accessed biological and environmental information encoded in archival tissues and hard parts (i.e., natural tags) to investigate population structure, connectivity, fish growth, and movement, all of which are important factors in understanding the population dynamics of this estuarine fish and the appropriate strategies for their future management. Specifically:

- (i) I identified strong philopatry among *A. butcheri* populations, with continental-scale genetic structuring across southern Australian estuaries and local-scale structuring across southeastern Australian estuaries (Chapter 2, Fig. 6.1). The limited gene flow, including across spatially adjacent locations, reflects a combination of various geographical barriers and the nature of *A. butcheri* both as an estuarine-dependent species and partial migrant (i.e., cases of lone migrations detected).
- (ii) The overall maximum classification of *A. butcheri* to their estuary of capture was 95% and consisted of a combination of otolith elemental composition, otolith isotopic composition, otolith shape, and genetics. In this chapter (Chapter 3), I found that increasing the number of natural tagging techniques (i.e., markers) integrated did not consistently increase their effectiveness in identifying population structure (Fig. 6.1), which highlights the importance of appropriate marker selection for elucidating population structure. Ultimately, the high discrimination among estuaries further reflected the fine-scale structuring and limited connectivity among sampled populations.
- (iii) I identified strong climatic effects on growth of *A. butcheri*, with a regional-scale, annuallagged climate effect (i.e., El Niño Southern Oscillation, ENSO) best explaining the variability in annual growth (Chapter 4). Furthermore, I detected limited evidence of spatial synchrony in growth, which occurred among estuaries that were considerably separated by latitude and longitude (Fig. 6.1). Findings highlight the impact of ENSO on population dynamics and the negative growth response and subsequent vulnerability to intensifying climatic events.

(iv) Variable movement patterns were detected both within and across southern Australian estuaries, comprising primarily of stable otolith signals reflecting resident fish and larger oscillating signals with notable spikes of strontium and barium elemental concentrations that reflect changes in the environment and likely movements throughout or out of the estuarine environment (Chapter 5, Fig. 6.1). Findings were not consistent across the species' distribution, with variations in profiles and movement indicators more frequent in larger, open systems and intermittently closed systems with small estuaries, and residents more abundant in intermittently closed systems with large estuaries. Variations in movement patterns across systems suggest that different management approaches may be necessary across different systems and regions, and in some cases the spatial scale possibly extends beyond estuaries into rivers and coastal waters.

In this closing chapter, I will further examine the main outcomes of my research and discuss management implications and future directions.



Fig. 6.1 Schematic representation of chapter findings, thesis outcomes and conclusion.

6.1 High genetic structuring among A. butcheri populations

As little was known of gene flow across this species entire distribution, I assessed genetic structuring and gene flow at a continental scale by accessing a natural tag that functions effectively over a large spatial scale and across a generational timeline. Through the next-generation sequencing of 33,493 Single-Nucleotide Polymorphisms (SNPs), I identified genetic patterns across A. butcheri's distribution range (3,200 km longitudinal range) that are likely the result of the interplay between large-scale marine barriers and the species' life history characteristics associated with estuarine dependency and habitat use (Chapter 2). Broad-scale structuring and the finding of regional clusters generally reflected major biogeographical provinces present across Australia (Li et al., 2013; Waters et al., 2003; Bennet and Pope 1953), with similar geographical breaks in gene flow previously found in marine sparids (Burridge and Versace 2006; Bertram et al., 2023). Large open water bodies, unsuitable habitats and strong ocean currents further explain the genetic differentiation identified at this spatial scale, as observed for other species (Islam et al., 2022; Kurata et al., 2022; Volk et al., 2021). The limited gene flow at the local-scale across south-eastern Australian estuaries is likely a reflection of the species' estuarine dependence (Doubleday et al., 2015; Partridge and Jenkins 2002), with cases of movement between spatially adjacent estuaries or long-distance fish movement via lone migrants reflecting the species ability to move outside of estuaries, and the coexistence of migratory and resident life cycles in the same population (i.e., partial migration) (Tracey et al., 2020; Gillanders et al., 2015). Nonetheless, most individuals likely remain in or return to their estuary of origin to reproduce, and the spatial proximity of estuaries was not necessarily a reflection of increased gene flow. Structuring of populations was also shown through the integration of natural tagging techniques (Chapter 3), with discrimination among estuaries not only present across a generational timescale, but also an ecological timescale using otolith-based approaches (i.e., chemistry and shape).

6.2 The integrative power of natural markers

By combining both molecular and non-molecular approaches that reflect different ecological and evolutionary processes at varying spatiotemporal ranges, I found that integrating markers increased the accuracy and resolution of the results (Reis-Santos *et al.*, 2018; Abaunza *et al.*, 2008) and discrimination among *A. butcheri* populations. Through a combination of otolith elemental composition, otolith shape, and genetics, approximately 95% of fish were correctly classified to their estuary of capture. Although population genetics provided stronger discrimination among estuaries out of all individual markers analysed (Izzo *et al.*, 2017), its resolution was improved with the addition of otolith-based techniques, likely reflecting the different sources of variability each marker

provides. For example, factors likely influencing otolith chemistry include environmental variables such as water pollution (i.e., increased otolith lead and zinc concentrations) (Chapter 3; Søndergaard *et al.*, 2015; McKinley *et al.*, 2012) and water temperature (i.e., increased stable carbon and oxygen isotopes, and overall rate of biomineralisation) (Chapter 3; Elsdon and Gillanders 2002; Stanley *et al.*, 2015). The discrimination of populations using otolith shape reflects the species known variability in size and growth across its distribution (i.e., ontogeny) (Cheshire *et al.*, 2013; Norriss *et al.*, 2002). However, the low discrimination of this marker compared to genetics and otolith chemistry reflects its lower resolution of environmental and genetic influences among populations. Additionally, not all marker integrations were beneficial (e.g., otolith isotope composition with otolith shape produced lower classification accuracy than either used independently), which highlights the importance of appropriate marker selection based on target species, environmental heterogeneity, and spatial scale.

6.3 Climate-induced growth variability

I aimed to provide information that could guide management to maximise the future resilience of A. butcheri by improving our understanding of the variability of growth among estuaries and the effects of changing environmental conditions. Using 31,006 otolith increment measurements from 4,792 fish, I applied a mixed effects modelling approach that assessed intrinsic and extrinsic effects at both a regional-scale (El Niño Southern Oscillation, ENSO) and local-scale (temperature and water flow). I found that the annual-lagged ENSO effect explained the most variability in annual growth, a climatic phenomenon previously shown to trigger both negative (Reis-Santos et al., 2021; Martino et al., 2019) and positive growth responses (Leahy and Robins 2021; Stocks et al., 2014) in other fish species likely altering local abiotic conditions and influencing fish growth via changes in productivity. The annual-lagged local-scale effects mirror similar growth responses of A. butcheri and other species to other location-specific environmental variables (e.g., rainfall, salinity, and wind stress) (Izzo et al., 2016; Morrongiello et al., 2021) and complement the regional-scale effects by likely altering environmental conditions that, in this case, trigger a delayed growth response. Furthermore, this delayed response was also shown in some cases of spatial synchrony in growth occurring across longitudinally separated estuaries in the years following shifts in ENSO phases between El Niño conditions (i.e., hot and dry) and La Niña conditions (i.e., cold and wet); consistent with a climateinduced growth response previously shown in other aquatic animals (Tanner et al., 2020; Ong et al., 2018, 2019; Matta et al., 2010). I also identified prolonged spatial synchrony (i.e., over multiple 11year sliding windows) occurring along latitudinally separated and environmentally heterogenous estuaries (Uvanović et al., 2023; Williams et al., 2020; Ong et al., 2018), further highlighting how

climate factors and extreme weather events play a major influencing role in this species' growth even across dramatically different bioregions. The regional-scale effects and the spatial synchrony in growth reflects the potential impact and vulnerability of the species across the entire distribution to intensifying climatic events.

6.4 Characterising fish movement using otoliths

Considering the variations in environmental conditions, and despite gene flow among southern Australian estuaries being limited, I investigated if movement within estuaries and adjacent environments (i.e., coastal waters and rivers) varied. By assessing 239 otolith chemical profiles (i.e., reflecting the life history of each fish) from eight estuaries across southern Australia, I identified two main patterns; namely one where chemical signals showed small range oscillations reflecting more stable environments and resident behaviour (Schmidt et al., 2014; Elsdon and Gillanders 2005; Howland et al., 2001), and another comprised of large range oscillating signals with notable spikes in elemental concentrations during early and adult life stages, reflecting increased movements throughout and possibly out of estuarine environments and into marine and/or freshwater systems (Reis-Santos et al., 2023; Avigliano et al., 2017; Gillanders et al., 2015), or along large salinity gradients within estuaries. The occurrence of these two patterns reflect the species ability to exhibit partial migration within populations (Tracey et al., 2020; Gillanders et al., 2015). However, the ratio of these movement types varied among estuaries of different sizes and features, with fish movement more frequent in open systems and intermittently closed systems with small estuaries, and residents more abundant in intermittently closed systems with large estuaries. Findings highlight the power of otolith chemistry in characterising fish movement and reflect the variable influence estuary-specific features and environmental conditions can have on movement patterns. Furthermore, the presence of populations with increased fish movement throughout or out of estuaries stresses the importance of location-specific management possibly extending beyond estuarine environments (e.g., into rivers where spawning might be more prevalent or into coastal waters where productivity is higher for select populations).

6.5 Management implications

Currently, assessments of stock status for *A. butcheri* are undertaken separately for nine management units at variable spatial scales distributed along southern Australia (i.e., Western Australia, South Australia, Victoria, Tasmania, and New South Wales) and these involve different indicators which are not applied consistently across assessments (i.e., they often consider one, or combinations, of catch, targeted effort, catch per unit effort, age and size compositions, estimated biomass, and harvest rate) (SAFS 2020). In this thesis, the high structuring of populations and limited

connectivity across southern Australian estuaries identified using a range of different approaches (i.e., genetics, multi-marker, growth dynamics, and movement patterns) highlights the importance of adaptive management and considering the appropriate scale for managing this commercially and recreationally important species. Although my findings suggest estuary-specific management is appropriate in most cases for *A. butcheri*, based on high population structuring using genetic and otolith-based techniques (Chapter 2 and 3), cases of movement outside and among estuaries, as well as synchronised growth across the species' distribution (Chapter 4) suggest a more complex management approach could be considered. For example, based on otolith chemical profiles (Chapter 5), management units that extend beyond estuaries and into rivers or adjacent coastal waters may be more appropriate for populations inhabiting open systems (e.g., Coorong) and intermittently closed systems with small estuaries (e.g., Glenelg River and Hopkins River), with increased fish movement out of these estuaries more likely to occur ; whereas population genetics (Chapter 2) suggests broader management units that extend beyond the salinity gradient and among estuaries where gene flow is present (e.g., between Coorong and Robe, Harriet River and Eleanor River, and across Port River, West Lakes, and Onkaparinga River) may be more appropriate.

In terms of management units in South Australia, the two commercial fisheries that target *A*. *butcheri*; the State-wide Marine Scalefish Fishery (MSF), currently classified as 'sustainable' (Smart *et al.*, 2023); and the Lakes and Coorong Fishery (LCF), currently classified as 'depleted' (Earl 2023), show no gene flow between key fishing areas, specifically the Coorong (LCF) and upper Gulf St Vincent and Spencer Gulf (MSF). Therefore fisheries-specific management would be recommended for MSF, although gene flow between LCF (i.e., Coorong) and estuaries further south (i.e., Robe) suggests broader-scale management extending beyond this fishery could be considered. However, understanding the relative contributions of fish originating from these two areas, as well as the estuaries of the Inman and Hindmarsh Rivers (spatially adjacent to the Coorong but not considered in this study due to sampling limitations) to the fishable biomass within the spatial constraints of the LCF (on a generational timescale rather than evolutionary) will be important information to consider before realignment of current management boundaries.

Currently in Australia, few fisheries are managed and assessed in the context of climatic or environmental conditions (an exception is South Australia's LCF; PIRSA 2022). However, my results suggest that such adaptive management approaches should be considered given the regional-scale effect on yearly *A. butcheri* growth and negative growth response to increasing El Niño conditions (i.e., hot and dry) and La Niña conditions (i.e., cold and wet) (Chapter 4). Furthermore, the potential negative impacts of overexploitation on growth (i.e., growth overfishing), the species' predicted range contraction with increasing climate change, and the subsequent impacts of such outcomes on

the future resilience of populations highlights the importance of periodic reviews of the spatial scales of management to ensure they align with the spatial scales of biological populations (Nikolaou and Katsanevakis 2023; Martino *et al.*, 2019; Kraak *et al.*, 2019; Pecl *et al.* 2014). Overall, the complexity in *A. butcheri* population dynamics suggests management strategies may not be easily transferable to other species with similar behaviours and life histories (i.e., partial migrants), either in Australia (e.g., Mulloway [*Argyrosomus japonicus*] and Barramundi [*Lates calcarifer*]) or globally (e.g., Chinook Salmon [*Oncorhynchus tshawytscha*] and Atlantic Striped Bass [*Morone saxatilis*]). The effective management and conservation of such species are likely to be equally complex and variable, and may need to be considered on a case by case basis, further stressing the importance of tailored investigations.

6.6 Future directions

Given the potential future environmental impacts on fish and their resilience, investigating population dynamics not only spatially but temporally should be a primary focus of future research. Applying a temporal trend to tagging techniques (e.g., decadal to multidecadal) can provide an understanding of not only locations for which fish are collected relative to others, but how these targeted systems change over time, how management may be driving such changes, and how this may influence our understanding and interpretation of structure and connectivity (Denechaud et al., 2020; Brennan et al., 2019; Mohan and Walther 2014). I planned to assess such change by utilising historical fish liver samples to investigate genetic differentiation over time (Chapter 2), but this was unsuccessful due to poor tissue quality and limited contemporary sampling from the corresponding estuaries that historical samples were collected from. Given these circumstances, enhancing storage of organic material for future studies and collaborations should be encouraged as it can improve the quality of results when assessing population dynamics, including collection of tissue samples (e.g., muscle or fin clippings) when able to correctly store (e.g., ultra-low temperature freezers), or more feasibly the collection and storage of fish otoliths and scales, which require little preservation to assess age, growth, and chemical composition. Furthermore, collecting extrinsic information from target locations (e.g., water chemistry) to build environmental reference libraries and couple with natural tagging technique (e.g., otolith chemistry and growth) can also increase the resolution of findings, including individual movement patterns and population connectivity (Zampatti et al., 2021; Denechaud et al., 2020; Barnett-Johnson et al., 2008).

Studies validating the effectiveness of tagging techniques for individual species and their spatial range are also recommended, given that natural markers may not always reflect environmental signals and can be buffered by physiological influences, and this can limit their

broader application (Reis-Santos *et al.*, 2023, 2008; Izzo *et al.*, 2018; Sturrock *et al.*, 2014). Selecting the appropriate tagging techniques based on target species, environmental heterogeneity, and spatial distribution is crucial and recommended to accurately assess population dynamics and connectivity. For example, although strontium and barium are effective elements for tackling fish movement between freshwater and marine systems, they are less reliable in tracking movement within more homogeneous marine environments (Sturrock *et al.*, 2014; Brown and Severin 2009). Highly mobile and connected marine fish would benefit from more artificial tagging techniques or unique external markings to track movement (Campana *et al.*, 2011; Graham *et al.*, 2007). Likewise, deepwater species would benefit from molecular approaches given the homogeneity of their environments (McGill *et al.*, 2023), relative to estuarine and freshwater fish where chemical approaches (e.g., otolith elemental and isotopic composition) would better reflect environmental signatures.

Applying multiple markers where possible when assessing population structure and connectivity should be implemented into future studies. Although molecular and otolith chemical approaches can be costly, morphometrics (i.e., otolith shape) is a feasible and at times valuable approach for discriminating populations (Ana Vaz *et al.*, 2023; Smoliński *et al.*, 2020). Research has been developing new chemical approaches by harnessing other organic materials, such as eye lenses (Rosinski *et al.*, 2023; Young *et al.*, 2022; Nielsen *et al.*, 2016), vertebrae (Frazier *et al.*, 2023; McMillan *et al.*, 2018) and operculae (Charapata *et al.*, 2023, 2022), any of which could potentially increase the resolution of findings when coupled with other tagging techniques (e.g., molecular or artificial).

Additional techniques that can be valuable include the assessment of trophic dynamics within estuaries, as understanding trophic positions of species throughout their life history can play an important role in their management (Pethybridge *et al.*, 2018; Heymans *et al.*, 2016). Assessing stomach eDNA, stable isotope compositions of nitrogen (δ^{15} N values) and carbon (δ^{13} C values) in tissue and otoliths, and diet content could be used to investigate trophic structure (Lueders-Dumont *et al.*, 2022; de Sousa *et al.*, 2019; Grønkjær *et al.*, 2013; Jackson *et al.*, 2012;)

Lastly, I recommend increased collaborative efforts to enhance the quality and sustainability of research and accuracy of interpretations. I found that collaborating with commercial and recreational fishers, interstate universities and government agencies allowed me to limit contemporary mortalities (i.e., scavenging fish frames) and increase sampling distributions spatially as well as temporally (i.e., historical otolith and tissue collections). Such interdisciplinary partnerships support sustainable fisheries management and bridge the gap between research and management (Reis-Santos *et al.,* 2023; Carlson *et al.,* 2017)

6.7 Concluding remarks

Throughout this thesis, I show that *A. butcheri* populations are highly structured across their entire distribution range, with minimal connectivity among southern Australian estuaries and at local-scales reflecting strong philopatry and the species' estuarine-dependency. Through integrating multiple natural tagging techniques, I was able to increase the resolution of my findings, which further reflected local-scale structuring. Assessing growth variability uncovered growth responses to climatic events and synchronised growth among estuaries, while assessment of movement patterns using otolith chemistry further demonstrated variations in life history patterns among estuaries of different sizes and features. Ultimately, the approaches used here to investigate population dynamics successfully delineated *A. butcheri* populations and highlighted their unique characteristics and attributes. Furthermore, I have highlighted the importance of location-specific management for this species, as well as the vulnerability of such estuarine fish to our rapidly changing environments.

References

- Abaunza, P, Murta, AG, Campbell, N, Cimmaruta, R, Comesaña, AS, Dahle, G, García Santamaría, MT, Gordo, LS, Iversen, SA, MacKenzie, K, Magoulas, A, Mattiucci, S, Molloy, J, Nascetti, G, Pinto, AL, Quinta, R, Ramos, P, Sanjuan, A, Santos, AT, Stransky, C, Zimmermann, C (2008) Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. *Fisheries Research* 89, 196-209. doi:10.1016/j.fishres.2007.09.022
- Avigliano, E, Leisen, M, Romero, R, Carvalho, B, Velasco, G, Vianna, M, Barra, F, Volpedo, AV (2017) Fluvio-marine travelers from South America: Cyclic amphidromy and freshwater residency, typical behaviors in *Genidens barbus* inferred by otolith chemistry. *Fisheries Research* 193, 184-194. doi:10.1016/j.fishres.2017.04.011
- Barnett-Johnson, R, Pearson, TE, Ramos, FC, Grimes, CB, MacFarlane, RB (2008) Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. *Limnology and Oceanography* **53**, 1633-1642. doi:10.4319/lo.2008.53.4.1633
- Bennett, I, Pope, EC (1953) Intertidal zonation of the exposed rocky shores of Victoria, together with a rearrangement of the biogeographical provinces of temperate Australian shores. *Marine and Freshwater Research* 4, 105-159. doi:10.1071/mf9530105
- Bertram, A, Bell, J, Brauer, C, Fowler, A, Hamer, P, Sandoval-Castillo, J, Stewart, J, Wellenreuther, M, Beheregaray, LB (2023) Bioregional boundaries and genomically-delineated stocks in snapper (*Chrysophrys auratus*) from southeastern Australia. *Life Science Weekly* 492. doi:10.1101/2023.01.16.524335
- Brennan, SR, Cline, TJ, Schindler, DE (2019) Quantifying habitat use of migratory fish across riverscapes using space-time isotope models. *Methods in Ecology and Evolution* 10, 1036-1047. doi:10.1111/2041-210x.13191
- Brown, RJ, Severin, KP (2009) Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Canadian Journal of Fisheries and Aquatic Sciences* 66, 1790+.
- Burridge, CP, Versace, VL (2006) Population genetic structuring in *Acanthopagrus butcheri* (Pisces: Sparidae): does low gene flow among estuaries apply to both sexes? *Marine Biotechnology* 9, 33-44. doi:10.1007/s10126-006-6023-7
- Campana, SE, Dorey, A, Fowler, M, Joyce, W, Wang, Z, Wright, D, Yashayaev, I (2011) Migration pathways, behavioural thermoregulation and overwintering grounds of blue sharks in the Northwest Atlantic. *PLoS ONE*, *6*(2), e16854. doi:10.1371/journal.pone.0016854
- Cardinale, M, Doering-Arjes, P, Kastowsky, M, Mosegaard, H (2004) Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 61, 158-167. doi:10.1139/F03-151
- Carlson, AK, Phelps, QE, Graeb, BD (2017) Chemistry to conservation: using otoliths to advance recreational and commercial fisheries management. *Journal of Fish Biology* 90, 505-527. doi:10.1111/jfb.13155
- Charapata, P, Oxman, D, McNeel, K, Keith, A, Mansouri, F, Trumble, S (2022) Lifetime hormone profiles for a long-lived teleost: opercula reveal novel estimates of age-specific reproductive parameters and stress trends in yelloweye rockfish (*Sebastes ruberrimus*). *Canadian Journal of Fisheries and Aquatic Sciences* 79, 1712-1728. doi:10.1139/cjfas-2022-0048
- Charapata, P, Trumble, S, Smoliński, S (2023) Environmental and ecological changes influence lifetime trends of reproduction, stress, and stable isotopes reconstructed from female yelloweye rockfish opercula. *ICES Journal of Marine Science* 80, 1500-1515. doi:10.1093/icesjms/fsad078
- Cheshire, KJM, Ye, Q, Fredberg, J, Earl, J, 2013. Aspects of reproductive biology of five key fish species in the Murray Mouth and Coorong. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.

- de Sousa, LL, Silva, SM, Xavier, R (2019) DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA*, 1(3), 199-214. doi:10.1002/edn3.27
- Denechaud, C, Smoliński, S, Geffen, AJ, Godiksen, JA, (2020) Long-term temporal stability of Northeast Arctic cod (*Gadus morhua*) otolith morphology. *ICES Journal of Marine Science* 77, 1043-1054. doi:10.1093/icesjms/fsz259
- Doubleday, ZA, Izzo, C, Haddy, JA, Lyle, JM, Ye, Q, Gillanders, BM (2015) Long-term patterns in estuarine fish growth across two climatically divergent regions. *Oecologia* 179, 1079-90. doi:10.1007/s00442-015-3411-6
- Earl, J, 2023. Assessment of the South Australian Lakes and Coorong Fishery in 2021-2022. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Elsdon, TS, Gillanders, BM (2002) Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Canadian Journal of Fisheries and Aquatic Sciences* 59, 1796-1808. doi:10.1139/F02-154
- Elsdon, TS, Gillanders, BM (2005) Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. *Marine Ecology Progress Series* 285, 233-243. doi:DOI 10.3354/meps285233
- Frazier, BS, Vinyard, EA, Fields, AT, Driggers, WB, Grubbs, RD, Adams, DH, Drymon, JM, Gardiner, JM, Hendon, JM, Hoffmayer, E, Hueter, RE, Wells, RJD, Wiley, TR, Portnoy, DS (2023) Age, growth and maturity of the bonnethead *Sphyrna tiburo* in the U.S. Gulf of Mexico. *Environmental Biology of Fishes* 106, 1597-1617. doi:10.1007/s10641-023-01439-5
- Gillanders, BM, Izzo, C, Doubleday, ZA, Ye, Q (2015) Partial migration: growth varies between resident and migratory fish. *Biology Letters* 11. doi:10.1098/rsbl.2014.0850
- Graham, RT, & Roberts, CM (2007) Assessing the size, growth rate and structure of a seasonal population of whale sharks (*Rhincodon typus* Smith 1828) using conventional tagging and photo identification. *Fisheries Research*, *84*(1), 71-80. doi:10.1016/j.fishres.2006.11.026
- Grønkjær, P, Pedersen, JB, Ankjærø, TT, Kjeldsen, H, Heinemeier, J, Steingrund, P, Nielsen, JM, Christensen, JT, Gillanders, B (2013) Stable N and C isotopes in the organic matrix of fish otoliths: validation of a new approach for studying spatial and temporal changes in the trophic structure of aquatic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 70, 143-146. doi:10.1139/cjfas-2012-0386
- Heymans, JJ, Coll, M, Link, JS, Mackinson, S, Steenbeek, J, Walters, C, Christensen, V (2016) Best practice in ecopath with ecosim food-web models for ecosystem-based management. *Ecological Modelling* 331, 173-184. doi:10.1016/j.ecolmodel.2015.12.007
- Howland, KL, Tonn, WM, Babaluk, JA, Tallman, RF (2001) Identification of freshwater and anadromous inconnu in the Mackenzie River system by analysis of otolith strontium. *Transactions of the American Fisheries Society* 130, 725-741. doi:10.1577/1548-8659(2001)130<0725:lofaai>2.0.Co;2
- Hüssy, K (2008) Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects. *Journal of Experimental Marine Biology and Ecology* 364, 35-41. doi:10.1016/j.jembe.2008.06.026
- Islam, MRU, Tachihara, K, Imai, H (2022) Cryptic lineage and genetic structure of *Acanthopagrus pacificus* populations in a natural world heritage site revealed by population genetic analysis. *Diversity-Basel* 14, doi:10.3390/d14121117
- Izzo, C, Doubleday, ZA, Grammer, GL, Barnes, TC, Delean, S, Ferguson, GJ, Ye, QF, Gillanders, BM (2016) Multi-species response to rapid environmental change in a large estuary system: A biochronological approach. *Ecological Indicators* 69, 739-748. doi:10.1016/j.ecolind.2016.05.019
- Izzo, C, Reis-Santos, P, Gillanders, BM (2018) Otolith chemistry does not just reflect environmental conditions: A meta-analytic evaluation. *Fish and Fisheries* 19, 441-454. doi:10.1111/faf.12264
- Izzo, C, Ward, TM, Ivey, AR, Suthers, IM, Stewart, J, Sexton, SC, Gillanders, BM (2017) Integrated

approach to determining stock structure: implications for fisheries management of sardine, *Sardinops sagax*, in Australian waters. *Reviews in Fish Biology and Fisheries* 27, 267-284. doi:10.1007/s11160-017-9468-z

- Jackson, MC, Donohue, I, Jackson, AL, Britton, JR, Harper, DM, Grey, J (2012) Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PLoS ONE*, 7(2). doi:10.1371/journal.pone.0031757
- Kerr, LA, Hintzen, NT, Cadrin, SX, Clausen, LW, Dickey-Collas, M, Goethel, DR, Hatfield, EMC, Kritzer, JP, Nash, RDM, Hidalgo, M (2017) Lessons learned from practical approaches to reconcile mismatches between biological population structure and stock units of marine fish. *ICES Journal of Marine Science*, 74(6), 1708-1722. doi:10.1093/icesjms/fsw188
- Kraak, SBM, Haase, S, Minto, C, Santos, J (2019) The Rosa Lee phenomenon and its consequences for fisheries advice on changes in fishing mortality or gear selectivity. *ICES Journal of Marine Science* 76, 2179-2192. doi:10.1093/icesjms/fsz107
- Kurata, NP, Hickerson, MJ, Hoffberg, SL, Gardiner, N, Stiassny, MLJ, Alter, SE (2022) Riverscape genomics of cichlid fishes in the lower Congo: Uncovering mechanisms of diversification in an extreme hydrological regime. *Molecular Ecology* 31, 3516-3532. doi:10.1111/mec.16495
- Leahy, SM, Robins, JB (2021) River flows affect the growth of a tropical finfish in the wet-dry rivers of northern Australia, with implications for water resource development. *Hydrobiologia* 848, 4311-4333. doi:10.1007/s10750-021-04641-7
- Li, J, Foighil, DO, Park, JK (2013) Triton's trident: cryptic Neogene divergences in a marine clam (*Lasaea australis*) correspond to Australia's three temperate biogeographic provinces. *Molecular Ecology* 22, 1933-46. doi:10.1111/mec.12220
- Lueders-Dumont, JA, Forden, AG, Kast, ER, Mohan, JA, Walther, BD, Sigman, DM, Ward, BB (2022) Controls on the nitrogen isotopic composition of fish otolith organic matter: Lessons from a controlled diet switch experiment. *Geochimica et Cosmochimica Acta* 316, 69-86. doi:10.1016/j.gca.2021.09.030
- Martino, JC, Fowler, AJ, Doubleday, ZA, Grammer, GL, Gillanders, BM (2019) Using otolith chronologies to understand long-term trends and extrinsic drivers of growth in fisheries. *Ecosphere* 10, e02553. doi:10.1002/ecs2.2553
- Matta, ME, Black, BA, Wilderbuer, TK (2010) Climate-driven synchrony in otolith growth-increment chronologies for three Bering Sea flatfish species. *Marine Ecology Progress Series* 413, 137-145. doi:10.3354/meps08689
- McGill, L, McDevitt, AD, Hellemans, B, Neat, F, Knutsen, H, Mariani, S, Christiansen, H, Johansen, T, Volckaert, FAM, Coscia, I, Grant, WS (2023) Population structure and connectivity in the genus *Molvain* the Northeast Atlantic. *ICES Journal of Marine Science*, *80*(4), 1079-1086. doi:10.1093/icesjms/fsad040
- McKinley, AC, Taylor, MD, Johnston, EL (2012) Relationships between body burdens of trace metals (As, Cu, Fe, Hg, Mn, Se, and Zn) and the relative body size of small tooth flounder (*Pseudorhombus jenynsii*). *Science of The Total Environment* 423, 84-94. doi:10.1016/j.scitotenv.2012.02.007
- McMillan, MN, Huveneers, C, Semmens, JM, Gillanders, BM (2018) Natural tags reveal populations of conservation dependent school shark use different pupping areas. *Marine Ecology Progress Series* 599, 147-156. doi:10.3354/meps12626
- Mohan, JA, Walther, BD (2014) Spatiotemporal Variation of Trace Elements and Stable Isotopes in Subtropical Estuaries: II. Regional, Local, and Seasonal Salinity-Element Relationships. *Estuaries and Coasts* 38, 769-781. doi:10.1007/s12237-014-9876-4
- Nielsen, JH, Rasmus B. ; Heinemeier, Jan ; Bushnell, Peter G. ; Christiansen,, Jorgen S. ; Olsen, JR, Christopher Bronk ; Brill, Richard W. ; Simon, Malene ;, Steffensen, KFS, John F (2016) Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*). *Science* 353, 702-704. doi:10.1126/science.aaf1703
- Norriss, JV, Tregonning, JE, Lenanton, RCJ, Sarre, GA, 2002. Biological synopsis of the black bream,

Acanthopagrus butcheri (Munro) (Teleostei: Sparidae) in Western Australia with reference to information from other southern states. Department of Fisheries, Perth, Western Australia.

- Ong, JJL, Rountrey, AN, Black, BA, Nguyen, HM, Coulson, PG, Newman, SJ, Wakefield, CB, Meeuwig, JJ, Meekan, MG (2018) A boundary current drives synchronous growth of marine fishes across tropical and temperate latitudes. *Global Change Biology* 24, 1894-1903. doi:10.1111/gcb.14083
- Partridge, GJ, Jenkins, GI (2002) The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). *Aquaculture* 210, 219-230. doi:10.1016/S0044-8486(01)00817-1
- Pethybridge, HR, Choy, CA, Polovina, JJ, Fulton, EA (2018) Improving marine ecosystem models with biochemical tracers. *Annual Review of Marine Science* 10, 199-228. doi:10.1146/annurev-marine-121916-063256
- PIRSA (2020), 'Management Plan for Recreational Fishing in South Australia' Available at <u>https://pir.sa.gov.au/__data/assets/pdf_file/0003/296490/2020_Recreational_Fishing_Management_Plan.pdf</u> [Accessed 20/02/24]
- Reis-Santos, P, Condini, MV, Albuquerque, CQ, Saint'Pierre, TD, Garcia, AM, Gillanders, BM, Tanner, SE (2021) El Nino Southern Oscillation drives variations in growth and otolith chemistry in a top predatory fish. *Ecological Indicators* 121,doi:10.1016/j.ecolind.2020.106989
- Reis-Santos, P, Gillanders, BM, Sturrock, AM, Izzo, C, Oxman, DS, Lueders-Dumont, JA, Hüssy, K, Tanner, SE, Rogers, T, Doubleday, ZA, Andrews, AH, Trueman, C, Brophy, D, Thiem, JD, Baumgartner, LJ, Willmes, M, Chung, M-T, Charapata, P, Johnson, RC, Trumble, S, Heimbrand, Y, Limburg, KE, Walther, BD (2023) Reading the biomineralized book of life: expanding otolith biogeochemical research and applications for fisheries and ecosystem-based management. *Reviews in Fish Biology and Fisheries* 33, 411-449. doi:10.1007/s11160-022-09720-zReis-Santos, P, Tanner, SE, Aboim, MA, Vasconcelos, RP, Laroche, J, Charrier, G, Perez, M, Presa, P, Gillanders, BM, Cabral, HN (2018) Reconciling differences in natural tags to infer demographic and genetic connectivity in marine fish populations. *Scientific Reports* 8, 10343. doi:10.1038/s41598-018-28701-6
- Reis-Santos, P, Vasconcelos, RP, Ruano, M, Latkoczy, C, Günther, D, Costa, MJ, & Cabral, H (2008) Interspecific variations of otolith chemistry in estuarine fish nurseries. *Journal of Fish Biology*, 72(10), 2595-2614. Doi:10.1111/j.1095-8649.2008.01871.x
- Rosinski, CL, Glaid, J, Hahn, M, Fetzer, WW (2023) Natal origin differentiation using eye lens stable isotope analysis. *North American Journal of Fisheries Management* 43, 547-555. doi:10.1002/nafm.10875
- SAFS (2020), 'Black bream' Available at https://fish.gov.au/report/366-Black-Bream-2020 [Accessed 16/01/24]
- Schmidt, DJ, Crook, DA, Macdonald, JI, Huey, JA, Zampatti, BP, Chilcott, S, Raadik, TA, Hughes, JM (2014) Migration history and stock structure of two putatively diadromous teleost fishes, as determined by genetic and otolith chemistry analyses. *Freshwater Science* 33, 193-206. doi:10.1086/674796
- Smart, JJ, McGarvey, R, Feenstra, J, Drew, MJ, Earl, J, Durante, L, Beckmann, CL, Matthews, D, Matthews, JM, Mark, K, Bussell, J, Davey, J, Tsolos, A, Noell, C, (2023) Assessment of the South Australian Marine Scalefish Fishery in 2021/22. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, South Australia.
- Smoliński, S, Schade, FM, Berg, F (2020) Assessing the performance of statistical classifiers to discriminate fish stocks using fourier analysis of otolith shape. *Canadian Journal of Fisheries and Aquatic Sciences* 77, 674-683. doi:10.1139/cjfas-2019-0251
- Smoliński, S, Schade, FM, Berg, F (2020) Assessing the performance of statistical classifiers to discriminate fish stocks using Fourier analysis of otolith shape. *Canadian Journal of Fisheries and Aquatic Sciences* 77, 674-683. doi:10.1139/cjfas-2019-0251
- Søndergaard, J, Halden, N, Bach, L, Gustavson, K, Sonne, C, Mosbech, A (2015) Otolith Chemistry of Common Sculpins (*Myoxocephalus scorpius*) in a Mining Polluted Greenlandic Fiord (Black

Angel Lead-Zinc Mine, West Greenland). *Water, Air, & Soil Pollution* 226, 336. doi:10.1007/s11270-015-2605-1

- Stanley, RRE, Bradbury, IR, DiBacco, C, Snelgrove, PVR, Thorrold, SR, Killen, SS (2015) Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). *ICES Journal* of Marine Science 72, 2350-2363. doi:10.1093/icesjms/fsv070
- Stocks, JR, Gray, CA, Taylor, MD (2014) Synchrony and variation across latitudinal gradients: The role of climate and oceanographic processes in the growth of a herbivorous fish. *Journal of Sea Research* 90, 23-32. doi:10.1016/j.seares.2014.03.002
- Sturrock, AM, Trueman, CN, Milton, JA, Waring, CP, Cooper, MJ, Hunter, E (2014) Physiological influences can outweigh environmental signals in otolith microchemistry research. *Marine Ecology Progress Series* 500, 245-264. doi:10.3354/meps10699
- Tanner, SE, Giacomello, E, Menezes, GM, Mirasole, A, Neves, J, Sequeira, V, Vasconcelos, RP, Vieira, AR, Morrongiello, JR (2020) Marine regime shifts impact synchrony of deep-sea fish growth in the northeast Atlantic. *Oikos* 129, 1781-1794. doi:10.1111/oik.07332
- Tracey, SR, Hartmann, K, McAllister, J, Lyle, JM (2020) Home range, site fidelity and synchronous migrations of three co-occurring, morphologically distinct estuarine fish species. *Science of The Total Environment* 713, 136629. doi:10.1016/j.scitotenv.2020.136629
- Uvanović, H, Peharda, M, Pavin, N, Thébault, J, Mazzoldi, C, Mihanović, H, Župan, I (2023) Developing a sclerochronology network in the Adriatic Sea: Growth synchrony among populations of *Callista chione. Regional Studies in Marine Science* 64, doi:10.1016/j.rsma.2023.103009
- Vaz, A, Guerreiro, MA, Landa, J, Hannipoula, O, Thasitis, I, Scarcella, G, Sabatini, L, Vitale, S, Mugerza, E, Mahé, K, Reis-Santos, P, Tanner, SE, Stransky, C, Pardal, M, Martinho, F (2023) Otolith shape analysis as a tool for stock identification of two commercially important marine fishes: *Helicolenus dactylopterus* and *Merluccius merluccius*. *Estuarine, Coastal and Shelf Science* 293, doi:10.1016/j.ecss.2023.108471
- Volk, DR, Konvalina, JD, Floeter, SR, Ferreira, CEL, Hoffman, EA (2021) Going against the flow: Barriers to gene flow impact patterns of connectivity in cryptic coral reef gobies throughout the western Atlantic. *Journal of Biogeography* 48, 427-439. doi:10.1111/jbi.14010
- Waters, JM, Roy, MS (2003) Marine biogeography of southern Australia: phylogeographical structure in a temperate sea-star. *Journal of Biogeography* 30, 1787-1796. doi:10.1046/j.0305-0270.2003.00978.x
- Williams, J, Cottingham, A, Denham, A, Hall, NG, Potter, IC (2020) Relationship between spawning and egg and larval stages of a unique estuarine-resident species and environmental variables and prey. *Estuarine Coastal and Shelf Science* 246, doi:10.1016/j.ecss.2020.107039
- Young, MJ, Larwood, V, Clause, JK, Bell-Tilcock, M, Whitman, G, Johnson, R, Feyrer, F (2022) Eye lenses reveal ontogenetic trophic and habitat shifts in an imperiled fish, Clear Lake hitch (*Lavinia exilicauda chi*). Canadian Journal of Fisheries and Aquatic Sciences 79, 21-30. doi:10.1139/cjfas-2020-0318
- Zampatti, BP, Bice, CM, Jennings, PR (2010) Temporal variability in fish assemblage structure and recruitment in a freshwater-deprived estuary: The Coorong, Australia. *Marine and Freshwater Research* 61, 1298-1312. doi:10.1071/MF10024

Appendix – Outreach and presentations

Table A1 List of	presentations and	outreach ac	-tivities cor	nducted durir	ig my PhD
	presentations and	outreactrac		iuucicu uurii	is my i no

Year	Style	Organisation	Audience demographic
2020	Seminar	Ecology and Evolutionary Biology seminar, The University of Adelaide	Academics
2020	Article in newsletter	Australian Society of Fish Biology	Academics/industry/government
2021	Presentation	The University of Adelaide - Zoology II tutorial	Undergraduate students
2021	3 minutes thesis	School of Biological Sciences, The University of Adelaide	Academics
2021	Video competition	Visualise your thesis, The University of Adelaide	Academics
2021	Presentation	World Fisheries Congress, Adelaide, Australia	Academics/industry/government
2022	Presentation	South Australian Research and Development Institute	Industry/government
2022	Presentation	South Australian Research Advisory Committee, Fisheries Research and Development Corporation	Academics/Industry/government
2022	Presentation at annual conference	Australian Society of Fish Biology, Queensland, Australia	Academics/industry/government
2023	Presentation	International otolith symposium, Viña del Mar, Chile	Academics/industry/government

Project materials developed

Scientific papers resulting from the research:

Sarakinis, KG, P Reis-Santos, SC Donnellan, Q Ye, J Earl, BM Gillanders. 2024. Strong philopatry in an estuarine-dependent fish. Ecology and Evolution 47, e10989.

Sarakinis, KG, P Reis-Santos, Q Ye, J Earl, BM Gillanders. 2024. Combining natural markers to investigate fish population structure and connectivity. Estuarine, Coastal and Shelf Science 108920.