SPATIAL MANAGEMENT OF SOUTHERN GARFISH (*HYPORHAMPHUS MELANOCHIR*) IN SOUTH AUSTRALIA – STOCK STRUCTURE AND ADULT MOVEMENT

MA Steer, AJ Fowler, and BM Gillanders (Editors).

Final Report for the Fisheries Research and Development Corporation

FRDC Project No. 2007/029

SARDI Aquatic Sciences Publication No. F2009/000018-1
SARDI Research Report Series No. 333

ISBN 9781921563089

October 2009

Title: Spatial management of southern garfish (*Hyporhamphus melanochir*) in South Australia – stock structure and adult movement
Editors: MA Steer, AJ Fowler, and BM Gillanders.

South Australian Research and Development Institute
SARDI Aquatic Sciences
2 Hamra Avenue
West Beach SA 5024
Telephone: (08) 8207 5400
Facsimile: (08) 8207 5406
http://www.sardi.sa.gov.au

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

Printed in Adelaide: October 2009

Reviewers: Dr Craig Noell, Dr Milena Fernandes and an anonymous external referee

Approved by: Dr Tim Ward, Principal Scientist: Wild Fisheries, SARDI Aquatic Sciences

Signed: 

Date: 12 October 2009

Circulation: Public Domain

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

2007/029	**Spatial management of southern garfish (*Hyporhamphus melanochir*) in South Australia – stock structure and adult movement.**

PRINCIPAL INVESTIGATOR: Dr A. Fowler
ADDRESS: SARDI (Aquatic Sciences)
PO Box 120
Henley Beach SA 5022
Telephone: (08) 8207 5400

OBJECTIVES:

1. To determine the spatial scale and geographic nature of population structuring of southern garfish (*Hyporhamphus melanochir*) in South Australia using a number of otolith-based techniques; and
2. To utilise this information to develop a spatial management model for the South Australian fishery.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

PIRSA Fisheries have adopted the new spatial management units that were detailed in this report. SARDI's fisheries modelling team has also amended the garfish (GarEst) model that was developed as part of FRDC project no. 1999/145 (McGarvey and Feenstra (2004)) to align with the smaller management units.

Understanding stock structure is fundamental to developing the best protocol for spatial management of a fishery or stock. For southern garfish, *Hyporhamphus melanochir*, a recent genetic study revealed four regional stocks across southern Australia, i.e. Western Australia, west coast of South Australia, the South Australian gulfs and Victoria, and Tasmania, but no genetic differentiation between Gulf St. Vincent and Spencer Gulf populations. Historically, the stock assessment and management approach for this species has been to treat the two regional, gulf populations as separate, independent and self-recruiting. This division was based on the assumption that the two gulfs are considered semi-enclosed systems and only modest levels of intermixing between the two gulfs is required to homogenise the genetic stock. However, in reality the patterns of movement and migration both within and between the two gulfs are unknown. Thus, it is not known the extent to which such movement helps sustain different regional populations and the extent to which these are independent and discrete is poorly understood.

In recent years there have been significant concerns and management issues with this fishery as a result of a rapid decline in commercial catch rates that occurred from 2001 to 2003. This led to the formation of the Garfish Working Group, which involved representatives from the commercial and recreational fishing sectors, fishing industry representatives, fishery managers and scientists, to determine the extent of the problem and to propose possible management solutions. This group identified that the level of understanding of the life-history of garfish was poor, particularly with respect to movement patterns of the adult fish and their consequences for stock structure. This project aimed to rectify this lack of knowledge and understanding for southern garfish, so that management of the South Australian fishery can be applied at the appropriate spatial scale.

This study adopted a combined approach to delineate potential garfish sub-populations, and determine the extent of mixing within South Australian coastal waters, through the integration of multiple otolith-based techniques. Fish otoliths ('ear-bones') can be used as natural tags or 'fingerprints' as their chemistry, shape and internal structure are influenced by the ambient environment. Therefore fish living in different environments can be discriminated on the basis of the chemical or physical properties of their otoliths. This study examined regional, age-related, differences in the chemical composition of the otolith, in terms of their trace element (Chapter 2) and stable isotope (Chapter 3) concentrations, as well as exploring discrepancies in their overall shape (Chapters 4 and 5) and internal microstructure (Chapter 5). The combined results indicated that the population structuring of garfish is more complex than previously assumed and suggests that stocks can be discriminated at a much finer spatial scale.

Spatial differences in otolith chemistry and morphometrics indicated that there were several groups of garfish that had spent significant parts of their lives in different environments and that there was some level of restriction that prevented complete mixing among the regions. At least five regional divisions were identified in this study, each exhibiting various levels of intermixing, but which can be considered semi-discrete. Three of these were clearly defined as they exhibited negligible levels of inter-regional mixing: (1.) the West Coast (west of Marine Fishing Area 28); (2.) Northern Spencer Gulf (MFAs 19-23); and (3.) South-Western Spencer Gulf (MFAs 29-33). The remaining two, however, were not as distinct from each other; (4.) Northern Gulf St. Vincent (MFAs 34-36) and (5.) Southern Gulf St. Vincent (MFAs 40-44), but demonstrated a level of population structuring that would regard them as separate as a precautionary measure. This level of population discrimination is sufficient to suggest that the current management framework of two discrete, gulf-specific stocks should be restructured to align with these five smaller, semi-discrete, regional units.

These findings have provided a basis for PIRSA Fisheries managers to amend the current management arrangements for South Australia's southern garfish fishery. PIRSA Fisheries have adopted the new spatial management units that were detailed in this report. SARDI's fisheries modelling team has also amended the GarEst model that was developed as part of FRDC project no. 1999/145 (McGarvey and Feenstra (2004)) to align with the smaller management units. In doing so, the parameter estimates have improved and we have a better understanding of the spatial scale over which population processes occur. This has lead to more accurate estimates of output parameters and to an improved assessment regarding the current status of the garfish stock and its future management.

KEYWORDS: Hemiramphidae, otolith, trace elements, stable isotopes, morphometrics, population structure.

Acknowledgements

We gratefully acknowledge the Fisheries Research and Development Corporation for providing the base funds to carry out this project (2007/029). We also thank SARDI and the University of Adelaide for the logistic and administrative support through the course of the project, including the use of laboratories and access to facilities.

We thank Matt Lloyd (SARDI), Bruce Jackson (SARDI), David Schmarr (SARDI) and Jason Earl (SARDI/Flinders University) for valuable field and laboratory assistance. Dr Andrew Munro (University of Adelaide) and Angus Netting (Adelaide Microscopy) provided advice on the laboratory procedures associated with LA-ICP MS. We are particularly grateful for Dr Munro's help with reprocessing the obtained elemental data. Dr Benjamin Walther (University of Adelaide) demonstrated how to operate the micro mill and offered regular assistance. Dr Galen Halverson's (University of Adelaide) knowledge and enthusiasm for otolith carbonate and stable isotope analysis was unwavering and sincerely appreciated. Thanks also to commercial marine scalefish fishers, particularly Adam Barnes, Neil Evans, Glen Florance, Neil Schmucker and Mike Slattery for providing samples, sharing their extensive knowledge and allowing us to work with them at sea. Dr Keith Jones (PIRSA Fisheries) provided additional historic information that significantly aided the interpretation of the results. Sunil Sharma (SARDI) and Annette Doonan (SARDI) constructed the maps. Dr Carolyn Stewardson (FRDC) provided advice on formatting. The photo of the garfish used on the front cover was supplied by Brian Saunders.

This report was reviewed by Dr Craig Noell (SARDI) and Dr Milena Fernandes (SARDI) and an anonymous reviewer nominated by FRDC. This report was formally approved for release by Dr Tim Ward, Wild Fisheries Principal Scientist, SARDI Aquatic Sciences.

LIST OF ACRONYMS

ABARE	Australian Bureau of Agricultural and Resource Economics
ANOVA	Analysis of Variance
AUD	Australian Dollars
DFA	Discriminant Function Analysis
EFA	Elliptical Fourier Analysis
FRDC	Fisheries Research and Development Corporation
GSV	Gulf St. Vincent
KI	Kangaroo Island
LA-ICPMS	Laser Ablation-Inductively Coupled Plasma Mass Spectrometer
MANOVA	Multivariate Analysis of Variance
MSF	Marine Scalefish Fishery
MSFMC	Marine Scalefish Fishery Management Committee
Nd:YAG	Neodymium-doped: Yttrium Aluminium Garnet
NGSV	Northern Gulf St. Vincent
NIST	National Institute of Standards and Technology
NSG	Northern Spencer Gulf
PIRSA	Primary Industry and Resources South Australia
PO.DAAC	Physical Oceanography Distributed Active Archive Centre
SA	South Australia
SARDI	South Australian Research and Development Institute
SG	Spencer Gulf
SST	Sea Surface Temperature
SWGSV	South Western Gulf St. Vincent
SWSG	South Western Spencer Gulf
VPBD	Vienna Peedee Belemnite
WC	West Coast

1 GENERAL INTRODUCTION

AJ Fowler and MA Steer

1.1 Overview

This is the final report to the Fisheries Research and Development Corporation (FRDC) project 2007/029 *"Spatial management of southern garfish (*Hyporhamphus melanochir*) in South Australia – stock structure and adult movement"*. The report is divided into six chapters. This chapter (Chapter 1) is the General Introduction that outlines the structure of the report, provides background information relevant to the project and presents the main objectives of the study.

Chapters 2 and 3 determine the level of structure within South Australia's garfish population through chemical analysis of adult otoliths, focussing specifically on spatial variation in trace element concentration and stable isotopic values, respectively. Chapter 4 infers stock structure through otolith shape analysis and morphometrics. Chapter 5 investigates spatial variation in otolith growth through the analysis of the otolith microstructure in juvenile garfish. Chapter 6 is the General discussion. Collectively, this report integrates all otolith data into a single analysis to develop a 'stock structure' model for garfish in South Australia, discusses relevant implications for management and outlines future research directions.

1.2 Background

1.2.1 South Australia's garfish fishery

The southern garfish (*Hyporhamphus melanochir*) is one of the most significant inshore fishery species of southern Australia, with fisheries in Victoria, Tasmania, South Australia and Western Australia. Historically, the national commercial catch for this species has been dominated by that from South Australia where the catch has usually exceeded 400 t per annum, with an approximate value of AUD$2 million (ABARE 2008). The recent commercial catches in both Victoria and Tasmania have also been significant at marginally less than 100 t per State, but lower in Western Australia at less than 50 t. This species is also a favourite with the recreational sector, which in 2000/01 provided catches of 133 t in South Australia, 25.5 t in Victoria, 45 t in Western Australia and <2 t in Tasmania (Henry and Lyle 2003).

In South Australia, the garfish fishery is principally located in Spencer Gulf and Gulf St. Vincent (Fig. 1.1) and is managed as part of the multi-species, multi-gear Marine Scalefish Fishery (MSF) through a complex of input and output controls. Licensed commercial fishers target garfish using power haul nets (30-32 mm mesh and up to 600 m in length) and dab nets. Haul net fishers account for the majority of the commercial catch and their fishing activities are restricted by regulation to waters <5 m depth. There are also many areas around the State that are either permanently or seasonally closed to net fishing. Recreational fishers are also permitted to use dab nets but predominantly use traditional hook and line and fish from boats and shore-based platforms throughout the State. Current output controls for garfish caught in South Australia include a minimum legal length of 230 mm total length and a recreational bag and boat limit of 60 and 180 fish, respectively. Commercial catches from both gulfs are similar, whereas recreational landings are higher in Gulf St. Vincent as a consequence of a greater number of recreational fishers residing in metropolitan Adelaide (McGarvey et al. 2006).

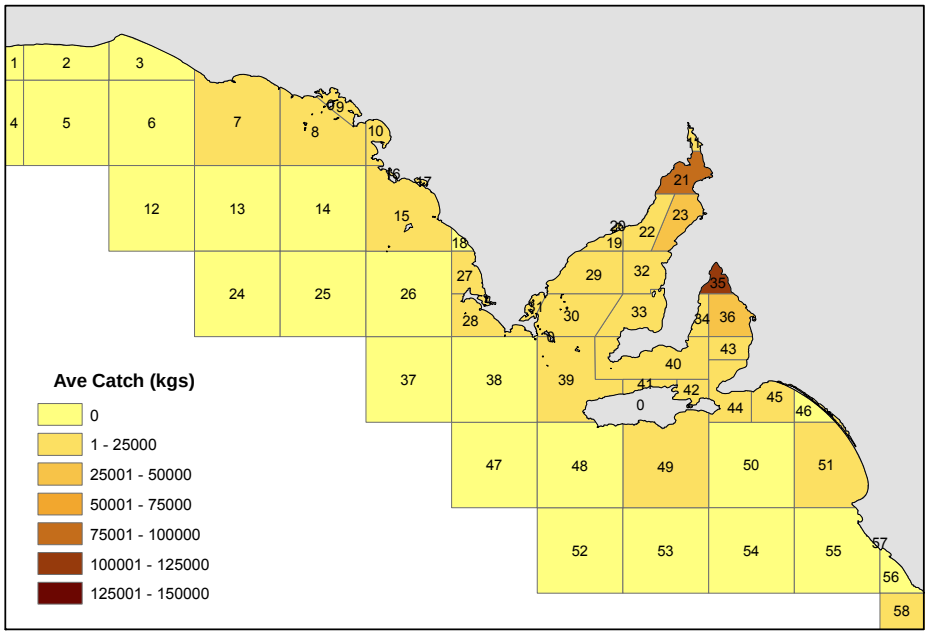

Figure 1.1. Spatial distribution of catches in South Australia's commercial garfish fishery averaged from 2005/06 to 2007/08. Numbered boxes delineate Marine Fishing Areas.

With an estimated total catch of 500-600 t per year across both sectors in South Australia, clearly the southern garfish is a prized and invaluable natural resource and is currently considered one of the four priority species within the commercial MSF, along with King George whiting (*Sillaginodes punctata*), snapper (*Chrysophrys auratus*) and calamary (*Sepioteuthis australis*). In recent years there have been significant concerns and management issues with the garfish fishery as a result of a dramatic decline in commercial catch rates that occurred from 2001 to 2003 (McGarvey et al. 2006). A critical management

response was subsequently initiated that culminated in a net buy-back scheme that reduced net fishing effort by approximately 45%, as well as permanently closing large parts of the State's inshore waters to commercial net fishing. During 2005/06 and 2006/07 the State committed approximately $436,000 towards collecting new biological information to assist in producing a detailed stock assessment to ensure our understanding of the stock status. Furthermore, the Marine Scalefish Fishery Management Committee (MSFMC) formed the Garfish Working Group to determine the extent of the problem and to propose possible and alternate management solutions. This group involved representatives from the commercial and recreational sectors, fishing industry representatives, fishery managers and scientists. One of the most significant revelations that emerged from the discussions of the MSFMC was that there was no understanding of the movement patterns of the southern garfish. As such, the consequences of adult movement on the stock structure of this species were unknown.

1.2.2 Stock discrimination

Understanding stock structure is fundamental to developing the best protocol for spatial management of a fishery or stock. For southern garfish a recent genetic study had revealed that there was evidence for only four regional stocks across southern Australia, i.e. Western Australia, the west coast of South Australia, the South Australian gulfs and Victoria, and Tasmania (Donnellan et al. 2002). As such, there was no genetic differentiation apparent between the populations of Gulf St. Vincent and Spencer Gulf. This is consistent with the view held by commercial fishing representatives on the Garfish Working Group who suggested that schools of garfish migrate between the gulfs. Such movement would therefore provide significant opportunity for the mixing of fish within and between the two gulfs. Yet, despite this, the current stock assessment and management approach for this species has been to treat the two regional, gulf populations as separate, independent, and self-recruiting. Clearly, this indicates that there is a lack of understanding of movement and migration of southern garfish that limits our understanding of the life history and stock structure of the species. Such knowledge is essential for effective and efficient fishery management, as indicated in the South Australian Marine Scalefish Fishery Management Plan (Noell et al. 2006).

Addressing the issues of fish movement and stock structure for marine scalefish species is often done with tag/recapture studies that use external tags (see Hancock 1989). However, there are several reasons why such studies are unlikely to be tractable for garfish in South Australia. First, such studies rely on there being a good spatial coverage of fishing effort in order to provide the tag returns. However, netting closures that were introduced in 2005 have now eliminated the possibility of obtaining tag returns from large parts of the State's waters

that are of interest for garfish. Second, garfish would be a difficult species to work with using traditional tag/recapture methods, as they are extremely fragile and highly susceptible to post-release mortality (Jones et al. 2002). In such circumstances alternative and less direct methods are required to determine fish movement. Numerous researchers around the world are now using the information that is locked away in the chemistry and structure of the otoliths of fish to address issues of stock structure and fish movement (Campana 1999).

The otoliths of fish are natural chronometers that record an extraordinary amount of information about the life history of fish and the environments they have experienced throughout their lives (Campana 1999, Panfili et al. 2002). They grow on a daily basis at a rate determined by the environmental conditions in which the fish is living at that time (Campana and Neilson 1985). As they grow, the otoliths incorporate various trace elements and isotopes into their crystalline structure at rates that are determined by the local ambient concentrations, water temperature and salinity (Elsdon and Gillanders 2002, 2003, 2004). Furthermore, the shape of otoliths can differ among conspecific fish that experience different environmental conditions (Begg and Brown 2000). Thus, otoliths can act as natural tags or 'fingerprints', that can be used to determine patterns of movement between different water masses and to relate fish back to the places where they originated (Gillanders and Kingsford 1996, Thorrold et al. 1998, Campana et al. 2000). This approach was used in several recent studies on snapper in both South Australia (Fowler et al. 2005) and Victoria (Hamer et al. 2003), which identified the places of origin and described their age-related dispersion to other regions extending over distances of up to several hundred kilometres. Such information was valuable for the determination of stock structure and the formulation of appropriate management protocols. This study will adopt similar principles aiming to access and interpret the information retained in the otoliths to determine the spatial scale over which southern garfish move, and the consequences of such movement for stock structure in South Australia. This will be specifically achieved through the examination of regional, age-related, differences in the chemical composition of garfish otoliths, in terms of their trace element and stable isotope concentrations, as well as exploring discrepancies in their overall shape and internal microstructure.

1.2.3 South Australia's physico-chemical oceanography

The success of studies using otolith chemistry to infer population structure and movement patterns depends on the physico-chemical characteristics of regional waters being sufficiently different to produce distinctive chemical signatures (Fowler et al. 2004). Temperature and salinity are the principle environmental factors that govern otolith chemistry (Campana 1999,

Elsdon and Gillanders 2002, 2003, 2004) and these two factors vary considerably in South Australia's coastal waters (Middleton and Bye 2007). This variation is largely due to the coastline's complex geography, penetrating waters of the Southern Ocean and seasonal meteorology (Middleton and Bye 2007).

South Australia's coastline is dominated by two large, relatively shallow gulfs; i.e. Spencer Gulf and Gulf St. Vincent. Spencer Gulf, the larger of the two gulfs, is a triangular body of water approximately 300 km long and 80 km wide at its mouth. Gulf St. Vincent is also triangular in shape and is 210 km long and 45 km wide (Petrusevics 1993). Both gulfs are subjected to the same weather conditions throughout the year and are influenced by similar oceanographic processes. They both exhibit extreme seasonal shifts, where they are semi-enclosed bodies of water in summer that dissipate and become mixed by the penetrating continental shelf waters in winter (Middleton and Bye 2007). These regime shifts are influenced by a combination of prevailing winds, which are predominantly from the south-east in summer and south-west in winter, and the formation of strong sea surface temperature and salinity fronts (Nunes Vaz et al. 1990; de Silva Samarasinghe 1998). The frontal systems typically develop at the mouths of the gulfs in late spring, persist throughout summer and collapse late autumn (Petrusevics 1993) (Fig. 1.2). Both gulfs are also substantially more saline than surrounding shelf waters because of high evaporation rates, low precipitation and lack of inflow of fresh water from creeks and rivers (Fig. 1.3). As a result, each exhibits a latitudinal temperature and salinity gradient increasing northwards (Petrusevics 1993), a feature that classifies them as inverse estuaries (Nunes Vaz et al. 1990). Smaller, semi-enclosed bays located on the west coast of the State (e.g. Baird Bay and Venus Bay) also exhibit similar inverse estuarine characteristics (Jones 1990).

There are numerous population centres situated along the coastlines of both gulfs that are also likely to alter local water chemistry as a result of anthropogenic activities. Areas of high industrial activity, such as metropolitan Adelaide in Gulf St. Vincent and Whyalla, Port Broughton and Port Pirie in northern Spencer Gulf are known to be significant point sources of heavy metals (Edwards et al. 2001). The gulfs also act as sinks for a variety of coastal urban wastes including treated effluent, sludge, rural storm-water runoff, and thermal waste (McLaren and Wiltshire 1984). The water chemistry in South Australia's coastal environments is therefore likely to be sufficiently different to produce detectable differences in fish otoliths.

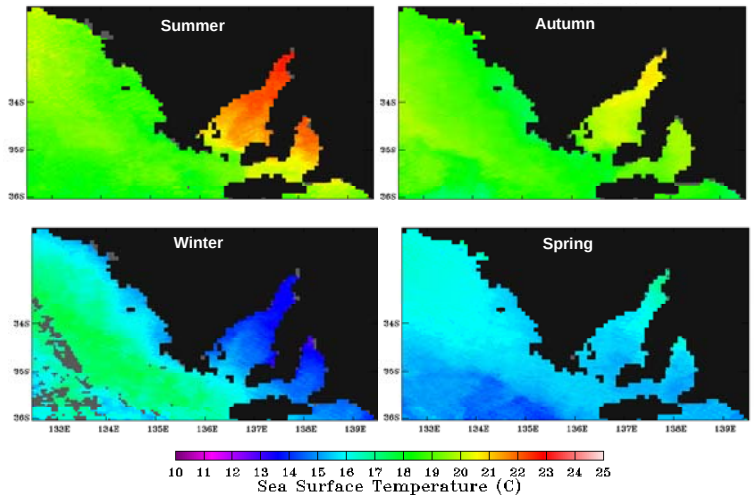

Figure 1.2. South Australia's mean seasonal sea surface temperature derived from satellite imagery http://podaac.jpl.nasa.gov/poet.

Figure 1.3. Schematic composite of estimates of salinity in South Australia acquired from published sources, i.e. Nunes and Lennon (1986) (black isohalines), Nunes Vaz et al. (1990) (grey isohalines), Bye and Kämpf (2008) (stippled isohalines), Jones (1990) (West Coast estimate).

1.3 Need

Knowledge of adult fish movement and stock structure is fundamental to identifying the appropriate spatial scale at which the processes of population replenishment work, and thus the spatial units to which fishery management should be applied. The management of the garfish fishery of South Australia has recently been at a heightened sensitivity due to serious concerns about the sustainability of the resource. Yet, a comprehensive understanding of fundamental aspects of the life history and population biology of this species is lacking, which significantly impedes identifying the most appropriate spatial management structure. There is a lack of understanding of the movement patterns of the adult fish, and the influence of that movement on the stock structure. Thus, it is not known the extent to which such

movement helps sustain different regional populations and the extent to which these populations are separated. There is a need to rectify this lack of knowledge and understanding for southern garfish, so that the management of the South Australian fishery can be applied at the appropriate spatial scale.

1.4 Objectives

There are two overall project objectives. These are:

1. To determine the spatial scale and geographic nature of population structuring of southern garfish (*Hyporhamphus melanochir*) in South Australia using a number of otolith-based techniques; and
2. To utilise this information to develop a spatial management model for the South Australian fishery.

1.5 References

ABARE (2008) *Australian Fisheries Statistics 2007*, Canberra, June.

Begg, G.A., and Brown, R.W. (2000) Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. *Transactions of the American Fisheries Society* 129: 935-945.

Bye J.A.T., and Kämpf, J. (2008) Physical Oceanography. *In:* Shepherd, S.A., Bryars, S., Kirkegaard, I., Harbison, P., and Jennings, J.T. (eds) Natural History of Gulf St. Vincent. Royal Society of South Australia. 496 pp.

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

Campana, S.E., Chouinard G.A., Hanson J.M. and Fretchet A. (2000). Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research* 46: 343-357.

Campana, S.E. and Neilson, J.D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences.* 42: 1014-1032.

Donnellan S., Haigh L., Elphinstone M., McGlennon D. and Ye, Q. (2002) Genetic discrimination between southern garfish (*Hyporhamphus melanochir*) stocks of Western Australia, South Australia, Victoria and Tasmania. In 'Fisheries biology and habitat ecology of southern garfish (*Hyporhamphus melanochir*) in southern Australian waters. (eds. Jones, G.K., Ye, Q., Ayvazian, S., Coutin, P.). FRDC Final Report 97/133.

Edwards J.W., Edyvanne K.S., Boxall V.A, Hamann M., and Soole K.L. (2001). Metal levels in seston and marine fish flesh near industrial and metropolitan centres in South Australia. *Marine Pollution Bulletin* 42: 389-396.

Elsdon T.S., and Gillanders B.M. (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.

Elsdon T.S., and Gillanders B.M. (2003) Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecology Progress Series* 260, 263-272.

Elsdon T.S., and Gillanders B.M. (2004) Fish otolith chemistry influenced by exposure to multiple environmental variables. *Journal of Marine Biology and Ecology* 313: 269-284.

Fowler, A.J., Gillanders B.M. and Hall K.C. (2004) Adult migration, population replenishment and geographic structure for snapper in South Australia. FRDC Final Report 2002/001, 152 pp.

Fowler, A.J., Gillanders B.M. and Hall K.C. (2005). Relationship between elemental concentration and age from otoliths of adult snapper (*Pagrus auratus*, Sparidae): implications for movement and stock structure. *Marine and Freshwater Research* 56: 661-676.

Gillanders, B.M., Able, K.W., Brown J.A., Egglestone D.B. and Sheridan P.F. (2003). Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. *Marine Ecology Progress Series* 247: 281-295.

Gillanders, B.M. and Kingsford M.J. (1996). Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. *Marine Ecology Progress Series* 141: 13-20.

Hamer, P.A., Jenkins G.P., Gillanders B.M. (2003). Otolith chemistry of juvenile snapper *Pagrus auratus* in Victorian waters: natural chemical tags and their temporal variation. *Marine Ecology Progress Series* 263: 261-273.

Hancock, D. A. (1989). (Ed.) Tagging - Solution or Problem? Proceedings No. 5 Australian Society for Fish Biology Tagging Workshop, 21-22 July, Sydney 1988. 208 pp.

Henry G.W., and Lyle J.M. (2003) The National Recreational and Indigenous Fishing Survey. FRDC Final Report 23/249. 105 pp.

Jones, G.K. (1990). Growth and mortality in a lightly fished population of garfish (*Hyporhamphus melanochir*), in Baird Bay, South Australia. *Transactions of the Royal Society of South Australia.* 114(1): 37-45.

Jones G.K., Ye Q., Ayvazian S. and Coutin P.C (2002) Fisheries Biology and Habitat Ecology of Southern Sea Garfish (*Hyporhamphus melanochir*) in South Australian Waters. In 'Fisheries biology and habitat ecology of southern garfish (*Hyporhamphus melanochir*) in southern Australian waters. (eds. Jones, G.K., Ye, Q., Ayvazian, S., Coutin, P.). FRDC Final Report 97/133. 322pp.

McGarvey R., Fowler A.J., Feenstra J.E., and Jackson W.B. (2006) Garfish (*Hyporhamphus melanochir*) Fishery. Fishery Assessment Report to PIRSA. SARDI Aquatic Sciences Publication No. RD04/0152-2, 55pp.

McLaren N.E., and Wiltshire, D.J. (1984). Northern Spencer Gulf marine biology study. Report for the South Australian Department of Environment and Planning.

Middleton, J.F., and Bye J.A.T. (2007). A review of the shelf-slope circulation along Australia's southern shelves: Cape Leeuwin to Portland. *Progress in Oceanography* 75: 1-41.

Noell C., Presser J. and Jones K. (2006) Management Plan for the South Australian Marine Scalefish Fishery. PIRSA. South Australian Fisheries Management Series No. 45, 68 pp.

Nunes Vaz, R.A. and Lennon, G.W. (1986). Physical property distributions and seasonal trends in Spencer Gulf, South Australia: an inverse estuary. *Australian Journal of Marine and Freshwater Research.* 37: 39-53.

Nunes Vaz, R.A., Lennon G.W., and Bowers D.G. (1990). Physical behaviour of a large, negative or inverse estuary. *Continental Shelf Research*. 10(3): 277-304.

Panfili, J., Pontual, H. (de)., Troadec, H. Wright P.J. (eds), (2002). *Manual of Fish Sclerochronology*. Brest, France: Ifremer-IRD coedition: 464pp.

Petrusevics, P.M. (1993). SST fronts in inverse estuaries, South Australia - Indicators of reduced gulf-shelf exchange. *Australian Journal of Marine and Freshwater Research* 44: 305-323.

Thorrold, S.R., Jones C.M., Swart P.K., and Targett P.E. (1998). Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. *Marine Ecology Progress Series* 173: 253-265.

2 AGE-RELATED MOVEMENT PATTERNS AND POPULATION STRUCTURING IN SOUTHERN GARFISH (*HYPORHAMPHUS MELANOCHIR*) INFERRED FROM OTOLITH CHEMISTRY.

MA Steer, AJ Fowler, BM Gillanders.

2.1 Introduction

In recent years, otolith chemistry has been used to address issues of stock structure and adult movement (reviewed in Campana 2005). Otoliths are natural chronometers that record an extraordinary amount of information about the life history of fish and the environments that they have experienced throughout their lives (Campana 1999). They grow continually at variable rates determined by endogenous and exogenous factors. As they grow, the otoliths incorporate a suite of trace elements into their crystalline structure at rates determined by the local ambient concentrations, water temperature and salinity (Elsdon and Gillanders 2002). Otoliths also exhibit discernable growth bands which correspond to daily, seasonal or annual time frames from which fish can be accurately aged (Campana and Thorrold 2001). Because the chemical composition of seawater varies spatially and temporally and the otolith material is metabolically inert once deposited (Campana 1999), the otoliths retain a chronological record of the environments experienced by the fish throughout its life (Secor and Rooker 2000). It is therefore possible to discriminate among groups of fish that have spent at least parts of their lives in different environments on the basis of the chemical composition, or 'elemental fingerprint', of their otoliths (Gillanders and Kingsford 1996; Thorrold et al. 1997, Rooker et al. 2001, Fowler et al. 2005). It is also possible to retrospectively reconstruct age-related movement patterns and to disentangle their role in structuring stocks.

The physical and chemical oceanography of South Australian coastal waters is complex (Middleton and Bye 2007). The two gulfs are substantially more saline than surrounding shelf waters because of high evaporation rates, low precipitation and lack of inflow of fresh water from creeks and rivers. As a result, each exhibits a strong latitudinal temperature and salinity gradient increasing northwards (Petrusevics 1993), a feature that classifies them as inverse estuaries (Nunes Vaz et al. 1990). In addition, adjacent shelf waters are seasonally affected by longshore currents and wind-forced upwelling events that penetrate the mouths of the gulfs (Middleton and Bye 2007). Such diverse oceanographic processes along a relatively short stretch of the South Australian coastline bodes well for otolith chemistry studies. The overall objective of this study was to undertake trace element analysis of otoliths of garfish sampled at different spatial scales to infer patterns of movement, delineate potential sub-populations, and determine the extent of mixing within South Australian coastal waters.

2.2 Materials and Methods

2.2.1 Sample collection

A range of 7 - 24 adult garfish from the 2+ age class were collected in June–August 2007 from three sites within each of six regions along the South Australian coast: Northern Gulf St. Vincent (NGSV), South West Gulf St. Vincent (SWGSV), Kangaroo Island (KI), Northern Spencer Gulf (NSG), South West Spencer Gulf (SWSG) and the West Coast of Eyre Peninsula (WC) (Table 2.1, Fig. 2.1). Sites were separated by distances of <60 km and regions >60 km. Fish were dab-netted throughout the night from shallow (<4 m) protected bays characterised by sandy substrate interspersed with patches of algae and seagrass. Once captured, fish were placed into labelled, sealed, plastic bags, stored on ice and processed within 8 hr. At processing, both sagittae were removed using plastic forceps rinsed with deionised (Milli-Q) water, cleaned of adhering tissue, air-dried and stored in Eppendorf microcentrifuge tubes.

Table 2.1. The number of adult garfish (Age 2+) sampled for trace element analysis.

Region	Site	n
West Coast	Baird Bay	11
	Streaky Bay	20
	Venus Bay	17
Northern SG	Port Broughton	16
	Port Pirie	15
	Wood Point	24
South West SG	Louth Bay	10
	Proper Bay	15
	Tumby Bay	17
Northern GSV	Middle Beach	12
	Port Parham	14
	Port Wakefield	10
South West GSV	Edithburgh	18
	Port Vincent	10
	Stansbury	10
Kangaroo Is.	American River	9
	Emu Bay	8
	Shoal Bay	14

2.2.2 Otolith preparation

One otolith from each fish was randomly selected and prepared for analysis. Each otolith was embedded in epoxy (Struers epofix) resin that was doped with indium (In) at ~ 30 μg g^{-1} as a resin indicator. A transverse section of approximately 400 μm thickness was taken through the primordium of each otolith using a slow speed gem saw with twin diamond blades

(Struers Accutom – 2), continuously lubricated with Milli-Q water. Sections were polished down to the inner core on both sides with 9 μm then 3 μm aluminium oxide lapping film rinsed with Milli-Q water. The polished sections were triple rinsed in Milli-Q water. Twelve randomly selected sections were fixed to an acid-washed (10% HNO_3) microscope slide with indium doped (~ 200 μg g^{-1}) crystal bond® thermoplastic cement. Each slide was sonicated in Milli-Q water for 3 min, triple rinsed with Milli-Q water, and air-dried for 24 hr under a laminar flow hood, before being stored in separate sealed plastic bags.

2.2.3 Trace element analysis

Sections were analysed using a laser ablation inductively coupled plasma mass spectrometer (LA-ICP-MS) at Adelaide Microscopy, University of Adelaide. The system consisted of a New Wave UP-213 high performance (Nd:YAG) ultraviolet laser ablation system connected to an Agilent 7500cs ICP-MS. Each slide with 12 otolith sections was placed in a sealed perspex ablation chamber with a helium atmosphere (0.82 L min^{-1}) and viewed remotely via a microscope objective lens connected to a computer monitor. The laser was programmed to follow a transect path from the otolith core to the outer edge of the dorsal margin. This sampling axis was chosen as it generally provided clear opaque annuli. To eliminate any chance of surface contamination, all transects were pre-ablated using an 80 μm laser beam diameter, at a pulse rate of 5 Hz ablating continuously while the stage moved at 10 μm s^{-1}. After each pre-ablation the chamber was backfilled with Argon gas for ~ 2 – 3 mins at a rate of 0.1 L min^{-1} to eliminate any background gases that may have contained contaminants. The laser was re-programmed to sample along the pre-ablated scar with a refined beam diameter of 30 μm, at a pulse rate of 5 Hz, an energy of ~ 0.7 – 0.8 mJ and a scan speed of 5 μm s^{-1}. The concentrations of elements were recorded every 3.34 s. The 18 elemental isotopes chosen for analysis were ^{7}Li, ^{23}Na, ^{24}Mg, ^{25}Mg, ^{55}Mn, ^{56}Fe, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{85}Rb, ^{88}Sr, ^{115}In, ^{138}Ba, ^{139}La, ^{208}Pb as well as ^{43}Ca that was measured for use as the internal standard. Prior to each laser activation the elemental isotopes were measured in the blank sample gas for ~30 s. This allowed the sample chamber to stabilise and provided mean background counts of the analysed isotopes which were then subtracted from the sample counts for each ablation. The ablation chamber was purged with argon gas for 60 s after each transect.

To eliminate possible biases associated with instrument drift the sequence of otoliths analysed was randomised. Concentrations of elements were calibrated against the National Institute of Standards and Technology (NIST) 612 glass standard. This standard was analysed twice at the beginning of the sampling session and twice after every 12 ablations to further eliminate

short-term instrument drift by linear interpolation. Calcium concentration was assumed to be constant at 388,000 μg g^{-1}, based on the published values for a certified reference material of otolith (Yoshinaga et al. 2000). For an ablated crater size of diameter of 30 μm, the estimated detection limits (μg g^{-1}) for each of the screened elements was estimated as the quantity of analyte required to produce a signal equivalent to three times the standard deviation of the blank. These detection limits were estimated as: ^{7}Li 0.02, ^{23}Na 1.82, ^{24}Mg 0.06, ^{25}Mg 0.4, ^{43}Ca 79.26, ^{55}Mn 0.28, ^{56}Fe 35.7, ^{57}Fe 3.71, ^{59}Co 0.03, ^{60}Ni 0.09, ^{63}Cu 0.06, ^{64}Zn 0.11, ^{85}Rb 0.02, ^{88}Sr 0.10, ^{115}In 0.02, ^{138}Ba 0.01, ^{139}La 0.01, ^{208}Pb 0.04. All elemental data were expressed as molar ratios to ^{43}Ca. An Excel macro was used to reprocess elemental data and to subtract background measurements.

2.2.4 Relating trace element profile to fish age

The elemental concentration profiles were matched to fish age using the opaque zones in the otolith macrostructure as temporal references (Fowler et al., 2005). After each otolith was ablated using LA-ICPMS a digital image of the section was recorded using Optimas Pro Image Analysis software. Using this image, a transect was drawn adjacent to the ablation scar, and the width of each annual increment was measured along it, between the outer edges of the consecutive translucent zones. The elemental concentration profiles were then divided into three life-history stages; juvenile (first 50 μm of the transect), year 1, and year 2, according to the measured distance along the transect (Fig. 2.2). Only chemical results from the otolith core to the second opaque zone were considered.

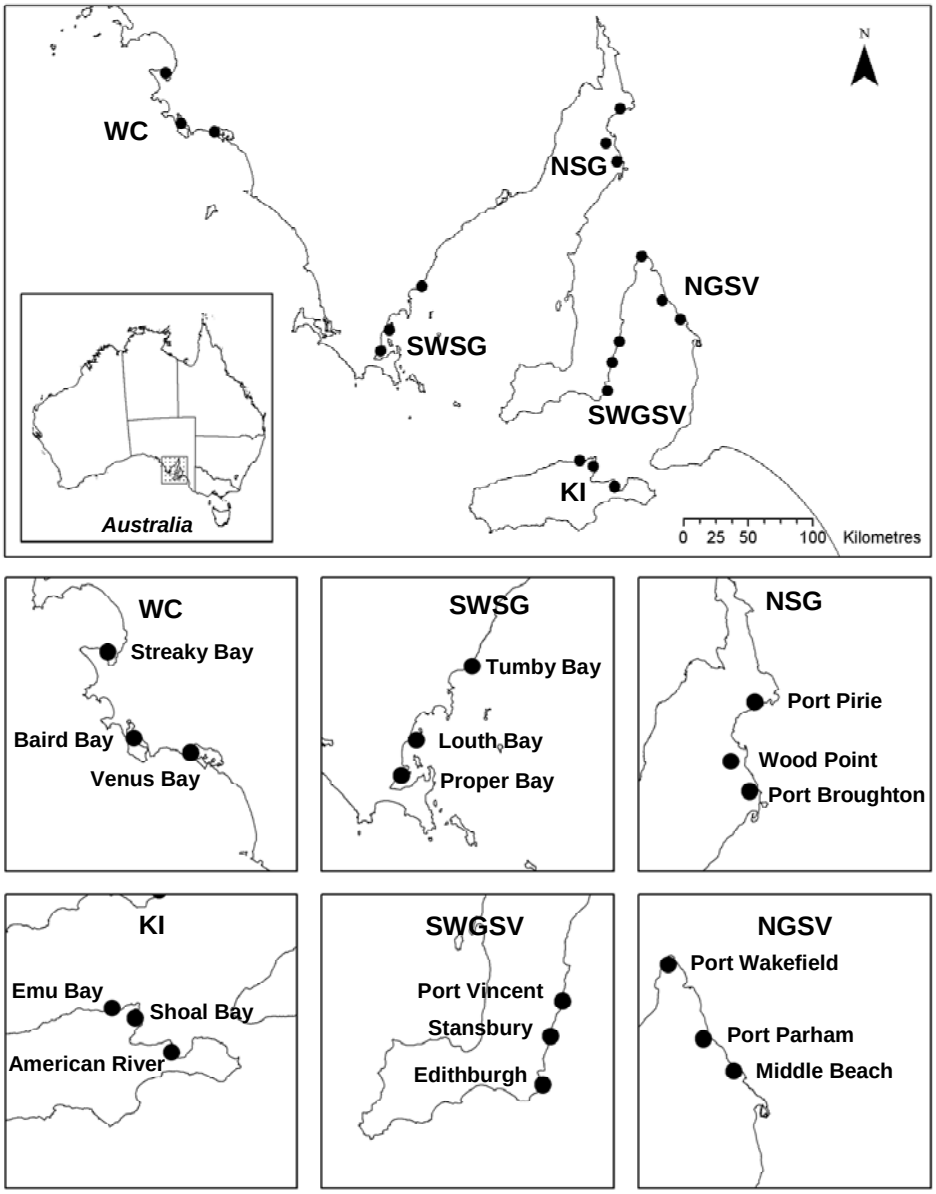

Figure 2.1. Map of South Australia showing the 18 sample sites within six regions: West Coast (WC); South West Spencer Gulf (SWSG); Northern Spencer Gulf (NSG); Kangaroo Island (KI); South West Gulf St. Vincent (SWGSV); and Northern Gulf St. Vincent (NGSV).

2.2.5 Data analyses

Of the 18 screened elements, Li, Mg, Mn, Sr, and Ba exceeded the detection limits of the analytical equipment and were, therefore, considered the most appropriate for subsequent analyses. The focus of the data analyses was to compare the age-related, otolith elemental profiles between sites nested within regions and among regions. To achieve this, it was necessary to simplify the transect data. The sequential elemental data from each otolith were smoothed with a 9-point running median followed by a 9-point running mean from which an age-related annual mean was calculated. This provided three age-related means, for each

otolith, one for the core of the otolith and the remaining two for each consecutive year in the life of the fish. The data for each element were analysed using a repeated-measures analysis of variance, comparing the age-related mean concentrations between the two spatial scales. The data were then combined across all five elements to form a multivariate dataset that was analysed using both a repeated-measures multivariate analysis of variance, and discriminant function analysis (DFA). Three variables required transformation to satisfy the assumptions of normality and homogeneity of variances. Li and Mg were both log-transformed according to $\log_{10}(X+1)$ and ^{138}Ba was inverse transformed according to $1/(X+1)$.

Three separate DFAs were carried out to assess whether the site of capture of each garfish could be reliably determined from the mean age-related trace element concentrations. The first DFA incorporated the mean concentration of each of the five elements measured within the juvenile portion of the otolith, the second from the whole of the first annulus, and the third incorporating the elemental concentrations from the second year of growth. These three functions were carried out separately to identify age-related differences in otolith chemistry. Due to the large number of predictors (five variables by three levels each), which exceeded the number of samples in the smallest group, a forward step-wise procedure of DFA was used to determine the most important predictors for the discriminant group membership. The entry of predictors to the analysis was determined by the statistical entry of Wilk's lambda (Λ) with a $p_{(entry)} = 0.05$ and $p_{(exit)} = 0.25$. The data were analysed separately for each increment to assess changes in the pattern of predicted group membership with increasing age. The performance of discriminant functions was evaluated using Cohen's Kappa (κ) statistic, which provides an objective means of calculating the chance-corrected percentage of agreement between actual and predicted group memberships. Values of κ range from 0 to 1, with 0 indicating the discriminant function yields no improvement over chance, and 1 indicating perfect agreement (Titus et al. 1984).

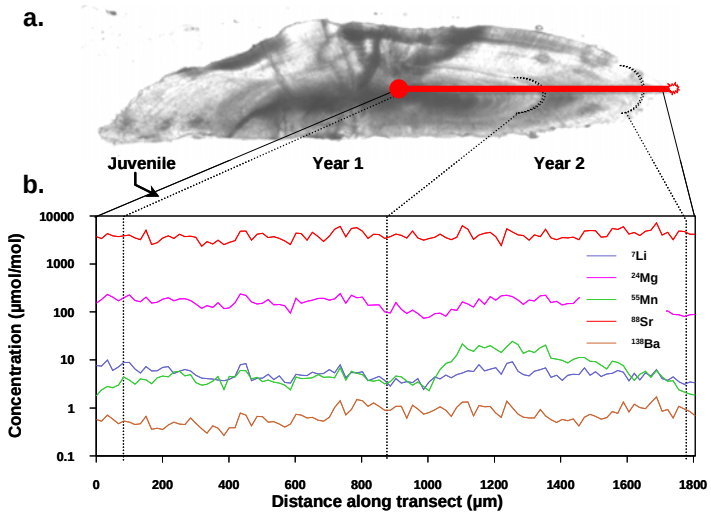

Figure 2.2. An example of the results obtained from LA-ICP-MS analysis (a.) transverse section of an age 2+ garfish sagitta indicating the sampling transect of the laser; (b.) continuous chemical profile of the five chosen trace elements along the chronological age structure of the otolith.

2.3 Results

2.3.1 Single element analyses

Garfish otoliths incorporated trace elements into their calcium carbonate structure at variable concentrations throughout the lifetime of the fish, as significant variation was detected in the age-related mean concentrations of Sr, Mn, Ba, Mg and Li (Table 2.2). The magnitude of this variation, however, was not consistent among the elements. Age-related inconsistency in chemical composition was evident among sites within regions for Li, Sr, Mn and Ba and among regions for Mg (Table 2.2). Sites within South West Spencer Gulf and Kangaroo Island displayed considerable variation in mean Li concentration, particularly during the fish's second year of growth (Fig. 2.3). Mean Sr concentration was inconsistent across all sites, with American River and Shoal Bay in Kangaroo Island and Port Broughton in Northern Spencer Gulf displaying the greatest age-related differences (Fig. 2.3). The highest average Sr levels were found in otoliths collected from Baird Bay, with concentrations that exceeded 4,500 µmol/mol. The otoliths from fish from Middle Beach in Northern Gulf St. Vincent and Port Pirie in Northern Spencer Gulf had higher mean concentrations of Mn during their second year of growth at 16.2 and 8.6 µmol/mol, respectively. Mean Mn concentration was <5.0 µmol/mol for the majority of the remaining sites (Fig. 2.3). Age-related differences in Ba concentration exceeded 1.5 µmol/mol in garfish otoliths collected from American River in Kangaroo Island, Port Broughton in South West Spencer Gulf and Port Wakefield in South West Gulf St. Vincent. Of these, mean Ba concentration was elevated in the juvenile portion of the otolith for American River fish, whereas concentrations were elevated within the

second annulus for garfish collected from the other sites (Fig. 2.3). Mean concentration of Mg was similarly elevated within the second annulus for all sampled fish, with the exception of those collected from Northern Spencer Gulf (Fig. 2.3).

Table 2.2. Results of repeated-measures analyses of variance testing for spatial differences in the age-related chemical concentration of garfish otoliths, for five separate trace elements. ns: not significant, *p < 0.05, ** p < 0.01, ***p < 0.001.

Source		Sr		Ba		Mn		Li		Mg	
	df	MS	F	MS	F	MS	F	MS	F	MS	F
Within-Subjects											
Age	2	1.40E+07	140.6***	0.06	5.45*	52.57	7.00**	0.01	1.88^{ns}	<0.01	42.70***
Age*Region	10	3.50E+06	3.50***	0.06	5.45***	54.03	7.20***	<0.01	6.59***	1.50E-05	2.11*
Age*Site(Region)	24	2.10E+05	2.10**	0.02	2.26*	20.8	2.77***	<0.01	1.89**	6.70E-06	0.92^{ns}
Error	444	9.90E+04		0.01		7.5		<0.01		7.20E-06	
Between-Subjects											
Region	5	6.10E+06	27.77***	0.13	4.83***	180.5	14.74***	0.11	9.20***	6.60E-05	3.88**
Site(Region)	12	2.50E+07	4.80***	0.05	1.70^{ns}	89.14	7.30***	<0.01	1.04^{ns}	1.90E-05	1.12^{ns}
Error	222	9.70E+07		0.02		12.2		0.01		1.70E-05	

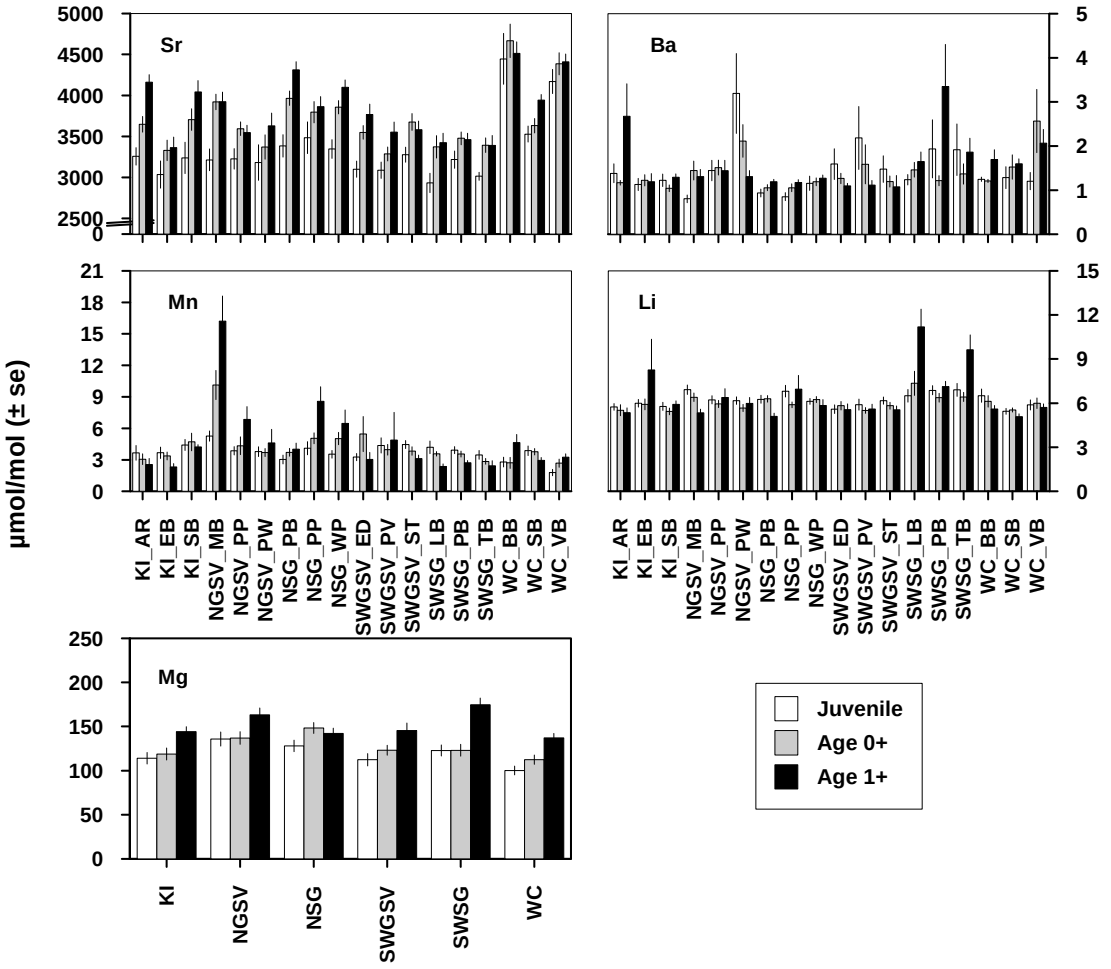

Figure 2.3 Age-related concentrations (µmol/mol relative to ^{43}Ca ± se) of Li, Mg, Mn, Sr, and Ba in southern garfish otoliths collected from 18 sites within six regions in South Australia.

2.3.2 Multi-element analyses

A multivariate analysis of variance, which combined the concentrations of all of the five trace elements into a single matrix, yielded similar results to the single element analysis, where otoliths had elements at various concentrations depending on their age and site (Table 2.3). Discriminant function analyses further confirmed this result by statistically separating sites on the basis of their discriminant function scores.

Table 2.3 Results of a repeated-measures multivariate analysis of variance testing for spatial, age-related differences in the chemical composition of garfish otoliths. ns: not significant, *p < 0.05, ** p < 0.01, ***p < 0.001.

Source	Value	df	df_{error}	F
Between-Subjects				
Region	0.91	25	218	9.87***
Site(Region)	0.74	60	1110	3.23***
Within-Subjects				
Age	0.71	10	213	50.84***
Age*Region	0.76	50	1085	3.91***
Age*Site(Region)	0.95	120	2220	1.94***

2.3.3 Fine-scale spatial discrimination

For the juvenile portion of the otolith four discriminant functions were described resulting in a combined χ^2 value of 203.47 (df = 68, p < 0.001). Of these, the first two functions accounted for 76.4% of the between-group variability. The first discriminant function separated Baird Bay and Venus Bay from the other sites (Fig. 2.4a), as well as separating Port Pirie in Northern Spencer Gulf from South West Spencer Gulf sites (Fig. 2.4c). Mean ^{88}Sr concentration was identified as the main element responsible for this separation. Mean ^{138}Ba concentration contributed to the separation of Middle Beach from Port Wakefield in Northern Gulf St. Vincent on the second discriminant function axis (Fig. 2.4b). With the exception of these two sites garfish collected from the remaining sites within Gulf St. Vincent had otoliths with similar chemical profiles. Such similarity, as illustrated by the considerable overlap of the 95% confidence ellipses, compromised the model's classification success with only 23.3% of the otoliths being correctly assigned to the site from where they had been sampled. According to Cohans' kappa statistic (κ) this classification was 16% better than chance. Classification success was greatest for Wood Point (NSG) and Venus Bay (WC) at 54% and 53%, respectively (Table 2.4).

Four discriminant functions were described for the otolith material that related to the Age 1 part of the otoliths, with a combined χ^2 value of 261.91 (df = 68, $p < 0.001$). The first two functions explained 55.5% and 26.2% of the between-group variability, respectively. The first discriminant function separated Baird Bay and Venus Bay from the other sites (Fig. 2.4d), and Northern Spencer Gulf sites from South West Spencer Gulf sites (Fig. 2.4f). Mean Sr concentration remained the main element responsible for this separation. Mean Mn concentration contributed to greater separation of Middle Beach from the other sites in the second discriminant function and also contributed to the separation of the two Spencer Gulf regions (Fig. 2.4e, f). With the exception of Middle Beach, the chemical profiles of otoliths collected from the Gulf St. Vincent and Kangaroo Island sites were similar. The model's classification success did not improve, marginally declining to 22.5% (κ = 17%). Classification success exceeded 50% for Port Wakefield, Middle Beach and Baird Bay (Table 2.5).

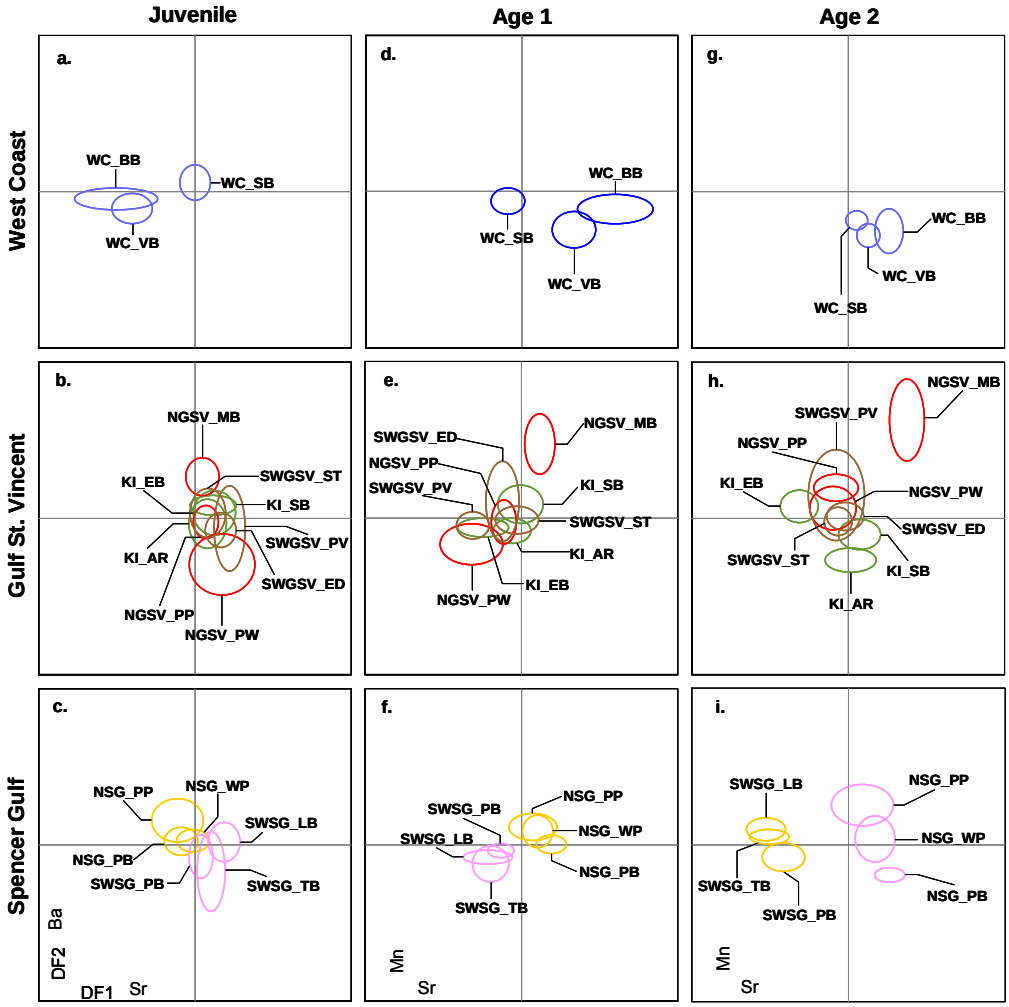

Figure 2.4. Age-related discriminant function centroids with 95% confidence ellipses for southern garfish otoliths collected from 18 sites within six regions in South Australia, based on five trace elements (Li, Mg, Mn, Sr, and Ba). Figures were separated for the West Coast, Spencer Gulf and Gulf St. Vincent for clarity.

The third discriminant function analysis, which incorporated otolith elemental concentrations from the second year of growth, was described by five discriminant functions with a combined χ^2 value of 412.10 (df = 85, $p < 0.001$). The first two functions accounted for 39.1% and 33.6% of the between group variability, respectively. Compared to the previous analyses this one produced the greatest separation between the sites (Fig. 2.4g). Middle Beach remained clearly separated from the rest of Gulf St. Vincent and considerable separation was observed between the three Kangaroo Island sites (Fig. 2.4h). Mean concentration of Mn accounted for the majority of this divergence. South West Gulf St. Vincent sites remained similar to each other. The north-south Spencer Gulf division was further emphasised and the three sites within Northern Spencer Gulf had increased in their dissimilarity (Fig. 2.4i). The magnitude of this dissimilarity reflected a latitudinal gradient which was primarily driven by differences in the mean concentration of ^{88}Sr.

The level of classification of individuals to the site from which they originated was higher at 32.1% ($\kappa = 22\%$). Considerable variation remained among the sites. In many cases garfish were incorrectly classified as originating from either an adjacent site or from one in close proximity (e.g. 31% of garfish collected from Port Broughton were statistically assigned to Wood Point, which is <20 km north) (Fig. 2.1; Table 2.6). Some individuals were classified as being broadly distributed among both gulfs. For example, 60.5% of the fish collected from Edithburgh in South West Gulf St. Vincent were incorrectly assigned to sites within Spencer Gulf (Table 2.6).

Table 2.4. Classification success of the discriminant function analysis incorporating all five trace element concentrations within the core (juvenile) of the garfish otolith. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the 18 sites (columns).

Juveniles	WC_BB	WC_SB	WC_VB	SWSG_LB	SWSG_PB	SWSG_TB	NSG_PB	NSG_PP	NSG_WP	SWGSV_ED	SWGSV_PV	SWGSV_ST	KI_AR	KI_EB	KI_SB	NGSV_MB	NGSV_PP	NGSV_PW
WC_BB	**45**	18	18	0	9	0	0	0	9	0	0	0	0	0	0	0	0	0
WC_SB	0	**30**	5	0	0	0	5	0	30	5	0	0	0	0	15	5	0	5
WC_VB	18	0	**53**	0	0	0	6	6	6	12	0	0	0	0	0	0	0	0
SWSG_LB	0	0	0	**0**	0	0	0	0	70	10	0	0	0	0	10	10	0	0
SWSG_PB	0	7	0	0	**7**	0	0	13	53	0	0	0	0	0	0	7	0	13
SWSG_TB	0	0	0	0	14	**0**	0	14	43	0	0	0	0	0	0	0	0	29
NSG_PB	0	6	19	0	6	0	**19**	13	31	6	0	0	0	0	0	0	0	0
NSG_PP	7	7	7	0	0	0	13	**33**	20	7	0	0	0	0	0	7	0	0
NSG_WP	4	13	8	4	0	0	0	4	**54**	0	0	0	0	0	4	0	0	8
SWGSV_ED	0	11	0	0	0	0	0	0	39	**28**	0	0	0	0	0	0	0	22
SWGSV_PV	0	0	0	0	0	0	0	0	10	30	**0**	0	0	0	10	20	0	30
SWGSV_ST	0	30	0	0	0	0	0	0	40	0	0	**0**	0	0	10	10	0	10
KI_AR	0	22	11	0	0	0	0	0	22	11	0	0	**0**	0	22	0	0	11
KI_EB	0	0	0	0	0	0	0	13	50	25	0	0	0	**0**	13	0	0	0
KI_SB	7	29	7	7	0	0	0	7	7	14	7	0	0	0	**7**	7	0	0
NGSV_MB	0	8	0	0	0	0	0	0	42	0	0	0	0	0	8	**42**	0	0
NGSV_PP	0	21	0	0	7	0	7	0	43	7	7	0	0	0	0	0	**0**	7
NGSV_PW	10	0	0	0	10	0	0	0	20	20	10	0	0	0	0	0	0	**30**

Table 2.5. Classification success of the discriminant function analysis incorporating all five trace element concentrations within the first annulus (Age 1) of the garfish otolith. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the 18 sites (columns).

Age 1	WC_BB	WC_SB	WC_VB	SWSG_LB	SWSG_PB	SWSG_TB	NSG_PB	NSG_PP	NSG_WP	SWGSV_ED	SWGSV_PV	SWGSV_ST	KI_AR	KI_EB	KI_SB	NGSV_MB	NGSV_PP	NGSV_PW
WC_BB	**55**	0	9	0	9	0	18	0	9	0	0	0	0	0	0	0	0	0
WC_SB	0	**35**	5	0	10	0	5	0	10	25	0	0	0	0	0	0	0	10
WC_VB	18	18	**47**	0	0	0	12	0	6	0	0	0	0	0	0	0	0	0
SWSG_LB	0	30	0	**20**	40	0	0	0	10	0	0	0	0	0	0	0	0	0
SWSG_PB	0	20	7	20	**27**	0	0	0	27	0	0	0	0	0	0	0	0	0
SWSG_TB	0	0	0	14	43	**0**	0	0	29	0	0	0	0	0	0	0	0	14
NSG_PB	6	6	6	0	6	0	**19**	0	50	0	0	0	0	0	6	0	0	0
NSG_PP	0	0	0	0	13	0	7	**0**	47	13	0	0	0	0	7	7	0	7
NSG_WP	0	8	4	4	8	0	17	0	**38**	8	0	0	0	0	0	13	0	0
SWGSV_ED	0	22	0	0	11	0	0	0	39	**6**	6	0	0	0	6	6	0	6
SWGSV_PV	0	40	0	0	0	0	0	0	10	10	**10**	0	0	0	0	0	0	30
SWGSV_ST	0	20	0	0	0	0	0	0	70	0	10	**0**	0	0	0	0	0	0
KI_AR	0	33	11	0	22	0	0	0	11	11	11	0	**0**	0	0	0	0	0
KI_EB	0	38	0	13	25	0	0	0	13	13	0	0	0	**0**	0	0	0	0
KI_SB	7	36	7	0	0	0	0	0	21	21	0	0	0	0	**0**	7	0	0
NGSV_MB	0	0	0	0	0	0	0	0	33	8	0	0	0	0	0	**58**	0	0
NGSV_PP	0	50	0	0	21	0	0	0	14	0	0	0	0	0	0	7	**0**	7
NGSV_PW	0	0	0	0	10	0	0	0	20	0	10	0	0	0	0	0	0	**60**

Table 2.6. Classification success of the discriminant function analysis incorporating all five trace element concentrations within the second annulus (Age 2) of the garfish otolith. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the 18 sites (columns).

Age 2	WC_BB	WC_SB	WC_VB	SWSG_LB	SWSG_PB	SWSG_TB	NSG_PB	NSG_PP	NSG_WP	SWGSV_ED	SWGSV_PV	SWGSV_ST	KI_AR	KI_EB	KI_SB	NGSV_MB	NGSV_PP	NGSV_PW
WC_BB	**18**	0	36	0	0	0	9	0	18	9	0	0	9	0	0	0	0	0
WC_SB	5	**70**	5	0	0	0	0	0	0	5	0	5	5	0	0	0	5	0
WC_VB	0	6	**59**	0	0	0	12	0	6	6	0	0	6	0	6	0	0	0
SWSG_LB	0	0	0	**60**	10	10	0	0	0	20	0	0	0	0	0	0	0	0
SWSG_PB	0	7	0	0	**60**	0	0	0	0	7	0	0	0	13	7	0	7	0
SWSG_TB	0	0	0	57	29	**0**	0	0	0	0	0	0	0	0	0	0	14	0
NSG_PB	6	13	0	0	0	0	**44**	0	6	19	0	0	0	0	13	0	0	0
NSG_PP	0	13	0	20	0	0	0	**0**	33	7	0	0	0	0	0	20	7	0
NSG_WP	0	13	0	8	0	0	33	0	**13**	8	0	0	0	4	4	17	0	0
SWGSV_ED	0	0	6	11	0	0	0	0	6	**39**	0	28	0	0	6	0	6	0
SWGSV_PV	0	10	10	10	0	0	0	0	0	20	**10**	10	0	0	10	10	10	0
SWGSV_ST	0	0	0	0	10	0	0	0	20	30	0	**40**	0	0	0	0	0	0
KI_AR	0	0	33	0	22	0	0	0	11	22	0	0	**0**	0	11	0	0	0
KI_EB	0	0	0	13	13	0	0	0	13	0	13	13	0	**38**	0	0	0	0
KI_SB	7	7	0	0	7	0	14	0	36	0	0	0	0	0	**14**	0	14	0
NGSV_MB	0	0	0	0	0	0	8	17	0	0	0	0	0	0	0	**58**	17	0
NGSV_PP	0	0	0	7	14	0	0	14	7	21	0	7	0	0	0	14	**14**	0
NGSV_PW	0	10	10	10	0	0	0	0	10	20	10	10	0	0	10	10	0	**0**

2.3.4 Broad-scale regional discriminations

Although significant variation in otolith chemistry was detected among sites within regions (Fig. 2.3), the level of replication at the site level was relatively low and rates of reclassification success across each age class were poor. It was, therefore, necessary to reduce the spatial resolution of the analysis to investigate broad-scale regional trends in an attempt to identify larger, more manageable spatial units. Once again, three age-related DFA's were carried out to describe the lifetime movement of the 2-year old garfish.

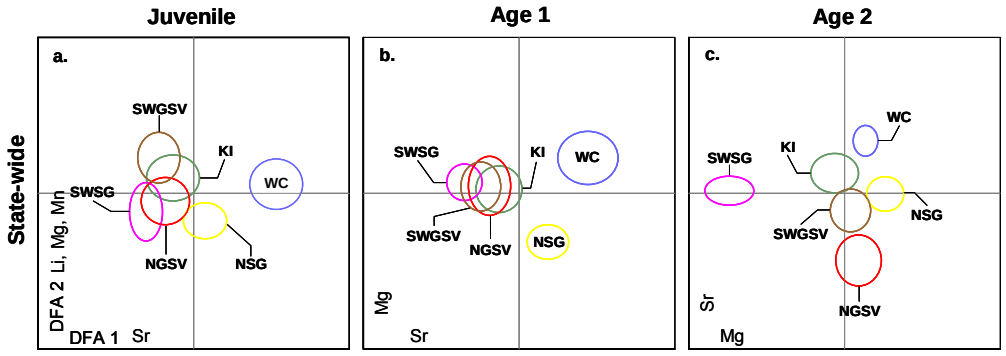

Figure 2.5. Age-related discriminant function centroids with 95% confidence ellipses for southern garfish otoliths collected from six regions in South Australia, based on five trace elements (Li, Mg, Mn, Sr, and Ba).

For the juvenile portion of the otoliths three discriminant functions (χ^2 = 95.89, df = 15, p < 0.001) identified clear regional dissimilarities in the chemical composition, with the first two explaining 92.6% of the variation. Garfish collected from the West Coast and Northern Spencer Gulf distinctly separated from the remaining regions on the basis of differences in mean Sr concentrations (Fig. 2.5a). This separation was further emphasised at age 1 (χ^2 = 135.26, df = 25, p < 0.001) (Fig. 2.5b). Otoliths collected from garfish from the remaining regions displayed different chemical profiles throughout the juvenile phase with South West Gulf St. Vincent almost completely separating from Northern Gulf St. Vincent and South West Spencer Gulf and Kangaroo Island separating from Southwest Spencer Gulf (Fig. 2.5a). These otolith chemistry differences were not maintained throughout age 1 as there was considerable regional convergence within Gulf St. Vincent and South West Spencer Gulf (Fig. 2.5b). By age 2 all six regions were clearly separated (Fig. 2.5c). This divergence was described by four discriminant functions (χ^2 = 216.42, df = 20, p < 0.001), of which the first two explained 86.3% of the between group variability. A clear latitudinal gradient was evident in Gulf St. Vincent, with the Northern and Kangaroo Island regions straddling the South West region. This separation was largely a function of differences in Sr and Mg in the otoliths. The dissimilarity between Spencer Gulf regions was also accentuated and the West Coast fish remained relatively independent (Fig. 2.5c). More than half (51.7%, κ = 41%) of the garfish included in the analysis were successfully categorised by the model. Classification success was >70% for South West Spencer Gulf and West Coast fish, whereas only 6.5% of Kangaroo Island fish were correctly classified. The majority of fish from this region were misclassified as either Northern Spencer Gulf (32.3%) or the West Coast (25.8%) (Table 2.7). The geographic separation of these two regions with Kangaroo Island is extreme (>360 km).

Table 2.7. Classification success of the discriminant function analysis incorporating all five trace element concentrations from each age-division of the garfish otoliths. The data presented are the percentage of otoliths from the region of origin (row) classified to each of the six regions (columns).

Juvenile	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**54**	2	29	13	0	2
SWSG	6	**3**	59	13	0	19
NSG	16	4	**69**	9	0	2
SWGSV	3	8	47	**37**	0	5
KI	16	7	36	36	**0**	7
NGSV	14	14	53	14	0	**6**

Age 1	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**52**	4	23	13	6	2
SWSG	3	**63**	16	9	3	6
NSG	13	13	**69**	2	0	4
SWGSV	5	11	32	**29**	8	16
KI	26	13	23	26	**10**	3
NGSV	17	17	28	6	3	**31**

Age 2	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**71**	0	13	16	0	2
SWSG	3	**78**	3	16	0	0
NSG	22	9	**42**	9	0	18
SWGSV	11	8	16	**58**	3	5
KI	26	13	32	23	**6**	0
NGSV	6	11	11	19	3	**50**

2.4 Discussion

South Australian garfish incorporated elements at various concentrations into their otoliths depending on their age and site from where they were sampled. Garfish collected from sites separated by <60 km displayed significantly different chemical signatures in their otoliths, especially during their second year of growth, indicating that they had inhabited different, discrete water bodies. This was particularly evident for garfish collected from sites located within the West Coast, Northern Spencer Gulf, Northern Gulf St. Vincent and Kangaroo Island. This level of discrimination, however, was not as evident for garfish collected from South West Spencer Gulf and South West Gulf St. Vincent. Otoliths from these fish shared similar chemical profiles and as such could not be accurately attributed to a specific site within their region.

From a broader regional perspective, garfish within Spencer Gulf were clearly partitioned into northern and south western components and this separation was evident throughout the first two years of their lives. Spencer Gulf is a large triangular body of water approximately 300 km long and 80 km wide at its entrance and exhibits a strong latitudinal temperature and salinity gradient increasing northwards (Petrusevics 1993). The otolith elemental profiles of garfish collected from the northern and south western extremes of this gulf clearly indicated that they had experienced different environmental regimes, particularly on the basis of marked differences in the mean concentration of Sr. Sr concentration has frequently been used as a proxy for changes in salinity and relied on to reconstruct migratory patterns for a range of diadromous species that move between freshwater, estuarine and marine habitats (Secor et al. 1995, Jessop et al. 2002, Milton and Chenery 2005). Given the clear environmental gradient and differences in otolith chemistry it is likely that these two regions within Spencer Gulf, are replenished by independent recruitment events and are relatively self-sustaining. This is also likely to be the case with garfish collected from the West Coast.

Such division was not as clear within Gulf St. Vincent as there was considerable similarity in the otolith chemistry in juvenile and age 1 garfish collected from all sites with the exception of Middle Beach. Otoliths collected from Middle Beach garfish had exceptionally high levels of Mn (>15 μmol/mol), at least triple the concentration of the majority of otoliths from the other sites. Elevated concentration of Mn in seawater is typically associated with industrial wastewater discharge (Daby 2006). It is likely that the separation of Middle Beach from the remaining Gulf St. Vincent sites is due to its proximity to Barker Inlet, a shallow, tidal inlet north of Metropolitan Adelaide that is contaminated with pollutants from coastal industries and high urbanisation (Edwards et al. 2001). There is, however, evidence to suggest that Mn

accumulation in otoliths is physiologically regulated and subsequently not influenced by ambient concentration but rather through trophic linkages (Thorrold et al. 1998, Elsdon and Gillanders 2003). Garfish feed predominantly on seagrass (*Zostera* spp.) (Earl 2007), and estuarine seagrass constituents typical of those found in the Middle Beach area have demonstrated significant transfer of metals such as Mn in other fish species (Pentreath 1976, Sanchez-Jerez et al. 2002). Previous analyses of sediments have indeed shown that the mangrove substrates within this area contained elevated levels of Mn (Harbison 1986). Despite differences in the metabolic pathways and processes of trace element accumulation into the otolith matrix, garfish collected from Middle Beach had a distinct 'elemental fingerprint' that clearly separated them from the other Gulf St. Vincent sites.

The discrimination of 1 year old garfish within remaining Gulf St. Vincent sites is uncertain. It is possible, that component populations similar to Middle Beach garfish exist at these sites, but cannot be identified as the chemical composition of the water they inhabit may be homogeneous and therefore cannot produce a clear chemical signature within their otoliths. Like Spencer Gulf, Gulf St. Vincent is a semi-enclosed system, but is comparably smaller at 210 km long and 45 km wide at its entrance. Consequently, it is subjected to a higher level of mixing (de Silva Samarasinghe 1998). Alternatively, the age 1 garfish within these sites may have recruited from a common spawning ground and comprise a single population, a proportion of which migrate to Kangaroo Island within their second year. Southern garfish occurs in close association with shallow seagrass beds, particularly in sheltered bays and estuaries and there is anecdotal evidence that suggests that they spawn over these beds, either depositing their eggs directly onto seagrass blades or the eggs become entangled within the vegetation (Ling 1958, Jordan et al. 1998, Noell 2005). Approximately 75% of Gulf St. Vincent seagrass meadows exist within the northern and south west regions of the gulf (Edyvane 1999), however it is unknown what proportion of these meadows supports spawning garfish.

Clearly, the results obtained from the trace element analysis suggest that the stock structuring of South Australian garfish is more complex than previously assumed. Instead of two self-sustaining stocks partitioned into the two major gulf systems it seems that garfish stocks can be discriminated at a much finer spatial scale. The detection of significantly different age-related chemical signatures among sites within some regions highlighted the spatial complexity of the resource. Assessment and management of spatially complex stocks is challenging, because the scale at which the biology of the target species varies is smaller than the scale at which the data are commonly acquired (Prince 2005). Assessing garfish stock components within individual bays (sites) is too fine-scale, exceeding the spatially explicit

level of reporting that currently exists within the management framework of the South Australian Marine Scalefish Fishery (Noell et al. 2005). In an attempt to reduce some of the complexity and broaden the focus of the study to identify more manageable spatial units the age-related analysis was re-run to include region as the primary spatial factor. The results obtained were comparable with those from the fine-scale spatial analysis. The separation of the two population components within Spencer Gulf was still evident along with the clear discrimination of West Coast garfish. There was relatively good separation among juveniles in Gulf St. Vincent, particularly for those fish collected from the southwest and northern parts of the Gulf, however, this contracted to a relatively homogenous group during the remainder of the fishes' first year of life. By the time the garfish reached two years of age the Gulf St. Vincent population had partitioned into three distinct regional components. It is unlikely that this reflects an active convergence of stock during age 1 as the juvenile and age 2 portion of the otolith share chemical signatures specific to their regions of origin. This pattern is more likely a result of physical oceanographic processes that operate on a seasonal basis within the Gulf and the subsequent variation in water chemistry.

Both Spencer Gulf and Gulf St. Vincent are subjected to the same weather conditions throughout the year and share similar oceanographic attributes. They both exhibit extreme seasonal shifts, and are characterised as semi-enclosed coastal bodies of water in summer that dissipate and become well mixed by the penetrating continental shelf waters in winter (Middleton and Bye 2007). These regime shifts are influenced by a combination of prevailing systemic winds, which are predominantly from the south-east in summer and south-west in winter, and the formation of strong sea surface temperature and salinity fronts (Nunes Vaz et al. 1990; de Silva Samarasinghe 1998). The frontal systems typically develop at the mouths of the gulfs in late spring, persist throughout summer and collapse in late autumn (Petrusevics 1993). During this time the water chemistry within the 'enclosed' gulfs is heterogeneous as a result of localised wind-driven gyres, regions of up- and down-welling, input from populated coastal areas and the accentuated temperature and salinity gradient (Bullock 1975, Petrusevics 1993). Fish inhabiting localised areas within the gulfs will therefore incorporate strong, region specific, elemental signatures during the warmer months which subsequently breakdown, or become masked, during the cooler months when the gulfs are flushed by shelf waters. As southern garfish spawn from October to March (Noell 2005), this dynamic oceanographic process can reconcile the distinct regional separation of spring/summer juveniles and the apparent autumn/winter convergence of age 1 fish. In their second summer the fish appear to diverge back to their region of origin, providing further evidence that the previous apparent convergence is a consequence of the seasonal oceanographic processes. It is therefore, likely, that the regional population structuring of South Australian garfish evident

during the juvenile life-history stage is stable, at least for the first two years of the fishes' life, and exhibits minimal inter-regional mixing. As this study only explored population structuring and movement patterns from the juvenile life-history stage until the fish entered the fishery at age 1+, the extent of any subsequent migration remains unknown.

A recent genetic investigation, using mitochondrial DNA, sub-divided South Australian garfish into two management units; one including the West Coast and the other constituting both gulf systems (Donnellan et al. 2002). This indicated that there is a degree of mixing among population components within the gulfs and given the essentially coastal distribution of garfish and their close association with seagrass habitats it is most likely to approximate a one-dimensional "stepping stone" model in which neighbouring sub-populations exchange genes (Kimura and Weiss 1964). However, only a few migrants per generation are required to maintain genetic homogeneity and as such genetic studies may over-estimate the size of management units (Patterson et al. 2004). This stepping stone movement pattern appears to hold true in this study as in many cases a proportion of garfish were incorrectly classified as originating from either an adjacent site or from one in close proximity. Despite this level of interconnectivity, each of these component populations fulfils Begg and Waldman's (1999) working definition of a stock, where they can be described as "semi-discrete groups of fish with some definable attributes of interest to managers". Their definable attributes, in this case, are their region-specific elemental signatures within their otoliths.

The identification of multiple semi-discrete population components for South Australia's garfish fishery indicates that its management may have to be restructured to align with the smaller spatial units and the stock assessment process, which requires that the sophisticated computer-based fisheries model be adjusted to cater for this new information. Furthermore, the various user-groups and stakeholders will have to adjust to the flow-on effects of any restructure of the fishery. Given the magnitude of the potential changes, it is recommended that the current evidence obtained from trace element analysis of the otoliths, be supported by further investigation using alternative, complementary, stock discriminatory methods such as stable isotope analysis (Valle and Herzka 2008) and otolith morphometrics (Campana and Casselman 1993). Integrating the results from each method into a multiple 'holistic' stock identification approach will maximise the likelihood of correctly delineating South Australian garfish stocks (Begg and Waldmann 1999). This will ultimately lead to the formulation of a 'stock structure' model that becomes an accepted empirical framework for South Australia's garfish fishery.

2.5 References

Bullock D.A. (1975) The general water circulation of Spencer Gulf, South Australia, in the period February to May. *Transactions of the Royal Society South Australia* 99(1), 43-53.

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

Campana S.E. (2005) Otolith science entering the 21st century. *Marine and Freshwater Research* 56, 485-495.

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

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

Daby D. (2006) Coastal pollution and potential biomonitors of metals in Mauritius. *Water, Air Soil and Pollution* 174, 63-91.

de Silva Samarasinghe J.R. (1998) Revisiting upper Gulf St. Vincent in South Australia: the salt balance and its implications. *Estuarine Coastal and Shelf Science* 46, 51-63.

Donnellan S., Haigh L., Elphinstone M., McGlennon D. and Ye, Q. (2002) Genetic discrimination between southern garfish (*Hyporhamphus melanochir*) stocks of Western Australia, South Australia, Victoria and Tasmania. In 'Fisheries biology and habitat ecology of southern garfish (*Hyporhamphus melanochir*) in southern Australian waters. (eds. Jones, G.K., Ye, Q., Ayvazian, S., Coutin, P.). FRDC Final Report 97/133.

Earl J. (2007) Feeding biology of the southern garfish (*Hyporhamphus melanochir,* Hemiramphidae) in Gulf St. Vincent, South Australia. Honours Thesis (Flinders University, Adelaide, South Australia).

Edwards J.W., Edyvane K.S., Boxall V.A., Hamann M. and Soole, K.L. (2001) Metal levels in seston and marine fish flesh near industrial and metropolitan centres in South Australia. *Marine Pollution Bulletin* 42(5), 389-396.

Edyvane K.S. (1999) Conserving marine biodiversity in south Australia. Part 2 - identification of areas of high conservation value in South Australia. SARDI Research Report Series no. 39.

Elsdon T.S., and Gillanders B.M. (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.

Elsdon T.S. and Gillanders B.M. (2003) Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecology Progress Series* 260, 263-272.

Elsdon T.S., Wells B.K., Campana S.E., Gillanders B.M., Jones C.M., Limburg K.E., Secor D.H., Thorrold S.R. and Walther B.D. (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.

Fowler A.J., Gillanders B.M. and Hall K.C. (2005) Relationship between elemental concentration and age from otoliths of adult snapper (*Pagrus auratus*, Sparidae): implications for movement and stock structure. *Marine and Freshwater Research* 56, 661-676.

Gillanders B.M. and Kingsford M.J. (1996) Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. *Marine Ecology Progress Series* 141, 13-20.

Harbison P. (1986) Mangrove muds - a sink and a source for trace elements. *Marine Pollution Bulletin* 17(6), 246-250.

Jessop B.M., Shiao J.C., Iizuka Y. and Tzeng W.N. (2002) Migratory behaviour and habitat use by American eel *Anguilla rostrata* as revealed by otolith microchemistry. *Marine Ecology Progress Series* 233, 217-229.

Jordan A.R., Mills D.M., Ewing G. and Lyle J.M. (1998) Assessment of inshore habitats around Tasmania for life-history stages of commercial finfish species. Fisheries Research and Development Corporation (Project No. 94/037). Canberra, Australia.

Kimura M. and Weiss G.H. (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* 49, 561-576.

Ling J.K. (1958) The garfish, *Reporhamphus melanochir* (Cuvier and Valenciennes) (Hemiramphidae), in South Australia: breeding, age determination, and growth rate. *Australian Journal of Marine and Freshwater Research* 9, 60-110.

Middleton J.F and Bye J.A.T. (2007) A review of the shelf-slope circulation along Australia's southern shelves: Cape Leeuwin to Portland. *Progress in Oceanography* 7, 1-41.

Milton D.A. and Chenery S.R. (2005) Movement patterns of barramundi *Lates calcarifer*, inferred from $^{87}Sr/^{86}Sr$ and Sr/Ca ratios in otoliths, indicate non-participation spawning. *Marine Ecology Progress Series* 301, 279-291.

Noell C.J. (2005) Early life stages of the southern garfish *Hyporhamphus melanochir* (Valenciennes, 1846) and their association with seagrass beds. PhD Dissertation (University of Adelaide, Adelaide, South Australia).

Noell C., Presser J. and Jones K. (2006) Management Plan for the South Australian Marine Scalefish Fishery. PIRSA. South Australian Fisheries Management Series No. 45, 68 pp.

Nunes Vaz R.A., Lennon G.W. and Bowers D.G. (1990) Physical behaviour of a large, negative or inverse estuary. *Continental Shelf Research* 10(3), 277-304.

Patterson H.M., McBride R.S. and Julien N. (2004) Population structure of red drum (*Sciaenops ocellatus*) as determined by otolith chemistry. *Marine Biology* 144, 855-862.

Pentreath R.J. (1976) Some further studies on the accumulation and retention of ^{65}Zn and ^{55}Mn by the plaice , *Pleuronectes platessa* L. *Journal of Experimental Marine Biology and Ecology* 21, 179-189.

Petrusevics P.M. (1993) SST fronts in inverse estuaries, South Australia - Indicators of reduced gulf-shelf exchange. *Australian Journal of Marine and Freshwater Research* 44, 305-323.

Prince J. (2005) Combating the tyranny of scale for Haliotids: micro-management for micro-stocks. *Bulletin of Marine Science* 76(2), 557-577.

Rooker J.R., Secor D.H., Zdanowicz V.S. and Itoh T. (2001) Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. *Marine Ecology Progress Series* 218, 275-282.

Sanchez-Jerez P., Gillanders B.M. and Kingford M.J. (2002) Spatial variability of trace elements in fish otoliths: comparison with dietary items and habitat constituents in seagrass meadows. *Journal of Fish Biology* 61, 801-821.

Secor D.H., Henderson-Arzapalo A. and Piccoli P.M. (1995) Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *Journal of Experimental Marine Biology and Ecology* 192, 15-33

Secor D.H. and Rooker J.R. (2000) Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fisheries Research* 46, 359-371.

Thorrold S. R., Campana S.E., Jones C.M. and Swart P.K. (1997) Factors determining ^{13}C and ^{18}O fractionation in aragonite otoliths of marine fishes. *Geochimica et Cosmochimica Acta* 61(14); 2909-2919.

Thorrold S.R., Jones C.M., Swart P.K. and Targett T.E. (1998) Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. *Marine Ecology Progress Series* 173, 253-265.

Titus U., Mosher J.A and Williams B.K. (1984) Chance-corrected classification for use in discriminant analysis: ecological applications. *The American Midland Naturalist* 111, 1-7.

Valle S.R. and Herzka S.Z. (2008) Natural variability in $\delta^{18}O$ values of otoliths of young Pacific sardine captured in Mexican waters indicates subpopulation mixing within the first year of life. *ICES Journal of Marine Science* 65, 174-190.

Yoshinaga J., Nakama A., Morita M. and Edmonds J.S. (2000) Fish otolith reference material for quality assurance of chemical analyses. *Marine Chemistry* 69, 91-97.

3 STOCK DISCRIMINATION OF SOUTHERN GARFISH (*HYPORHAMPHUS MELANOCHIR*) BY STABLE ISOTOPE RATIO ANALYSIS OF OTOLITH CARBONATE.

MA Steer, G Halverson, AJ Fowler, BM Gillanders

3.1 Introduction

Stable isotopes in biogenic calcium carbonate have been used for various species as environmental recorders and palaeothermometers (Thorrold et al. 1997). Historically, studies have analysed the isotopic fractionation of oxygen ($\delta^{18}O$) in invertebrate shells (e.g. molluscs, foraminifera, coral skeletons) to reconstruct temperature and salinity regimes of modern and ancient oceans (Guilderson et al. 2004). This is based on the principle that oxygen isotopes are incorporated into biogenic carbonate in approximate equilibrium with ambient water and vary as a function of temperature and evaporation/precipitation rates. Similarly, carbon isotope ratios ($\delta^{13}C$) have been used as a proxy for metabolism, trophic position and to identify upwelling events as carbon fractionation is both physiologically and environmentally mediated (Tanaka et al. 1986; Høie et al. 2003; McConnauhey and Gillikin 2008). More recently, stable isotope ratio analysis of oxygen and carbon combined, has been applied to fish otoliths and successfully used to delineate geographical population structure (Edmonds and Fletcher 1997; Newman et al. 2000; Ashford and Jones 2007), infer patterns of habitat utilization (Dufour et al. 1998) and reconstruct migratory pathways (Nelson et al. 1989; Herzka 2005) for a number of economically important fish species worldwide.

The first studies based on stable isotope analysis used whole otoliths as the analyses were limited to relatively large quantities of deproteinated carbonate (Nelson et al. 1989; Edmonds and Fletcher 1997). Therefore, these analyses integrated the entire life-history of the fish and therefore only had the potential to reflect the home range of each individual (Newman et al. 2000). Recent advances in automated micro-milling techniques and improved mass spectrometry have enabled more detailed investigation into the isotopic variation within individual otoliths, permitting the accurate collection of multiple age-related carbonate samples in order to resolve the ontogenetic history of individual fish and their associated populations (Wurster et al. 1999; Weidman and Millner 2000). Similar principles have been applied to trace element analysis, where laser-ablation is used to investigate age-related variation in otolith elemental chemistry allowing the reconstruction of migratory pathways over the life-time of a fish (Fowler et al. 2005; Elsdon et al. 2008; Chapter 2). One of the major benefits of stable isotope analysis is that, unlike trace element analysis, it analyses the main structural constituents of the otolith (carbon and oxygen) and avoids potential biases and contamination issues associated with determining trace quantities of a range of elements

(Edmonds and Fletcher 1997). As such, the discriminatory resolution of using carbon and oxygen isotopes ratios is considered to be more powerful (Ashford and Jones 2007).

In Australia, stable isotope analysis has been successfully used to delineate stocks of pilchard (*Sardinops sagax*), pink snapper (*Pagrus auratus*), goldband snapper (*Pristipomoides multidens*), red emperor (*Lutjanus sebae*) and Rankin cod (*Epinephelus multinotatus*), all of which are widely distributed across large environmental gradients (Edmonds and Fletcher 1997, Edmonds et al. 1999, Newman et al. 2000, Stephenson et al. 2001). This study aims to use the same principles and methodology applied in previous studies to delineate the stock structure of southern garfish (*Hyporhamphus melanochir*) within South Australian coastal waters. But unlike the previous studies, it will adopt an age-related approach and analyse multiple parts of the otolith to investigate each individual's ontogenetic history.

Two large, shallow, gulfs (Spencer Gulf and Gulf St. Vincent) dominate the South Australian coastline (Fig. 2.1) and are substantially more saline than adjacent shelf waters due to high evaporation rates, low precipitation and a lack of fresh water inflow from creeks and rivers. Consequently, each gulf exhibits a strong north-south temperature and salinity gradient (Petrusevics 1993), the latter of which is reflected in an increasing northward oxygen isotope ratio of the seawater, analogous to the ^{18}O-enriched waters of Shark Bay, Western Australia (Edmonds et al. 1999). The majority of the commercial catch of southern garfish in South Australia is harvested from the northern reaches of the two gulfs, although in recent years commercial catch rates have markedly declined (McGarvey et al. 2006). Currently, the population structure of this fishery is not fully understood and therefore it is not known whether the key commercial fishing areas are self-replenishing or sustained by recruitment from adjacent areas. Understanding the replenishment process is essential for avoiding the possibility of key fishing areas becoming locally depleted. This study takes advantage of the temperature and salinity gradients within the two gulfs to examine whether South Australia's southern garfish population can be delineated on the basis of location-specific stable isotopic signatures that are retained within their otolith carbonate.

3.2 Materials and Methods

3.2.1 Sample collection

A range of 7 - 14 adult garfish from the 2+ age class were used for stable isotope analysis (Table 3.1). Refer to sections 2.2.1 and 2.2.2 for sample collection details and otolith preparation.

Table 3.1. The number of adult garfish (Age 2+) sampled for stable isotope analysis.

Region	Site	n
West Coast	Baird Bay	8
	Streaky Bay	8
	Venus Bay	12
Northern SG	Port Broughton	9
	Port Pirie	10
	Wood Point	7
South West SG	Louth Bay	9
	Proper Bay	9
	Tumby Bay	5
Northern GSV	Middle Beach	12
	Port Parham	14
	Port Wakefied	10
South West GSV	Edithburgh	10
	Port Vincent	9
	Stansbury	10
Kangaroo Island	American River	9
	Emu Bay	7
	Shoal Bay	9

3.2.2 Stable isotope analysis

Three, age-related, carbonate samples were milled from each otolith using a high-resolution, computerised, micromilling machine (New Wave Research MicroMill). A standardised nine-point grid-raster, drill pattern (350 x 350 μm), calibrated to drill to a depth of 325 μm, was used to mill an mean of 88.5 μg (se ± 2.4 μg, n = 191) of carbonate powder from the juvenile (core), first and second year growth zones of the otolith (Fig. 3.1). Each milled sample was carefully tapped onto a sheet of wax paper and transferred into a small, clean, metallic vial. After the collection of each sample, the otolith section, drill bit, wax paper and work surfaces were blown clear of remnant powder with compressed air. All samples were baked for ~ 4 hours at 80°C to remove any potential volatiles prior to stable isotope analysis. $\delta^{13}C$ and $\delta^{18}O$ isotope data were acquired simultaneously on a Fisons Optima dual inlet mass spectrometer attached to an Isocarb preparation device at the University of Adelaide. Micro-milled sample powder was reacted in a common, purified H_3PO_4 bath at 90ºC for 420 seconds. Evolved CO_2 was doubly-distilled cryogenically and measured against an in-house reference gas. $\delta^{18}O$ was corrected for equilibrium with H_2O during the reaction using the Craig (1957) equation and both $\delta^{13}C$ and $\delta^{18}O$ samples were calibrated to Vienna PeeDee Belemnite (PDB) reference standard using an in-house calcite standard. External error (1σ) based on house standards was better than ±0.05‰ for $\delta^{13}C$ and ±0.1 for $\delta^{18}O$. Standards were run in triplicate before each session and once after six consecutive samples. All measurements were reported in per mil (‰) units using the standard delta notation, $\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{reference}) - 1]* 1000$, which also applies to $\delta^{18}O$.

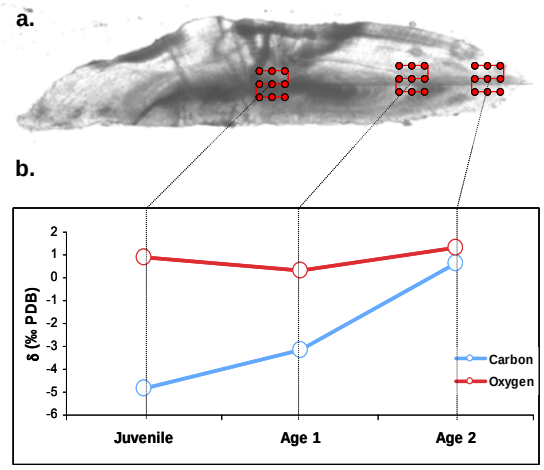

Figure 3.1. (a.) transverse section of an age 2+ garfish sagitta indicating the raster sampling pattern of the automated micro-milling machine; (b.) the carbon and oxygen isotope values along the chronological age structure of the otolith.

3.2.3 Data analyses

The focus of the data analysis was to compare age-related, stable isotopic signatures in garfish otoliths between sites nested within regions and among regions. As three age-related isotopic signatures were obtained from each individual fish the data were interdependent and, therefore repeated-measures (RM) analysis of variance was used to compare age-related mean concentrations of $\delta^{13}C$ and $\delta^{18}O$ between the two spatial scales. Residuals conformed to the assumptions of normality and homogeneity of variances and therefore no data transformations were required. The data were then combined into a multivariate dataset and analysed using a RM multivariate analysis of variance. As isotopic means were found to be significantly different, three linear discriminant function analyses (DFA) were undertaken to assess whether the site of capture of each garfish could be reliably determined from the mean age-related stable isotopic signatures. The first DFA incorporated the mean value of $\delta^{13}C$ and $\delta^{18}O$ within the juvenile (core) portion of the otolith, the second from the first annulus, and the third from the second annulus. The data were analysed separately for each life-stage to assess changes in the patterns of predicted group membership with increasing age. Equal prior probabilities were used and the error rate was estimated by cross-validation. The performance of each discriminant function was evaluated using Cohen's Kappa (κ) statistic, which provides an objective means of calculating the chance-corrected percentage of agreement between actual and predicted group memberships. Values of κ range from 0 to 1, with 0 indicating the discriminant function yields no improvement over chance, and 1 indicating perfect agreement (Titus et al. 1984).

3.2.4 Calculation of expected $\delta^{18}O$ values

The expected $\delta^{18}O$ values of seawater were calculated for each of the six regions and the three life history stages using Grossman and Ku's (1986) fractionation equation for aragonite;

$$T\ (°C) = 20.6 - 4.34 * (\delta^{18}O_{otolith} - \delta^{18}O_{water}) \quad [3.1.]$$

Estimated monthly mean time series of SST (°C) for each of the six regions (Table 3.2) were derived from satellite images acquired through the online PO.DAAC Ocean ESIP Tool (POET) at the Physical Oceanography Distributed Active Archive Centre (PO.DAAC), NASA Jet Propulsion Laboratory, Pasadena, CA. (http://podaac.jpl.nasa.gov/poet). Estimates are based on data gathered with Advanced Very High Resolution Radiometers (AVHRR) on the National Oceanic and Atmospheric Administration's (NOAA) polar-orbiting weather satellites. The SST time series data were partitioned to correspond with the otolith carbonate sample and the relative age of the garfish, so that mean monthly SST from December 2004 to February 2005 represented the juvenile life stage; from October 2005 to November 2005 (Spring 05) represented the first year of growth; and from October 2006 to November 2006 (Spring 06) represented the second year of growth (Table 3.2).

The calculation of expected $\delta^{18}O$ values assumes constant salinity. As detailed salinity data were unavailable for the period of this study; estimates of regional salinity were obtained from the literature (Table 3.2). Linear regression analyses were used to examine the relationship between age-specific, water corrected $\delta^{18}O$ values ($\delta^{18}O_{otolith} - \delta^{18}O_{water}$) and regional salinity estimates.

Table 3.2. Age resolved estimates of sea surface temperature for each of the six regions as derived from satellite imagery http://podaac.jpl.nasa.gov/poet. Estimates of salinity were sourced from: Jones (1990) [a], Nunes Vaz et al. (1990) [b], Nunes and Lennon (1986) [c], de Silva Samarasinghe et al. (2003) [d].

Region	Area (Long, Lat)	Salinity (‰)	SST Juvenile (Summer 04/05)	SST Age 1 (Spring 05)	SST Age 2 (Spring 06)
WC	134.11E, 33.09S 134.32E, 33.17S	42[a]	19.9	16.5	16.5
SWSG	136.01E, 34.34S 136.07E, 34.45S	36[b]	20.3	16	15.8
NSG	137.40E, 33.06S 137.47E, 33.30S	41[c]	20.9	16.23	16.1
KI	137.37E, 35.26S 137.51E, 35.42S	37[b]	20.00	15.7	15.3
SWGSV	137.52E, 34.46S 137.56E, 35.06S	37[d]	20.5	15.8	15.6
NGSV	138.00E, 34.21S 138.16E, 34.37S	38[d]	21.35	16	15.8

3.3 Results

3.3.1 Variation in $\delta^{18}O$

$\delta^{18}O$ values from garfish otoliths ranged from -2.4‰ to 3.4‰. Significant differences were detected in mean $\delta^{18}O$ among sites within regions and as a function of age (Table 3.3). Age-related variation was consistent across all otoliths (Table 3.3, Fig. 3.2). The greatest variation in mean $\delta^{18}O$ was for otoliths from West Coast garfish, with values ranging from 0.8‰ to 2.5‰. Garfish from Baird Bay consistently had significantly higher values (by ~ 1‰) than the other two West Coast sites across all three ages (Fig. 3.2). All remaining sites had similar oxygen isotopic signatures within their respective regions and were generally within 0.4‰ of each other.

Table 3.3. Results of the repeated-measures analysis of variance testing for spatial differences in age-related δ18O of garfish otoliths. ns: not significant, *p < 0.05, ** p < 0.01, ***p < 0.001.

Source	df	$\delta^{18}O$ MS	F
Between-Subjects			
Region	5	11.86	22.21***
Site(Region)	12	2.6	4.86***
Error	127	0.53	
Within-Subjects			
Age	2	1.64	5.4**
Age*Region	10	0.16	0.51^{ns}
Age*Site(Region)	24	0.15	0.50^{ns}
Error	254	0.3	

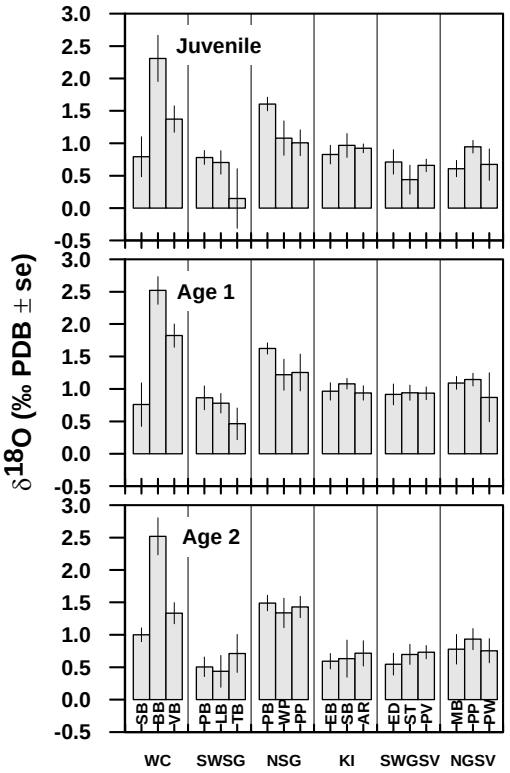

Figure 3.2. Age-resolved spatial variation in oxygen isotope values of garfish otoliths.

Significant regional differences in mean $\delta^{18}O$ were also detected (Table 3.3) with otoliths collected from West Coast and Northern Spencer Gulf garfish consistently exhibiting higher values than those collected from the other regions. Mean $\delta^{18}O$ values were similar for garfish collected from regions within Gulf St. Vincent (i.e. KI, NGSV and SWGSV) and South West Spencer Gulf and these similarities were consistent throughout the life-time of the fish (Fig. 3.3). There were no significant correlations between $\delta^{18}O$ values and estimated SST

(Pearson's: Juvenile: r = -0.49, p = 0.33; Age 2: r = 0.82, p = 0.06; Age 2: r = 0.75, p = 0.84) with observed values ($\delta^{18}O_{aragonite}$) clearly conflicting with the calculated expected ($\delta^{18}O_{water}$) values for each age division (Fig. 3.3). There were, however, significant positive linear relationships between water-corrected $\delta^{18}O$ values and regional salinity estimates, with salinity explaining >94% of the variation (Fig. 3.3).

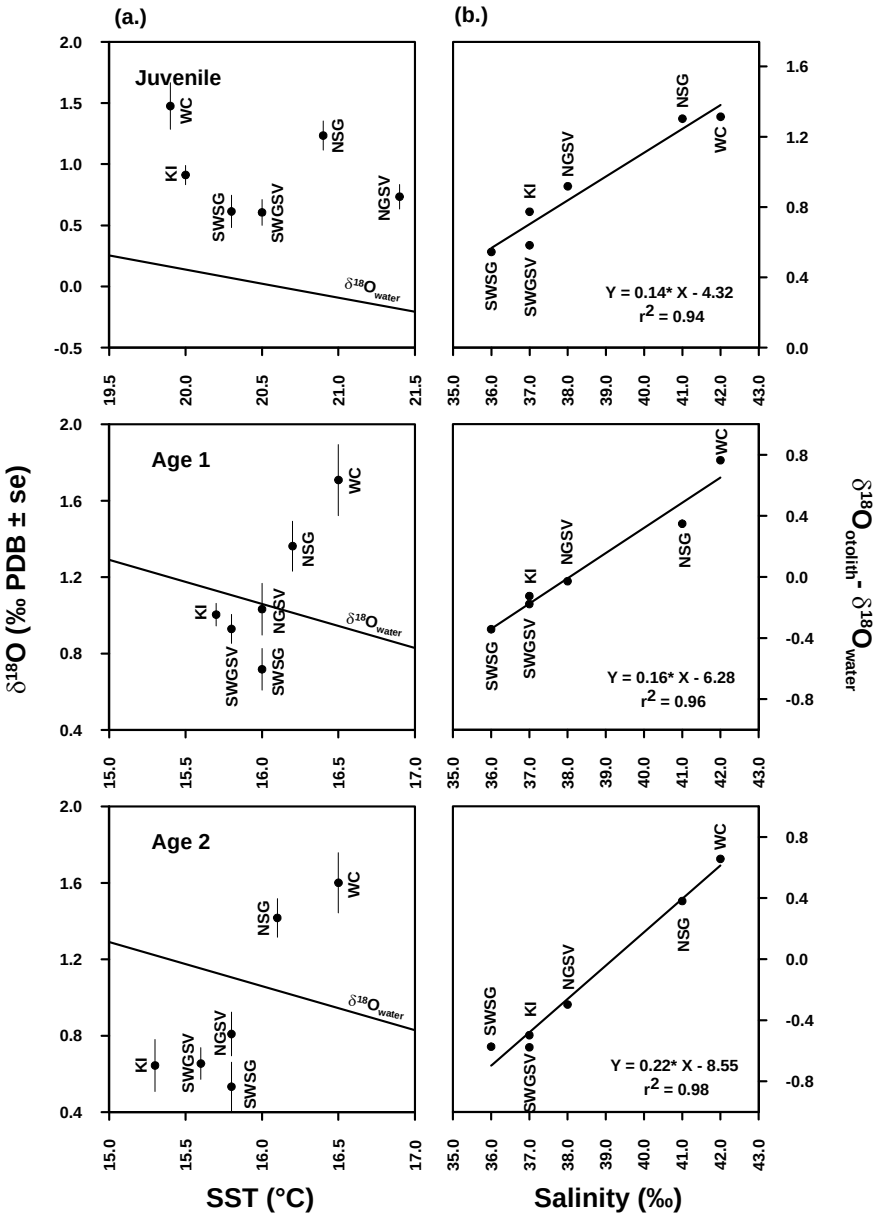

Figure 3.3. (a.) Age-resolved, regional, mean oxygen isotope values against estimates of sea surface temperature. For comparative purposes, the expected $\delta^{18}O$ of the water based on Grossman and Ku's (1986) equation was calculated. (b.) Water-corrected $\delta^{18}O$ values regressed with regional salinity estimates for each of the age-divisions.

3.3.2 Variation in $\delta^{13}C$

$\delta^{13}C$ values from garfish otoliths were highly variable ranging from -7.6‰ to 2.5‰. Significant differences were detected in mean $\delta^{13}C$ among sites within regions and varied as a function of age (Table 3.4). Age-related variation was not consistent across all regions (Table 3.4, Fig. 3.4). The greatest within-region differences in mean $\delta^{13}C$ were seen in otoliths collected from Kangaroo Island, where fish from Emu Bay had significantly lower $\delta^{13}C$ values than the other two sites (Fig. 3.4). The magnitude of these differences increased with age, changing from 2.8‰ during the juvenile phase to 4.9‰ by age 2. Similar age patterns were seen for most sites within Northern Gulf St. Vincent, South West Spencer Gulf and Northern Spencer Gulf, with mean $\delta^{13}C$ values differing up to 2.6‰, 3.4‰ and 4.3‰, respectively (Fig. 3.4). Significant variation in $\delta^{13}C$ was also evident among sites within South West Gulf St. Vincent and the West Coast, but the pattern of variation was inconsistent with age.

Table 3.4. Results of the repeated-measures analysis of variance testing for spatial differences in age-related δ13C of garfish otoliths. ns: not significant, *p < 0.05, ** p < 0.01, ***p < 0.001.

Source		$\delta^{13}C$	
	df	MS	F
Between-Subjects			
Region	5	68.26	17.12***
Site(Region)	12	42.05	10.54***
Error	127	3.99	
Within-Subjects			
Age	2	303.6	177.2***
Age*Region	10	3.5	2.04*
Age*Site(Region)	24	3.48	2.03**
Error	254	1.71	

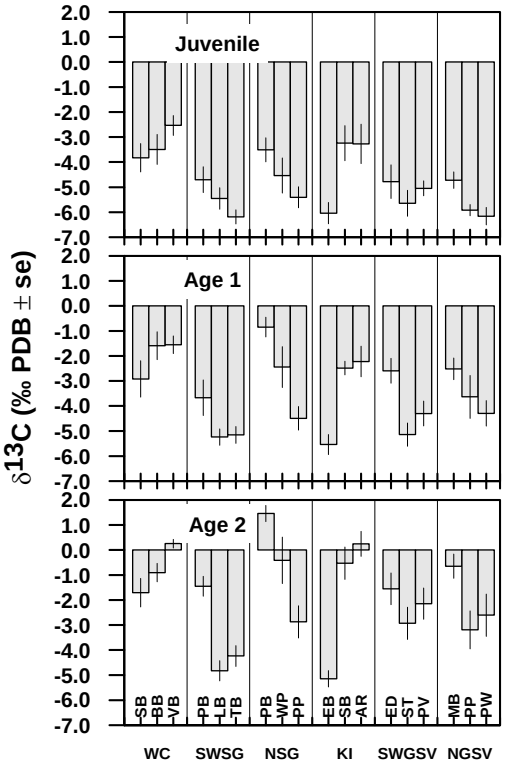

Figure 3.4. Age-resolved spatial variation in carbon isotope values of garfish otoliths.

3.3.3 Fine-scale spatial discrimination

Combining $\delta^{13}C$ and $\delta^{18}O$ values into a single multivariate matrix, yielded similar results to the univariate analyses, where the isotopic signatures of the otoliths differed significantly among sites within regions and between regions as a function of age (Table 3.5). Significant spatial variation in the isotopic signature was detected within the juvenile portion of garfish otoliths ($\Lambda = 0.409$, $p < 0.001$), however the percentage of individuals correctly classified to their site of origin was poor (17.5%) and according to Cohen's κ this classification was only 7.2% better than chance. Classification rates exceeded 60% for fish collected from Baird Bay and Venus Bay on the West Coast and rarely exceeded 20% for the remaining fish (Table 3.6). Garfish collected from Emu Bay (KI), Middle Beach (NGSV), Port Broughton (NSG) and Tumby Bay (SWSG) all exhibited mean isotopic signatures that differed from other sites within their respective regions (Fig. 3.6). Mean isotopic values of fish collected from all three sites within the West Coast also exhibited clear spatial separation. The majority of this spatial separation was driven by $\delta^{13}C$ which accounted for 78.4% of the between-group variability.

Table 3.5. Results of a repeated-measures multivariate analysis of variance testing for spatial, age-related differences in the carbon and oxygen stable isotope values of garfish otoliths. ns: not significant, *p < 0.05, ** p < 0.01, ***p < 0.001.

Source	Value	df	df_{error}	F
Between-Subjects				
Region	0.43	10	252	13.30***
Site(Region)	0.81	24	254	7.19***
Within-Subjects				
Age	0.73	4	124	84.03***
Age*Region	0.17	20	508	1.10^{ns}
Age*Site(Region)	0.38	48	508	1.10^{ns}

Overall classification success was moderately improved to 19.7% ($\kappa = 16.3\%$) when the age 1 portion of the otolith was analysed. This analysis had higher discriminant power ($\Lambda = 0.387$, $p < 0.001$) and $\delta^{13}C$ continued to explain most (81.5%) of the between-group variation. Classification rates improved for Baird Bay (WC) and Shoal Bay (KI) at 80.0% and 63.6%, respectively, and exceeded 30% for garfish collected from Port Broughton and Port Pirie in Northern Spencer Gulf, Louth Bay in South West Spencer Gulf and Venus Bay on the West Coast (Table 3.7). All other sites remained below 15%. Increased isotopic separation was evident among sites at Kangaroo Island, South West Gulf St. Vincent, North and South West Spencer Gulf and the West Coast (Fig. 3.5).

Greater spatial separation was detected in the isotopic signatures when the age 2 portion of the otoliths were analysed (Λ = 0.313, p <0.001) (Fig. 3.5). Although still poor, overall classification improved to 25.6% (κ = 24.3%). $\delta^{13}C$ continued to explain the majority of the between-group variation on the first discriminant function (62.2%), however $\delta^{18}O$ increased its contribution on the second discriminant function (37.8%). Classification success rates remained above 60% for garfish collected from Baird Bay and Venus Bay on the West Coast and Louth Bay in South West Spencer Gulf (Table 3.8).

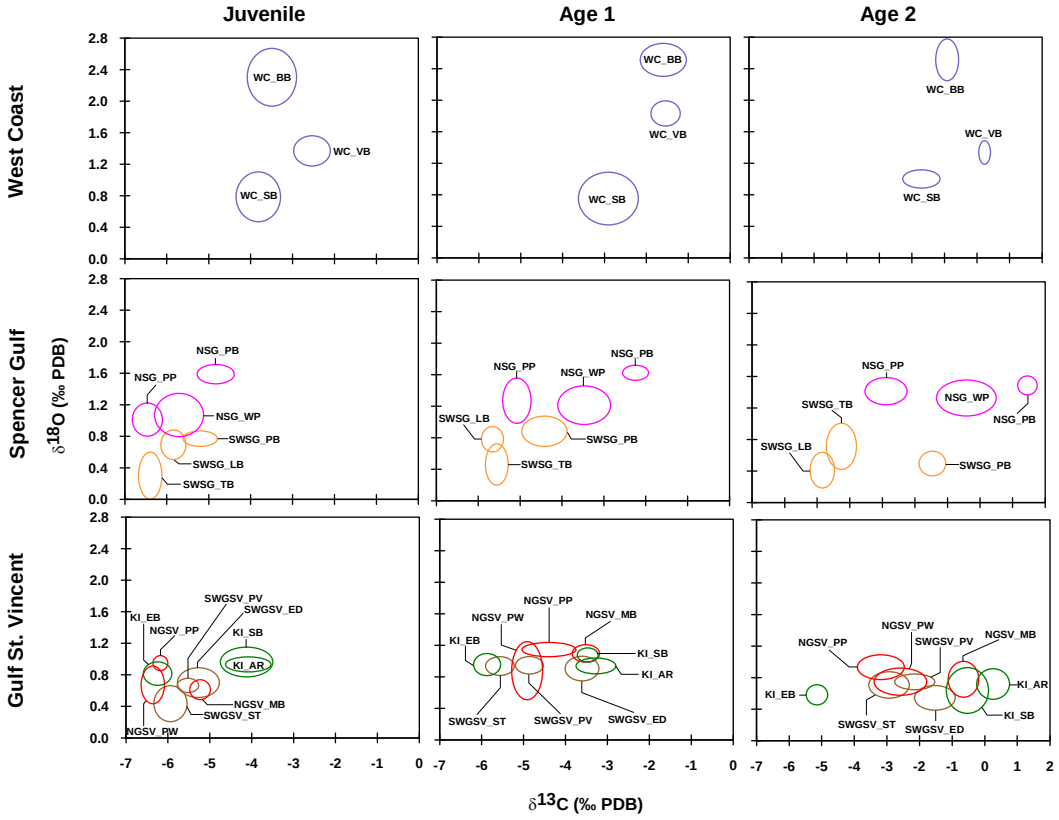

Figure 3.5. Age related mean $\delta^{18}O$ versus $\delta^{13}C$ (± 95% confidence ellipses) for otolith carbonate from southern garfish collected from 18 sites within six regions in South Australia. Figures were separated for the West Coast, Spencer Gulf and Gulf St. Vincent for clarity.

Table 3.6. Classification success of the discriminant function analysis incorporating carbon and oxygen isotope values from the core (juvenile) portion of the garfish otoliths. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the 18 sites (columns)

Juveniles	WC_BB	WC_SB	WC_VB	SWSG_LB	SWSG_PB	SWSG_TB	NSG_PB	NSG_PP	NSG_WP	SWGSV_ED	SWGSV_PV	SWGSV_ST	KI_AR	KI_EB	KI_SB	NGSV_MB	NGSV_PP	NGSV_PW
WC_BB	**63**	0	0	0	0	0	13	0	0	0	0	0	13	0	0	0	0	13
WC_SB	0	**0**	13	0	13	0	0	38	0	0	0	13	13	0	13	0	0	0
WC_VB	8	0	**67**	0	0	0	8	8	0	0	0	0	8	0	0	0	0	0
SWSG_LB	0	0	11	**0**	0	0	0	11	0	22	0	33	11	0	0	0	0	11
SWSG_PB	0	0	11	0	**0**	0	0	33	0	11	0	22	11	0	11	0	0	0
SWSG_TB	0	0	0	0	0	**20**	0	0	0	20	0	20	0	0	0	0	0	40
NSG_PB	0	0	56	0	0	0	**33**	11	0	0	0	0	0	0	0	0	0	0
NSG_PP	10	0	0	0	0	0	10	**20**	0	10	0	10	10	0	0	10	0	20
NSG_WP	0	0	29	0	0	0	14	29	**0**	0	0	29	0	0	0	0	0	0
SWGSV_ED	0	0	10	0	0	10	0	50	0	**10**	0	20	0	0	0	0	0	0
SWGSV_PV	0	0	0	0	22	0	0	11	0	44	**0**	11	11	0	0	0	0	0
SWGSV_ST	0	10	0	0	0	0	0	20	0	20	0	**50**	0	0	0	0	0	0
KI_AR	0	11	33	0	0	0	0	22	0	22	0	0	**11**	0	0	0	0	0
KI_EB	0	0	0	0	14	0	0	29	0	14	0	14	0	**0**	0	0	0	29
KI_SB	0	0	44	0	11	0	0	22	0	11	0	11	0	0	**0**	0	0	0
NGSV_MB	0	0	0	0	38	0	0	13	0	13	13	13	0	0	0	**13**	0	0
NGSV_PP	0	0	0	0	0	0	0	57	0	0	0	14	0	0	0	0	**0**	29
NGSV_PW	0	0	0	0	13	13	0	63	0	0	0	13	0	0	0	0	0	**0**

Table 3.7. Classification success of the discriminant function analysis incorporating carbon and oxygen isotope values from the first annulus (Age 1) of the garfish otoliths. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the 18 sites (columns)

Age 1	WC_BB	WC_SB	WC_VB	SWSG_LB	SWSG_PB	SWSG_TB	NSG_PB	NSG_PP	NSG_WP	SWGSV_ED	SWGSV_PV	SWGSV_ST	KI_AR	KI_EB	KI_SB	NGSV_MB	NGSV_PP	NGSV_PW
WC_BB	**80**	0	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WC_SB	0	**0**	0	0	0	10	10	30	0	10	0	0	10	0	30	0	0	0
WC_VB	17	0	**42**	0	0	0	8	0	8	8	0	0	0	0	17	0	0	0
SWSG_LB	0	10	0	**30**	0	10	0	10	0	0	0	40	0	0	0	0	0	0
SWSG_PB	0	13	0	13	**0**	0	0	63	0	0	0	0	13	0	0	0	0	0
SWSG_TB	0	0	0	29	0	**14**	0	0	0	0	0	29	0	0	0	0	0	29
NSG_PB	0	0	40	0	0	0	**40**	0	0	0	0	0	0	0	20	0	0	0
NSG_PP	0	9	9	0	0	9	0	**36**	0	0	0	27	9	0	0	0	0	0
NSG_WP	10	0	30	10	0	0	10	10	**0**	0	0	10	10	0	10	0	0	0
SWGSV_ED	0	36	9	0	0	0	0	18	0	**0**	0	0	0	0	36	0	0	0
SWGSV_PV	0	11	0	11	0	0	0	11	0	22	**0**	22	0	0	11	0	0	11
SWGSV_ST	0	10	0	30	0	0	0	40	0	0	0	**10**	0	0	0	0	0	10
KI_AR	0	11	0	0	0	0	11	22	0	11	0	0	**11**	0	33	0	0	0
KI_EB	0	0	0	33	0	0	0	33	0	0	0	17	0	**0**	0	0	0	17
KI_SB	0	9	0	0	0	0	0	9	9	9	0	0	0	0	**64**	0	0	0
NGSV_MB	0	0	20	10	0	0	10	0	10	10	0	0	0	0	30	**0**	0	10
NGSV_PP	0	0	0	11	0	0	11	22	0	0	0	22	11	0	22	0	**0**	0
NGSV_PW	0	10	20	0	0	10	0	30	0	0	0	20	0	0	10	0	0	**0**

Table 3.8. Classification success of the discriminant function analysis incorporating carbon and oxygen isotope values from the second annulus (Age 2) of the garfish otoliths. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the 18 sites (columns)

Age 2	WC_BB	WC_SB	WC_VB	SWSG_LB	SWSG_PB	SWSG_TB	NSG_PB	NSG_PP	NSG_WP	SWGSV_ED	SWGSV_PV	SWGSV_ST	KI_AR	KI_EB	KI_SB	NGSV_MB	NGSV_PP	NGSV_PW
WC_BB	**80**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
WC_SB	0	**30**	20	10	0	0	0	20	0	0	0	0	0	0	20	0	0	0
WC_VB	0	0	**58**	0	0	0	0	0	17	0	0	0	0	0	25	0	0	0
SWSG_LB	0	0	0	**70**	0	0	0	0	0	0	0	20	0	10	0	0	0	0
SWSG_PB	0	0	0	0	**20**	0	0	0	0	20	10	10	0	0	40	0	0	0
SWSG_TB	0	0	0	57	0	**0**	0	29	0	0	0	14	0	0	0	0	0	0
NSG_PB	0	0	20	0	0	0	**60**	0	10	0	0	0	10	0	0	0	0	0
NSG_PP	18	0	9	27	0	0	0	**27**	18	0	0	0	0	0	0	0	0	0
NSG_WP	9	0	27	18	0	0	36	0	**0**	0	0	0	0	9	0	0	0	0
SWGSV_ED	0	0	0	0	10	0	0	30	0	**0**	0	20	0	0	40	0	0	0
SWGSV_PV	0	0	0	10	0	0	0	20	0	0	**10**	20	0	0	40	0	0	0
SWGSV_ST	0	0	10	10	0	0	0	10	0	0	10	**20**	0	10	20	0	0	10
KI_AR	0	0	38	0	13	0	0	0	0	0	0	0	**13**	0	38	0	0	0
KI_EB	0	0	0	75	0	0	0	13	0	0	0	0	0	**13**	0	0	0	0
KI_SB	0	17	17	8	0	0	8	8	0	0	0	0	0	0	**33**	0	0	8
NGSV_MB	0	0	10	0	10	0	0	10	20	0	0	0	0	0	50	**0**	0	0
NGSV_PP	0	0	0	14	0	0	0	14	29	14	0	14	0	14	0	0	**0**	0
NGSV_PW	0	0	20	30	0	0	0	20	0	0	0	0	0	10	20	0	0	**0**

3.3.4 Broad-scale regional discrimination

Analysis of the fine-scale population structure revealed that many garfish were misclassified to adjacent sites, or sites in close proximity, particularly for garfish collected from Northern Spencer Gulf and South West Gulf St. Vincent. Consequently, it was necessary to reduce the spatial resolution of the analysis to explore broader, regional trends in an attempt to identify larger, more manageable spatial units.

Significant regional separation in the isotopic signatures was detected within the cores of the otoliths ($\Lambda = 0.660$, $p < 0.001$). Overall classification was better than the fine-scale results, but still poor, with 31.8% of all individuals successfully reclassified to their region of capture, a result that was 16.9% greater than chance. Classification rates were highest for fish collected from South West Gulf St. Vincent and the West Coast at 72.4% and 60.7%, respectively (Table 3.9). None of the fish collected from Northern Gulf St. Vincent and South West Spencer Gulf were correctly reclassified, the majority (>70%) were misclassified to South West Gulf St. Vincent. Some individuals were classified as being broadly distributed across the State. For example, 38.5% of fish collected from Northern Spencer Gulf and 28.0% from Kangaroo Island were incorrectly assigned to the West Coast,

representing an extreme separation of >360 km. In both cases, South West Spencer Gulf represents an intermediate region, bridging Northern Spencer Gulf and Kangaroo Island with the West Coast, however none of the misclassified garfish were assigned to this transitional region.

Similar results were found for the subsequent age groupings, where significant regional separation was detected (Age 1, $\Lambda = 0.712$, $p < 0.001$; Age 2, $\Lambda = 0.658$, $p < 0.001$) and overall classification rates remained poor (<32%). Similarly, there was limited evidence of any geographic pattern in some of the misclassified garfish, as they appeared to be classified on the basis of similar environments, rather than reflecting true migratory pathways (Table 3.9). In an attempt to mitigate this, the spatial resolution was further reduced to explore regional population structuring within the confines of each of the two gulfs separately.

Table 3.9. Classification success of the discriminant function analysis incorporating carbon and oxygen isotope values from each age-division of the garfish otoliths. The data presented are the percentage of otoliths from the region of origin (row) classified to each of the six regions (columns)

Juvenile	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**61**	0	14	7	14	4
SWSG	4	**0**	9	70	13	4
NSG	39	0	**27**	23	4	8
SWGSV	3	0	14	**72**	10	0
KI	28	0	16	36	**16**	4
NGSV	0	0	9	83	9	**0**

Age 1	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**56**	6	25	9	3	0
SWSG	0	**32**	0	60	8	0
NSG	55	13	**10**	16	7	0
SWGSV	3	17	20	**40**	13	7
KI	4	12	46	27	**4**	8
NGSV	14	10	31	38	3	**3**

Age 2	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**44**	3	25	16	13	0
SWSG	7	**52**	0	22	19	0
NSG	38	22	**34**	0	3	3
SWGSV	0	27	3	**33**	37	0
KI	7	32	29	11	**21**	0
NGSV	11	37	22	7	22	**0**

Clear regional separation was evident within Spencer Gulf with garfish collected from the South West exhibiting significantly different isotopic signatures than those collected from the North. Mean values of $\delta^{13}C$ and $\delta^{18}O$ in South Western garfish were generally 50% lower than their northern counterparts. This separation was consistent for each of the age groupings (Juv, $\Lambda = 0.787$, $p = 0.004$; Age 1, $\Lambda = 0.658$, $p < 0.001$; Age 2, $\Lambda = 0.610$, $p < 0.001$) (Fig. 3.6). Overall classification success rates were also relatively high and improved with age, increasing from 71.4% ($\kappa = 43.0\%$) for juveniles to 83.1% ($\kappa = 60.6\%$) by age 2. Classification success was marginally (~5%) better for fish collected from the South West.

The population structure of garfish within Gulf St. Vincent was not as clear. Significant regional separation was detected during the early juvenile phase ($\Lambda = 0.819$, $p = 0.006$), separating Kangaroo Island fish from the other two regions with a moderate level of success (52.0%). However, this was not maintained and the initial weak population structure dissipated with age (Age 1, $\Lambda = 0.955$, $p = 0.435$; Age 2, $\Lambda = 0.974$, $p = 0.703$) (Fig 3.6).

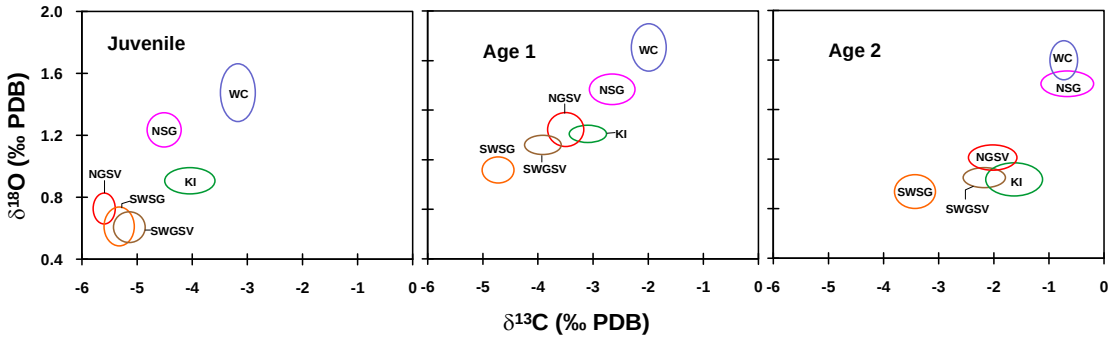

Figure 3.6. Age related mean $\delta^{18}O$ versus $\delta^{13}C$ (± 95% confidence ellipses) for otolith carbonate from southern garfish collected from six regions in South Australia.

3.4 Discussion

Variation in otolith $\delta^{18}O$ has been used for determining the population structure of commercially harvested fish species. Differences in oxygen isotope values among locations are generally explained by relative differences in water temperature. This thermodynamic relationship is typically inversely proportional, with highest mean $\delta^{18}O$ values indicating lowest mean water temperatures (Høie et al. 2003). So, if fish are highly migratory and freely move across temperature gradients then their mean oxygen isotopic signatures will be similar and suggest that the population constitutes a single, mixed stock. Alternatively, if fish occupy a specific location for most of their lives, then they would have a site-specific signature that would be indicative of a level of sub-population structure. This relationship, however, is only consistent if there is no significant variation in the isotopic composition of the ambient water (Ayvazian et al. 2004). Age-related correlations of $\delta^{18}O$ with mean SST for South Australian garfish did not reflect temperature in the manner that would be expected. Given that South Australia's gulfs are inverse estuaries and the shallow enclosed bays of the West Coast are relatively hypersaline, the oxygen isotopic composition of South Australia's coastal waters is highly variable. Therefore, the fundamental assumption of constant salinity that underpins the $\delta^{18}O$ thermodynamic relationship cannot be met and explains why a clear relationship between $\delta^{18}O$ and temperature was not detected. Salinity, however, accounted for regional variation in $\delta^{18}O$, where a change of +1‰ in salinity reflected an increase of approximately 0.17‰ in $\delta^{18}O$. This relationship was temporally stable and as a consequence provides evidence of regional sub-population structure within South Australia's garfish fishery.

Unlike $\delta^{18}O$, no relationship between $\delta^{13}C$ and SST was expected as otolith carbon is considered to be derived from a combination of ambient seawater and metabolic processes (Kalish 1991, Høie et al. 2003). Ontogenetic increases in mean $\delta^{13}C$ values were consistently

observed in all garfish, however the magnitude of these increases significantly differed as a function of where the fish were collected. This was particularly evident in garfish collected from Kangaroo Island, Northern and South Western Spencer Gulf. Such ontogenetic differences may be a result of concomitant changes in the fishes' metabolic rates and diet. Consistent ontogenetic increases in $\delta^{13}C$ have been observed in bluefin tuna (*Thunnus thynnus*), Australian salmon (*Arripis trutta*), jackass morwong (*Nemadactylus macroterus*) and blue grenadier (*Macruronus novaezelandiae*) on the presumption that younger fish have higher metabolic rates resulting in greater depletion of ^{13}C (Kalish 1991). Similarly ontogenetic changes in diet can also account for enriched otolith $\delta^{13}C$. Researchers generally assume that each trophic level is enriched by ~ 1‰ compared to its prey (Peterson and Fry 1987). Thus, as garfish shift from a planktivorous larval stage to an omnivorous diet with an increased dependence on seagrasses (Noell 2005) their otolith $\delta^{13}C$ signature would be expected to increase with age. Spatial differences in diet and feeding habits have previously been detected in adult garfish collected from Gulf St. Vincent (Earl 2007) and, as such, may also account for variation in the observed otolith $\delta^{13}C$ signatures. Regional variation in the magnitude of ontogenetic differences in otolith $\delta^{13}C$ may, therefore, reflect local variation in diet and metabolic processes.

Combined oxygen and carbon stable isotope values yielded distinct spatial isotope signatures at both the site and regional levels relevant to the issues of stock differentiation. Site-specific differences were most evident in garfish collected from the West Coast. All three West Coast bays where garfish were collected are semi-enclosed, with very narrow mouths and extensive tidal sand bars at their entrances. It is, therefore, likely that the high degree of site-fidelity of garfish within these bays is a result of the constraining geography. Garfish collected from Port Broughton (Northern Spencer Gulf), Proper Bay (South West Spencer Gulf), and Emu Bay (Kangaroo Island) also displayed some evidence of site-fidelity, but not to the same extent as the West Coast fish, as was exemplified by their relatively poor reclassification success. Reduced rates of reclassification success of garfish to these sites may be a result of two alternative processes. It may simply be a function of similarities in water chemistry as a result of these sites being more exposed and conducive to a greater level of water mixing. Alternatively, it may be a result of contrasting migratory behaviours where some garfish remain relatively site-attached while others disperse more widely, or at least occupy home ranges that exhibit some degree of overlap with neighbouring groups. Similar migratory strategies have been identified in the Australian herring (*Arripis georgiana*) (Ayvazian et al. 2004) and the Western Australian pilchard (*Sardinops sagax*) (Edmonds and Fletcher 1997), two species that are otherwise considered to be highly migratory. Determining partial

residency is dependent on fine-scale spatial sampling, i.e., how close the sampling areas are to each other, how large the sites are considered to be and the time-frame in which the samples were collected (Edmonds and Fletcher 1997). Given that our sampling occurred over a short period (three-months) and garfish were sampled over a large area in the present study, it is likely that the detection of the weak, fine-scale population structure is a feature of this fishery and that additional, site-specific population components may exist in other sheltered bays throughout the State.

Otolith chemical signatures can be considered powerful discriminators of groups of fish when differences exist, but are of negligible value when differences cannot be detected (Campana 1999). This inherently relies on the physio-chemical characteristics of regional waters being sufficiently different to produce distinctive chemical signatures (Fowler et al. 2005) and is likely to depend on the spatial scale of the investigation. For example, sites that are close to each other may share similar environmental conditions and water chemistry. It is possible that different fish populations inhabit adjacent sites but cannot be differentiated from each other on the basis of their similar chemical signatures. Similarly, sites that are separated by considerable distances but share similar geographic features and oceanography may also have comparable environments. In such situations, the distances between such sites may be large enough that it is highly unlikely that fish actively migrate between them. This seemed to be the case for southern garfish in South Australia that inhabited geographically separated regions characterised by elevated temperatures and abnormally high salinities, specifically those from the West Coast, Northern Spencer Gulf and Northern Gulf St. Vincent. In many cases, discriminant function analysis misclassified garfish to such similar environments, which subsequently reduced the investigation's overall statistical power. If garfish did actively migrate between these regions then the intermediate regions that bridge these geographic extremes would be expected to account for a proportion of the misclassified fish. This would be indicative of a large-scale movement gradient. However, for South Australian garfish there was no clear evidence that fish undertook large-scale migration between such regions, on the basis of the distribution pattern of misclassified fish. As such, garfish from these regions were considered to be independent of each other. In order to remove the confounding effect of these large-scale environmental similarities, the spatial scale of the analyses were constrained to explore movement patterns within each gulf separately.

Under these constraints, clear regional separation was evident in Spencer Gulf with garfish collected from the south west exhibiting significantly different isotopic signatures to those collected from the north. This separation was accentuated with age. The location of garfish spawning grounds and the extent of egg and larval dispersal is currently unknown. Garfish

eggs possess chorionic filaments enabling them to adhere to seagrasses and drift algae (Jordon et al. 1998; Noell 2005). Both embryonic development and the larval stage are relatively long, estimated at ~28 and 10 days, respectively (Jordon et al. 1998; Noell 2005). Therefore, if eggs become attached to drifting of floating vegetation, then the distance that larvae are transported from their origin of spawning could be significantly influenced by local oceanographic conditions (Noell 2005). Thus, at early life history stages garfish may show more similar isotopic signatures.

The same level of regional population structuring was not evident in Gulf St. Vincent. Significant regional separation was detected during the juvenile stage separating Kangaroo Island from the other two regions, but this level of differentiation decreased with age. As Gulf St. Vincent is considerably smaller than Spencer Gulf it is likely to be subjected to a higher level of mixing, diluting any region-specific signatures. Although the gulfs were considered as separate entities to reduce the confounding effect of environmental similarities, the possibility of population connectivity between the two gulfs cannot be excluded. This is particularly relevant for the two southern-most regions of the gulfs which may constitute an area of convergence. Approximately 25% of garfish collected from South West Gulf St. Vincent were misclassified to South West Spencer Gulf, however both regions also share similar water temperatures and salinities (Nunes and Lennon 1986, de Silva Samarasinghe and Lennon 1987), therefore, the degree of connectivity between the two gulfs remains unresolved.

From a fisheries management perspective, otolith oxygen and carbon stable isotope values indicated that the South Australian southern garfish fishery is comprised of multiple, regional, population components that persist through time, or at least through the first two years of the fish's lives. Both the West Coast and Northern Spencer Gulf regions constitute distinct population components that exhibit little inter-regional mixing and therefore may be considered as discrete management units. The South West Spencer Gulf region may also be considered a separate population component, however, its level of connectivity with Gulf St. Vincent is uncertain. With the exception of juvenile garfish from Kangaroo Island, there was no clear regional separation within Gulf St. Vincent.

3.5 References

Ashford, J.R. and Jones, C.M. (2007). Oxygen and carbon stable isotopes in otoliths record spatial isoloation of Patagonian toothfish (*Dissostichus eleginoides*). *Geochimica et Cosmochimica Acta* 71: 87-94.

Ayvazian, S.G., Bastow, T.P., Edmonds, J.S., How, J. and Nowara, G.B. (2004). Stock structure of Australian herring (*Arripis georgiana*) in southwestern Australia. *Fisheries Research* 67: 39-53.

Craig, H. (1957). Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta*. 12: 133-149.

De Silva Samarasinghe, J.R., Bode, L. and Mason, L.B. (2003). Modelled response of Gulf St. Vincent (South Australia) to evaporation, heating and winds. *Continental Shelf Research.* 23: 1285-1313.

Dufour, V., Pierre, C. and Rancher, J. (1998). Stable isotopes in fish otoliths discriminate between lagoonal and oceanic residents of Taiaro Atoll (Tuamotu Archipeligo, French Polynesia). *Coral Reefs* 17: 23-28.

Edmonds, J.S. and Fletcher, W.J. (1997). Stock discrimination of pilchards *Sardinops sagax* by stable isotope ratio analysis of otolith carbonate. *Marine Ecology Progress Series* 152: 241-247.

Edmonds, J.S., Steckis, R.A., Moran, M.J., Caputi, N. and Morita, M. (1999). Stock delineation of pink snapper and tailor from Western Australia by analysis of stable isotope and strontium/calcium ratios in otolith carbonate. *Journal of Fish Biology* 55: 243-259.

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R. and Walther, B.D. (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.

Fowler A.J., Gillanders B.M. and Hall K.C. (2005) Relationship between elemental concentration and age from otoliths of adult snapper (*Pagrus auratus*, Sparidae): implications for movement and stock structure. *Marine and Freshwater Research* 56, 661-676.

Grossman, E.L. and Ku, T.-L. (1986). Oxygen and carbon isotope fractionation in biogenic aragonite: temperature effects. *Chemical Geology.* 59: 59-74.

Guilderson, T.P., Schrag, D.P. and Cane, M.A. (2004). Surface water mixing in the Solomon Sea as documented by a high-resolution coral 14C record. *Journal of Climate* 17: 1147-1156.

Herzka, S.Z. (2005). Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. *Estuarine, Coastal and Shelf Science* 64: 58-69.

Høie, H., Folkvord, A. and Otterlei, E. (2003). Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (*Gadus morhua* L) otoliths. *Journal of Experimental Marine Biology and Ecology* 289: 41-58.

Jones, G.K. (1990). Growth and mortality in a lightly fished population of garfish (*Hyporhamphus melanochir*), in Baird Bay, South Australia. *Transactions of the Royal Society of South Australia.* 114 (1): 37-45.

Jordan A.R., Mills D.M., Ewing G. and Lyle J.M. (1998) Assessment of inshore habitats around Tasmania for life-history stages of commercial finfish species. Fisheries Research and Development Corporation (Project No. 94/037). Canberra, Australia.

Kalish, J.M. (1991). 13C and 18O isotopic disequilibria in fish otoliths: metaboloic and kinetic effects. *Marine Ecology Progress Series* 75: 191-203.

Knight, M.A., Doonan, A.M., and Tsolos, A. (2007). South Australia wild Fisheries Information and Statistics Report. SARDI Aquatic Sciences Publication No. F2007/000571-1

McConnaughey, T.A. and Gillikin, D.P. (2008). Carbon isotopes in mollusk shell carbonates. *Geo-Mar Lett* 28: 287-299.

McGarvey R., Feenstra J.E., Fowler A.J. and Jackson W.B. (2006) Garfish (*Hyporhamphus melanochir*) Fishery. Fishery Assessment Report to PIRSA for the Marine Sclaefish Fishery Management Committee. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, RD04/0152-2. SARDI Report Series No. 163.

Nelson, C.S., Northcote, T.G. and Hendy, C.H. (1989). Potential use of oxygen and carbon isotopic composition of otoliths to identify migratory and non-migratory stocks of the New Zealand common smelt: a pilot study. *New Zealand Journal of Marine and Freshwater Research.* 23: 337-344.

Newman, S.J., Steckis, R.A., Edmonds, J.S. and Lloyd, J. (2000). Stock structure of the goldband snapper *Pristipomoides multidens* (Pisces: Lutjanidae) from the waters of northern and western Australia by stable isotope ratio analysis of sagittal otolith carbonate. *Marine Ecology Progress Series* 198: 239-247.

Noell C.J. (2005) Early life stages of the southern garfish *Hyporhamphus melanochir* (Valenciennes, 1846) and their association with seagrass beds. PhD Dissertation (University of Adelaide, Adelaide, South Australia).

Noell C., Presser J. and Jones K. (2006) Management Plan for the South Australian Marine Scalefish Fishery. PIRSA. South Australian Fisheries Management Series No. 45, 68 pp.

Nunes, R.A. and Lennon, G.W. (1986). Physical property distributions and seasonal trends in Spencer Gulf, South Australia: an inverse estuary. *Australian Journal of Marine and Freshwater Research.* 37: 39-53.

Nunes Vaz R.A., Lennon G.W. and Bowers D.G. (1990) Physical behaviour of a large, negative or inverse estuary. *Continental Shelf Research* 10(3), 277-304.

Peterson, B.J. and Fry B. (1987). Stable isotopes in ecosystem studies. *Annual Reviews of Ecology and Systematics* 18: 293-320.

Stephenson, P.C., Edmonds, J.S., Moran, M.J. and Caputi, N. (2001). Analysis of isotope ratios to investigate stock structure of red emperor and Rankin cod in northern Western Australia. *Journal of Fish Biology* 58: 126-144.

Tanaka, N., Monaghan, M.C. and Rye, D.M. (1986). Contribution of metabolic carbon to mollusc and barnacle shell carbonate. *Nature* 320: 520-523.

Thorrold, S.R., Campana, S.E., Jones, C.M. and Swart, P.K. (1997). Factors determining 13C and 18O fractionation in aragonite otoliths of marine fishes. *Geochimica et Cosmochimica Acta* 61(14): 2909-2919.

Titus U., Mosher J.A and Williams B.K. (1984) Chance-corrected classification for use in discriminant analysis: ecological applications. *The American Midland Naturalist* 111, 1-7.

Weidman, C.R. and Millner, R. (2000). High-resolution stable isotope records from North Atlantic cod. *Fisheries Research* 46: 327-342.

Wurster, C.M., Patterson, H.M. and Cheatham, M.M. (1999). Advances in micromilling techniques: a new apparatus for acquiring high-resolution oxygen and carbon isotope values and major/minor elemental ratios from accretionary carbonate. *Computers and Geosciances* 25: 1159-1166.

4 GEOGRAPHIC VARIATION OF SOUTHERN GARFISH *HYPORHAMPHUS MELANOCHIR* IN SOUTH AUSTRALIAN WATERS BASED ON OTOLITH MORPHOMETRICS AND SHAPE ANALYSIS.

MA Steer

4.1 Introduction

Morphological and meristic characteristics of fish and their hard body parts have been commonly used to infer phenotypic stock structure for a range of marine and freshwater species (Blouw et al. 1988, Tuset et al. 2003; Stransky et al. 2007). Linear and shape morphometrics of otoliths have proven useful in stock discrimination studies as their growth is highly correlated to somatic growth, they are not resorbed or altered through time, and they remain unaffected by short-term changes in fish condition which may confound body morphometrics (Campana and Neilson 1985; Campana and Casselman 1993). Although it is expected that gross otolith morphology is genetically controlled and changes as the fish ages, geographical variation in shape is almost certainly environmentally driven by factors such as depth (Torres et al. 2000; Gauldie and Crampton 2002), water temperature (Lombarte et al. 2003) and food availability (Gagliano and McCormick 2004).

There have been a variety of otolith shape descriptors that have been used to discriminate putative fish stocks. These range from simple one-dimensional 'land-mark' methods, such as linear measurements and truss networks (Turan 2000; Begg et al. 2001), to more complicated two-dimensional 'outline methods', such as Fourier analysis (Stransky et al. 2007; Burke et al. 2008). Recent advances in digital technology and image analysis software have made it quicker and easier to collect multivariate, two-dimensional, shape data from digitised images with better precision and accuracy than more traditional methods (Cadrin and Friedland 1999). As such, otolith morphometric and shape analyses have been incorporated into more recent multidisciplinary stock discrimination studies (Bergenius et al. 2005; Turan 2006; Stransky et al. 2007; Burke et al. 2008) and are continuing to be developed (Parisi-Baradad et al. 2005; Piera et al. 2005).

This study aims to use otolith morphometrics and shape analysis to explore the population structure of southern garfish (*Hyporhamphus melanochir*) in South Australia. So far, five stock discrimination methods have either directly or indirectly addressed aspects of the population structuring of southern garfish. Collette (1974) was the first to report meristic differences in garfish across its entire geographic range (southern Australia) and was able to separate eastern and western populations on the basis of vertebral counts. An analysis of

stock structure based on mitochondrial DNA found low-level population sub-division across a similar geographic gradient, however was unable to differentiate genetic stocks within the South Australian gulfs (Donnellan et al. 2002). Similarly, Fowler et al. (2008) was unable to detect geographic differences in growth rates between the main commercial fishing areas in the two gulfs, and therefore could not exclude the possibility of large spatial scale mixing. More recently, otolith chemistry (i.e. trace elements and stable isotopes) studies suggested that South Australia's garfish population can be discriminated at a much finer spatial scale and provided evidence of regional population structuring within the gulfs that exhibit various levels of intermixing (Chapters 2 and 3). Collectively, these results introduce a level of uncertainty and indicate that further investigation is required to clarify the level of population structuring within the South Australian garfish fishery. This study aimed to address this uncertainty by exploring whether there is any geographic variation in the shape of garfish otoliths that can delineate any population sub-structuring.

4.2 Materials and Methods

4.2.1 Sample collection

Refer to section 2.2.1 for sample collection. One sagitta from each otolith pair was randomly selected, weighed to the nearest 0.01 mg, and used for shape and morphometric analysis. The remaining otolith from each pair was used in companion otolith chemistry studies (Chapters 2 and 3).

4.2.2 Image and shape analysis

Otolith images were digitised using a high-resolution video camera mounted onto a Leica stereo microscope at 10x magnification. Each otolith was positioned sulcus side up with the rostrum horizontally aligned. High-contrast images were produced under reflected light, so that the otolith was represented as a bright, white, two-dimensional object on a dark background. The image was digitally captured using Optimas (Version 6.5) image analysis software. As otoliths were randomly selected it was necessary to horizontally flip some images using standard image analysis techniques to ensure that they were all consistently aligned, reducing potential distortion errors within the normalisation process. The area (A), maximum length (L), maximum width (W) and perimeter (P) of each otolith were measured using the calibrated image analysis software. From these univariate measurements, five otolith morphometrics were calculated: the coefficent of form, which is a mean that estimates the irregularity of the surface area from a scale of 0 to 1, where 1 is a perfect circle; roundness

and circularlity, which provide information of the otolith's features to a perfect circle at a value of 1 and 4π (~12.6), respectively; rectangularity, which describes the variations in length and width with respect to area; and ellipticity, which indicates whether the changes in the axes are proportional (Tuset et al. 2003) (Table 4.1).

Table 4.1. Otolith morphometric indices (from Tuset et al. 2003).

Morphometric	Formulae
Coefficient of Form	$(4\pi A) / P^2$
Roundness	$(4 A) / (\pi L^2)$
Circularity	P^2 / A
Rectangularity	A / (L x W)
Ellipticity	(L – W) / (L + W)

The digitised otolith images were imported into the program 'Shape version 1.3', which used a 'chain coding' algorithm to extract the numerical contour of each otolith outline in preparation for elliptical Fourier analysis (EFA) (Iwata and Ukai 2002). EFA uses an orthogonal decomposition of a curve into a sum of harmonically related ellipses that when combined approximate the original otolith shape. Each harmonic is characterised by four coefficients (*A, B, C, D*), the higher the number of harmonics, the greater the accuracy of the outline description (Kuhl and Giardina 1982). The elliptical Fourier descriptors were calculated for each digitised otolith image using the 'Shape' software. This software also normalises the descriptors to make them invariant to otolith size and relative orientation. The number of harmonics required to sufficiently reconstruct the otolith outline was estimated from the mean Fourier power spectrum (FP) which provides a measure of the amount of 'shape information' described by the harmonic (Pothin et al. 2006, Mérigot et al. 2007). For the n^{th} harmonic the Fourier power is expressed as:

$$FP_n = (A_n^2 + B_n^2 + C_n^2 + D_n^2) / 2 \qquad [4.1.]$$

where A_n, B_n, C_n and D_n are the Fourier coefficients of the n^{th} harmonic. Then, the cumulated power percentage (FP_c) defined by:

$$FP_c = \sum_1^n FP_n \qquad [4.2.]$$

was calculated (Pothin et al. 2006). The number of harmonics that reach >99% of the total variation of FP is the total number of harmonics required for subsequent analyses (Pothin et

al. 2006). In this study, the total number of harmonics was determined from a random sub-sample of 30 otoliths. The first ten harmonics exceeded 99.99% of the mean cumulated power, indicating that the shape of garfish otoliths can be adequately summarised by 40 Fourier descriptors (10 harmonics x 4 coefficients) (Fig. 4.1). As each otolith was normalised by the 'Shape' program, the first three coefficients (A_1, B_1, and C_1) were constant for all outlines, therefore reducing the number of Fourier descriptors to 37.

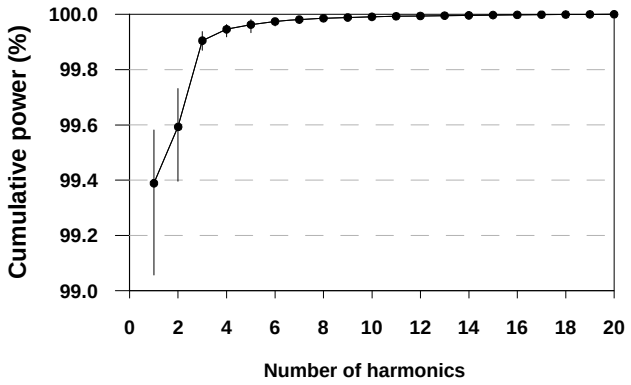

Figure 4.1. Variation in the mean cumulative power (%) of Fourier power in relation to the number of harmonics describing the overall shape of garfish otoliths. Vertical bars indicate minimum and maximum range of the cumulative power.

4.2.3 Data analyses

All garfish used in this study were from the same age class (2+) and as such were unlikely to display any ontogenetic differences in otolith shape. However, there was significant spatial variation in garfish size at both the site (Kruskal-Wallis: $\chi^2 = 76.64$, df = 17, $p < 0.001$) and region ($\chi^2 = 45.46$, df = 5, $p < 0.001$) level. Furthermore, all five otolith morphometrics were significantly (Pearson's; $p < 0.001$) correlated to garfish size. To ensure unbiased comparisons between groups, the effect of fish size was standardised by statistically removing the pooled among-group slope (*b)* of garfish total length on all otolith morphometrics (Thorpe 1975, Lleonart et al. 2000). This transformation is considered one of the best for removing allometric bias and is appropriate when there are differences in fish size among the groups examined (Thorpe 1975). Gender was not a potential confounding source of variation ($p > 0.05$).

Otolith morphometrics and the Fourier descriptors were treated separately and combined for comparative analysis. All data were normally distributed (Kolmogorov-Smirnov test of normality; $p > 0.05$), therefore no data transformations were necessary. Geographic variation in otolith shape was compared between sites nested within regions and among regions using

multivariate analysis of variance and discriminant function analysis (DFA). Multiple univariate analyses were used to explore the relative contribution of each of the shape indices and Fourier descriptors. As these tests were non-independent of each other, there is an increased probability of making type I errors. To account for this, the probability values were adjusted using the Bonferroni method. Post hoc Hochberg's multiple comparison tests were carried out where applicable to determine the nature of significant differences. DFA's were carried out to assess whether the site of capture of each garfish could be reliably determined from their shape descriptors. Due to the large number of predictors (five otolith morphometrics and 37 Fourier descriptors in the combined analysis), which exceeded the number of samples in the smallest group, step-wise DFA was used to determine the most important predictors for the discriminant group membership. The entry of predictors to the analysis was determined by the statistical entry of Wilk's lambda (Λ) with a $p_{(entry)} = 0.05$ and $p_{(exit)} = 0.25$. Classification success was calculated using jack-knifed cross-validation. The analysis was conducted at both the site and region levels. The performance of discriminant functions was evaluated using Cohen's Kappa (κ) statistic, which provides an objective means of calculating the chance-corrected percentage of agreement between actual and predicted group memberships. Values of κ range from 0 to 1, with 0 indicating the discriminant function yields no improvement over chance, and 1 indicating perfect agreement (Titus et al. 1984).

4.3 Results

4.3.1 Spatial variation in otolith shape

There was significant spatial variation in the shape of two-year old garfish otoliths, with multivariate analyses identifying significant variation among site within region for both the morphometric data and elliptical Fourier descriptors (Table 4.2). Of the five morphometric indices otolith ellipticity was the only descriptor to significantly differ among sites within regions, particularly among sites within Kangaroo Island, the West Coast and South West Gulf St. Vincent (Table 4.2, Fig. 4.2a). Otolith roundness, varied among regions, whereas otolith circularity, coefficient of form and rectangularity did not exhibit any spatial variation (Table 4.3). All otoliths, with exception of those collected from Kangaroo Island garfish, exceeded a mean roundness index of 0.59 with Northern Gulf St. Vincent garfish exhibiting the roundest (> 0.61) otoliths (Fig. 4.2b). One Fourier descriptor (A_4,) displayed significant variation among sites within regions and three among (D_1, A_3, B_{10},) regions (Table 4.4).

Table 4.2. Results of multivariate analyses of variance testing for spatial differences otolith shape using morphometric indices and Fourier descriptors separately. ns: not significant, *p < 0.05, ** p < 0.01, ***p < 0.001.

Variable	Value	df	df_{error}	F
Morphometrics				
Region	0.336	25	1100	3.174***
Site (Region)	0.353	60	1100	1.391*
Fourier descriptors				
Region	1.143	185	940	1.506***
Site (Region)	2.306	444	2340	1.254***

Table 4.3. Results of the univariate ANOVA testing for spatial differences in the shape of garfish otoliths, for five morphometric indices. * indicates significance for Bonferroni corrected p value (p < 0.013).

Source	Variable	MS	df	F
Region	Coeff. Form	0.25	5	2.58
	Roundness	0	5	5.39*
	Circularity	1.34	5	2.52
	Rectangularity	0	5	1.39
	Ellipticity	0	5	8.36*
Site (Region)	Coeff. Form	0.11	12	1.14
	Roundness	0	12	0.84
	Circularity	0.6	12	1.13
	Rectangularity	0	12	1.57
	Ellipticity	0	12	2.21*

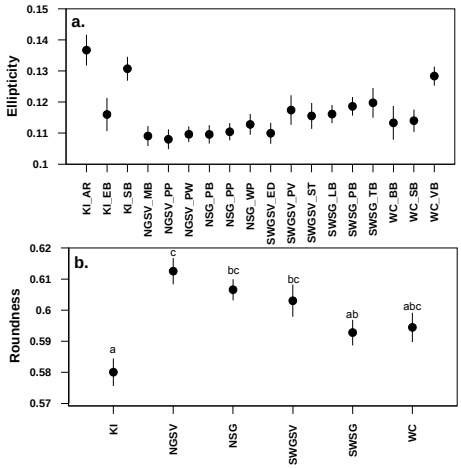

Figure 4.2. Spatial variation in otolith morphometrics. Lower case letters indicate results of *post hoc* comparison.

Table 4.4. Results of the univariate ANOVA testing for spatial differences in the shape of garfish otoliths, for the 37 Fourier descriptors. Only significant results for Bonferroni corrected p value (p < 0.0013) are shown.

Source	Variable	MS	df	F
Region	D1	4.70E-03	5	7.23
	A3	4.40E-04	5	5.42
	B10	2.00E-05	5	4.52
Site(Region)	A4	7.40E-05	12	3.48

4.3.2 Fine-scale spatial discrimination

Discriminant function analysis of the morphometric data detected significant variation in otolith shape among sites ($\Lambda = 0.645$, $p < 0.001$). Two discriminant functions explained all of the between-group variability, however only 18.1% of the individuals were successfully classified to their site of origin, a success rate (κ) that was only 5% better than chance (Table 4.5). Separation along the first discriminant function was mainly driven by differences in ellipticity, whereas otolith coefficient of form and circularity contributed equally to the second discriminant function (Fig. 4.3). Classification success was moderately improved to 24.8% ($\kappa = 20\%$) when the normalised elliptical Fourier descriptors were separately analysed. This analysis had higher discriminant power ($\Lambda = 0.333$, $p < 0.001$) and was explained by six functions, of which the first two explained 60.4% of the within-group variability. Both of these functions were separated by low order Fourier descriptors (D_1, B_3, and C_2, and D_2, respectively) indicating that sites were separated on the basis of their overall elliptical shape, rather than by complex morphologies. Combining both the morphometric and elliptical Fourier datasets further improved the model's discriminant power ($\Lambda = 0.175$, $p < 0.001$) and classification success (35.3%, $\kappa = 31\%$). The first two discriminant functions explained 52% of the variation and otolith ellipticity and low order Fourier descriptors continued to contribute to the separation of sites along these two axes. Classification success exceeded 50% for garfish collected from American River and Shoal Bay at Kangaroo Island and Venus Bay on the West Coast (Table 4.6). Despite poor classification success of the combined analysis, variation in otolith shape was evident for garfish collected from sites at Kangaroo Island, South West Gulf St. Vincent and the West Coast (Fig. 4.5).

Table 4.5. Summary of statistics from discriminant function analyses exploring fine-scale (site) and broad-scale (region) spatial differences in otolith shape incorporating morphometric data and ellipitical Fourier descriptors (EFD) separately and combined.

		Morph	EFD	Combined
Region	*Λ*	0.783	0.532	0.465
	P	<0.001	<0.001	<0.001
	Success (%)	29.4	39.9	44.5
	κ	0.13	0.27	0.32
Site	*Λ*	0.645	0.333	0.175
	P	<0.001	<0.001	<0.001
	Success (%)	18.1	24.8	35.3
	κ	0.05	0.2	0.31

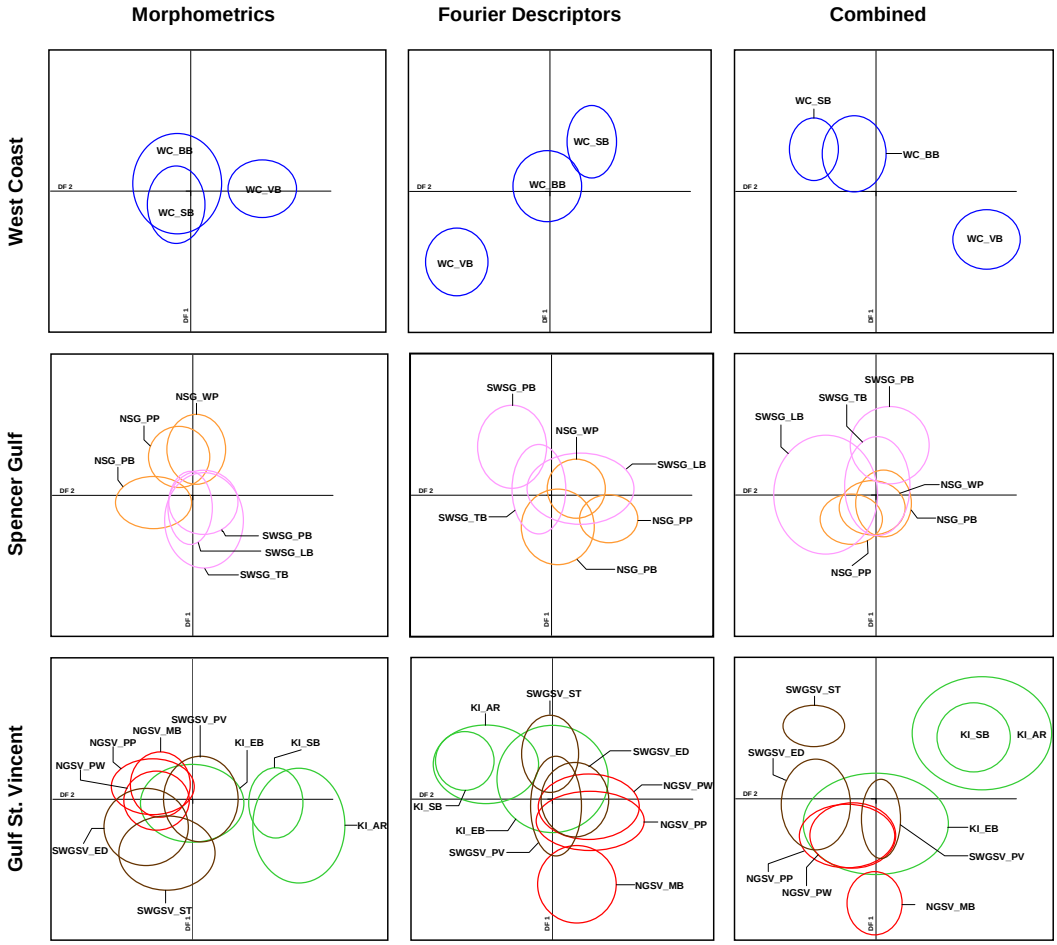

Figure 4.3. Discriminant function centroids with 95% confidence ellipses for southern garfish otoliths collected from 18 sites within six regions in South Australia, based otolith morphometrics and elliptical Fourier descriptors separately and combined. Figures were separated for the West Coast, Spencer Gulf and Gulf St. Vincent for clarity.

Table 4.6. Classification success of the discriminant function analysis incorporating all otolith morphometric indices and elliptical Fourier descriptors. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the 18 sites (columns)

Combined	WC_BB	WC_SB	WC_VB	SWSG_LB	SWSG_PB	SWSG_TB	NSG_PB	NSG_PP	NSG_WP	SWGSV_ED	SWGSV_PV	SWGSV_ST	KI_AR	KI_EB	KI_SB	NGSV_MB	NGSV_PP	NGSV_PW
WC_BB	**18**	27	9	0	0	0	18	9	0	18	0	0	0	0	0	0	0	0
WC_SB	5	**45**	5	0	10	0	5	0	15	5	0	0	0	5	0	0	5	0
WC_VB	0	0	**71**	0	0	0	0	6	6	0	0	0	0	6	12	0	0	0
SWSG_LB	0	10	0	**40**	20	0	0	0	0	10	10	0	0	0	0	10	0	0
SWSG_PB	0	7	0	0	**33**	0	0	7	13	0	0	7	7	13	13	0	0	0
SWSG_TB	0	0	14	0	0	**0**	0	0	29	14	0	0	14	14	0	0	14	0
NSG_PB	6	6	0	0	6	0	**44**	6	13	0	6	0	0	0	6	0	6	0
NSG_PP	7	7	0	0	0	0	14	**21**	43	0	0	0	0	0	0	7	0	0
NSG_WP	0	4	13	0	4	0	8	8	**33**	4	0	0	8	4	4	0	8	0
SWGSV_ED	0	12	6	6	0	0	6	0	18	**24**	0	12	0	0	0	6	6	6
SWGSV_PV	0	0	10	10	0	0	0	10	50	0	**20**	0	0	0	0	0	0	0
SWGSV_ST	0	30	0	0	0	0	20	0	0	20	0	**30**	0	0	0	0	0	0
KI_AR	0	0	0	11	0	0	0	0	11	0	0	0	**56**	0	22	0	0	0
KI_EB	0	0	13	0	0	0	0	0	25	0	13	0	0	**38**	13	0	0	0
KI_SB	7	7	7	0	0	0	0	0	14	7	0	0	7	0	**50**	0	0	0
NGSV_MB	0	0	17	0	0	0	8	8	0	0	0	0	0	0	0	**42**	17	8
NGSV_PP	0	0	0	7	7	0	0	0	21	14	0	7	0	0	0	7	**36**	0
NGSV_PW	0	10	0	0	10	0	10	20	10	10	10	0	0	0	0	10	10	**0**

4.3.3 Broad-scale spatial discrimination

Analysis of the morphometric data identified significant regional variation in otolith shape ($\Lambda = 0.783$, $p < 0.001$). Between-group variation was explained by two discriminant functions with otolith ellipticity and roundness accounting for the majority of the separation on the first discriminant axis and coefficient of form and circularity on the second axis (Fig. 4.4). Classification success was poor at 29.4%, a result that was 13% better than chance (Table 4.7). A separate discriminant analysis of the normalised Fourier descriptors improved classification success to 39.9% ($\kappa = 27\%$). Northern Gulf St. Vincent and Northern Spencer Gulf were the only two regions where classification success exceeded 50%. The model's power was also improved ($\Lambda = 0.532$, $p < 0.001$) and described five discriminant functions in which the first two accounted for 70.0% of the variation. Low-order Fourier descriptors (D_1, B_3, and C_5) accounted for the majority of the separation along the first axis, whereas higher order descriptors (B_{10}, B_8) accounted for separation along the second axis. These data indicate that, on mean, garfish otoliths from Kangaroo Island were more elliptical in shape in comparison to Northern Gulf St. Vincent garfish, and otoliths from the West Coast were more complex and sculptured compared to those from South West Spencer Gulf (Fig. 4.4). Combining both datasets provided the most powerful result ($\Lambda = 0.465$, $p < 0.001$), which

successfully reclassified 44.5% (κ = 32%) of garfish to their region of origin. Classification success ranged from 27.0% for South West Gulf St. Vincent garfish to 55.6% for Northern Spencer Gulf garfish (Table 4.7). The first two discriminant functions explained 64.0% of the variation, and ellipticity, roundness and D_1, and Fourier descriptors B_{10}, B_8 accounted for the majority of separation along each of the axes, respectively (Fig. 4.4).

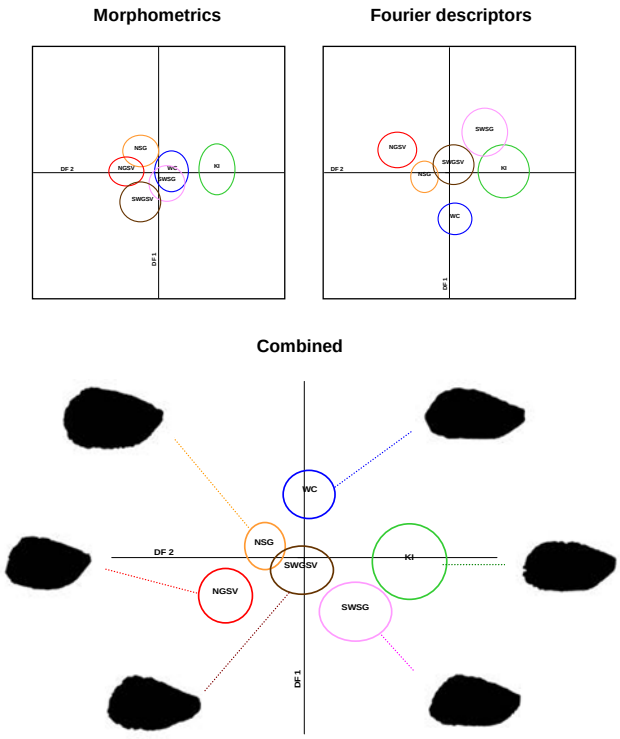

Figure 4.4. Discriminant function centroids with 95% confidence ellipses for southern garfish otoliths collected from six regions in South Australia, based on otolith morphometrics and elliptical Fourier descriptors separately and combined. Otolith silhouettes are representative of individuals at the extremities of each regional grouping.

Table 4.7. Classification success of the discriminant function analysis incorporating all otolith morphometric indices and elliptical Fourier descriptors separately and combined. The data presented are the percentage of otoliths from the region of origin (row) classified to each of the six regions (columns).

Combined	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**29**	0	33	8	23	6
SWSG	31	**0**	41	13	9	0
NSG	17	0	**57**	13	9	4
SWGSV	16	0	35	**32**	11	5
KI	32	0	23	7	**39**	0
NGSV	11	0	61	22	3	**3**

Morph.	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**46**	4	29	6	13	2
SWSG	9	**38**	19	13	13	9
NSG	20	4	**52**	9	6	9
SWGSV	16	14	35	**5**	11	19
KI	26	13	16	3	**36**	7
NGSV	11	11	14	8	0	**56**

EFD	WC	SWSG	NSG	SWGSV	KI	NGSV
WC	**50**	4	31	6	8	0
SWSG	9	**34**	22	9	13	13
NSG	17	6	**56**	7	7	7
SWGSV	14	14	24	**27**	11	11
KI	16	13	16	0	**45**	10
NGSV	11	14	14	6	8	**47**

4.4 Discussion

This study revealed high variation in the shape of garfish otoliths and weak signals for geographic separation. Despite significant site- and region-specific separation of garfish in discriminant space, overall classification success of fish to their site of capture was poor ranging from 18.1 – 35.3% and 29.4 – 44.5%, respectively. Elliptical Fourier analysis exhibited the greatest sensitivity in describing otolith shape compared to the linear morphometric descriptors, however, combining the two datasets into a single analysis improved discriminant power and classification success. Classification values of more than 75% are generally considered acceptable in stock discrimination studies (Friedland and Reddin 1994, Stransky et al. 2007, Villegas-Hernández et al. 2008) although there have been a number of studies that have inferred population sub-structuring despite poor to moderate classification scores (Tuset et al. 2003, Stransky 2005, Galley et al. 2006, Jónsdóttir et al. 2006). In these cases, inferences were based on fish being incorrectly classified to adjacent areas (Galley et al. 2006, Jónsdóttir et al. 2006), or by achieving improved results through expanding the spatial resolution of the study (Stransky 2005). Expanding the spatial resolution in this study from the site to regional level marginally improved classification success by 9.2% and there was limited evidence of any geographic pattern in the distribution of mis-classified garfish.

Movement of southern garfish is suggested to approximate a one-dimensional "stepping-stone" model in which neighbouring sub-populations exchange genes (Donellan et al. 2002). However, it does not appear to be limited strictly to adjacent sub-populations as in many cases garfish were misclassified to originate from distant regions. For example, 31% of garfish collected from the West Coast were misclassified as originating from Northern Spencer Gulf and only 4% from South West Spencer Gulf which bridges the two regions. A 'modified stepping-stone model' has been identified in the population structure of red drum (*Sciaenops occellatus*), where movement occurs beyond adjacent regions (Gold et al. 2001). It is possible that a similar pattern exists for South Australian garfish. A more likely alternative may be that similarities in otolith shape among regions are reflective of fish inhabiting, or moving through, similar environments (Stransky 2005, Peturnsdottir er al. 2006) and exhibiting comparable growth rates, as was detected by Fowler et al. (2008). Interpreting patterns of variation in otolith shape analysis within a species is extremely challenging since it is difficult to dissociate the interactive influence of genetics and the environment (Stransky et al. 2007). This is exemplified by Cadrin and Friedland (1999) who noted that "understanding the reason for subtle, but statistically significant, shape differences is abstract". Furthermore, shape analysis does not have the capability to determine whether

individuals are misclassified because of inherent methodological error or because the fish have legitimately strayed from another area (Campana and Casselman 1993).

The results of this study were sufficient to indicate some level of population structuring within South Australia's garfish fishery, as fish were correctly classified at rates that were 32% better than would be expected by chance. Six putative regional components, with varying levels of classification success, were detected based on differences in otolith shape. On mean, otoliths from the southern-most regions of the two gulfs were rounder and more elliptical in shape than their northern counterparts, whereas otoliths collected from West Coast garfish were the most complex and sculptured. Variation in otolith shape has been identified at similar spatial scales for a variety of marine fishes (Petursdottir et al. 2006, Pothin et al. 2006, Mérigot et al. 2007). The regional patterns in otolith shape observed in this study are in part consistent with the results from a companion otolith chemistry study, which identified the same six population components but with a greater degree of confidence (Chapter two). The major difference, however, is that unlike otolith chemistry which is inextricably linked to the physiochemical environment (Campana and Thorrold 2001), variation in otolith shape is also influenced by genetics (Cardinale et al. 2004). Given that there was no evidence of genetic differentiation in garfish collected from gulf waters (Donnellan et al. 2002), it is likely that there were underlying genetic similarities in otolith shape that contributed to reduced discriminant power.

The migratory capabilities of garfish from the genus *Hyporhamphus* is poorly understood. The southern garfish's most closely related congener, *H. australis*, is considered to form a single migratory stock, which moves up and down the New South Wales coast in response to the East Australian current (Hughes and Stewart 2006). Conversely, the river garfish, *H. regularis*, is confined to estuaries (Jones et al. 1996). The migratory pattern of the southern garfish appears to fall somewhere in-between, as there is evidence of weak regional sub-structuring within a genetically-uniform population. It is possible that this pattern is a result of co-existing migratory and residential behaviours (Secor 1999). Although sympatric migration and resident behaviour is more commonly documented for anadromous fish (e.g. Salmonids), it has also been identified for some marine species such as Atlantic cod (*Gadus morhua*) and Bluefin tuna (*Thunnus thynnus*). This is thought to be an evolutionary strategy that minimises the risks associated with inhabiting an unpredictable and variable environment (Secor 1999). The southern garfish is closely associated with Zosteracean seagrass, relying on it as a source of food (Earl 2007) and potential spawning habitat (Noell 2005). The most extensive seagrass meadows in South Australia occur in northern gulf waters and become discontinuous and patchy further south where the bottom habitat is predominantly calcareous

reef and algae (Edyvane 1999). In systems where there is discontinuous habitat suitability there is potential for groups of fish to disperse across unfavourable habitats and colonise new areas. This divergent migration may be influenced by ontogenetic shifts, population density, habitat value or an increased proclivity for dispersal (Secor 1999). Previous research has identified significant gender-specific differences in the spatial distribution and schooling behaviour of garfish during the spawning season (Ye et al. 2002). Females tended to form large schools in shallow (<5 m) waters, whereas males were more highly dispersive, patchy in distribution and occupied deeper (>5 m) offshore waters. Although no gender-specific differences in otolith shape were detected in the present study, alternate schooling behaviour between the sexes is indicative of divergent migration. Furthermore, South Australia's commercial garfish fishers have historically speculated that two behavioural types exist and have differentiated 'residential' and 'vagrant' garfish on the basis of their colour and external appearance (Florance pers. comm.). Garfish that exhibit brown hues, particularly on their ventral side, and are "dirty" in appearance are considered to be residential, whereas highly reflective, metallic blue coloured individuals are thought to have migrated from elsewhere.

Weak spatial differences in otolith shape indicated that groups of garfish had spent parts of their lives in different environments and that there was some level of restriction that prevented complete mixing among the regions. As such there is some evidence to suggest that the management framework that currently identifies two discrete stocks be restructured to align with these smaller spatial units. High levels of exploitation in any of these regions can potentially lead to localised depletion as unlike a completely mixed stock these regional population components have a lower compensatory potential of being replenished by fish emigrating from other regions (Turan 2006).

4.5 References

Begg, G.A., Overholtz, W.J. and Munroe, N.J. (2001). The use of internal otolith morphometrics for identification of haddock (*Melanogrammus aeglefinus*) stocks on Georges Bank. *Fishery Bulletin* 99: 1-14.

Bergenius, M.A.J., Begg, G.A. and Mapstone, B.D. (2005). The use of otolith morphology to indicate the stock structure of common coral trout (*Plectropomus leopardus*) on the Great Barrier Reef, Australia. *Fishery Bulletin* 104: 498-511.

Blouw, D.M., Saxon, S.D., Chadwick, E.M.P. (1988). Temporal variation of meristic traits within an Atlantic salmon (*Salmo salar*) stock, and implications for stock identification. *Canadian Journal of Fisheries and Aquatic Sciences.* 45: 1330-1339.

Burke, N., Brophy, D. and King, P.A. (2008). Shape analysis of otolith annuli in Atlantic herring (*Clupea harengus*): a new method for tracking fish populations. *Fisheries Research*.

Cadrin, S.X. and Friedland, K.D. (1999). The utility of image processing techniques for morphometric analysis and stock identification. *Fisheries Research* 43: 129-139.

Campana, S.E. and Neilson, J.D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences.* 42: 1014-1032.

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

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

Cardinale, M., Doering-Arjes, P., Kastowsky, M. and 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.

Collette, B.B. (1974). The garfishes (Hemiramphidae) of Australia and New Zealand. *Records of the Australian Museum.* 29(2): 11-105.

Donnellan S., Haigh L., Elphinstone M., McGlennon D. and Ye, Q. (2002) Genetic discrimination between southern garfish (*Hyporhamphus melanochir*) stocks of Western Australia, South Australia, Victoria and Tasmania. In 'Fisheries biology and habitat ecology of southern garfish (*Hyporhamphus melanochir*) in southern Australian waters. (eds. Jones, G.K., Ye, Q., Ayvazian, S., Coutin, P.). FRDC Final Report 97/133.

Earl J. (2007) Feeding biology of the southern garfish (*Hyporhamphus melanochir,* Hemiramphidae) in Gulf St. Vincent, South Australia. Honours Thesis (Flinders University, Adelaide, South Australia).

Edyvane, K.S. (1999). Conserving marine biodiversity in South Australia Part 2 - Identification of areas of high conservation value in South Australia. Urrbrae: South Australian Research and Development Institute.

Fowler, A.J., Steer, M.A., Jackson, W.B. and Lloyd, M.T. (2008). Population characteristics of southern sea garfish (*Hyporhamphus melanochir*, Hemiramphidae) in South Australia. *Marine and Freshwater Research* 59: 429-443.

Friedland, K.D. and Reddin, D.G. (1994). Use of otolith morphology in stock discriminations of Atlantic salmon (*Salm salar*). *Canadian Journal of Fisheries and Aquatic Sciences..* 51: 91-98.

Gagliano, M. and McCormick., M.I. (2004). Feeding history inflences otolith shape in tropical fish. *Marine Ecology Progress Series* 278: 291-296.

Galley, E.A., Wright., P. and Gibb, F.M. (2006). Combined methods of otolith shape analysis improve identication of spawning areas of Atlantic cod. *ICES Journal of Marine Science.* 63: 1710-1717.

Gauldie, R.W. and Crampton, J.S. (2002). An eco-morphological explanation of individual variability in the shape of the fish otolith: comparison of the otolith of *Hoplostethus atlanticus* with other species by depth. *Journal of Fish Biology*. 60 (5): 1204-1221.

Gold, J.R., Burridge, C.P. and Turner, T.F. (2001). A modified stepping stone model of populationstructure in red drum, *Sciaenops ocellatus* (Sciaenidae), from the northern Gulf of Mexico. *Genetica* 111: 305-317.

Hughes, J.M. and Stewart, D.J. (2006). Reproductive biology of three commercially important Hemiramphid species in south-east Australia. *Environmental Biology of Fishes* 75: 237-256.

Iwata, H. and Ukai, Y. (2002). SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptical Fourier descriptors. *The Journal of Heredity* 93(5): 384-385.

Jones, G.K., Baker, J.L., Edyvane, K. and Wright, G.J. (1996) Nearshore fish community of the Port River-Barker Inlet estuary, South Australia. I. Effect of thermal effluent on the fish community structure, and distribution and growth of economically important fish species. *Marine and Freshwater Research.* 47: 785-800.

Jónsdóttir, I., Campana, S.E. and Marteinsdottir, G. (2006). Otolith shape and temporal stability of spawning groups of Icelandic cod (*Gadus morhua* L.). *ICES Journal of Marine Science.* 63: 1501-1512.

Kuhl, F.P. and Giardina, C.R. (1982). Elliptical Fourier features of a closed contour. *Computer Graphics and Image Processing* 18: 236-258.

Lombarte, A., Torres G.J., and Morales-Nin, B. (2003). Specific *Merluccius* otolith growth patterns related to phylogenetics and environmental. *Journal of the Marine Biological Association, U.K.* 83: 277-281.

Mérigot, B., Letourner, Y. and Lecomte-Finiger, R. (2007). Characterization of local populations of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis/. *Marine Biology* 151: 997-1008.

Noell C.J. (2005) Early life stages of the southern garfish *Hyporhamphus melanochir* (Valenciennes, 1846) and their association with seagrass beds. PhD Dissertation (University of Adelaide, Adelaide, South Australia).

Parisi-Baradad, V., Lombarte, A., Garcia-Ladona, E., Cabestany, J., Piera, J. and Chic, O. (2005). Otolith shape contour anaylsis using affine transformation invariant wavelet transforms and curvature scale space representation. *Marine and Freshwater Research* 56: 795-804.

Petursdottir, G., Begg, G.A. and Marteinsdottir, G. (2006). Descrimination between Icelandic cod (*Gadus morhua* L.) populations from adjacent spawning areas based on otolith growth and shape. *Fisheries Research* 80: 182-189.

Piera, J., Parisi-Baradad, V., Garcia-Ladona, E., Lombarte, A., Recasens, L. and Cabestany, J. (2005). Otolith shape feature extraction oriented to automatic classification with open distributed data. *Marine and Freshwater Research* 56: 805-814.

Pothin, K., Gonzalez-Salas, C., Chabanet, P. and Lecomte-Finiger, R. (2006). Distinction between *Mulloidichthys flavolineatus* juveniles from Reunion Island and Mauritius Island (south-west Indian Ocean) based on otolith morphometrics. *Journal of Fish Biology* 69: 38-53.

Secor, D.H. (1999). Specifying divergent migrations in the concept of stock: the contingent hypothesis. *Fisheries Research* 43: 13-34.

Stransky, C., Baumann, H., Fevolden, S.-E., Harbitz, A., Høie, H., Nedreaas, K., Salberg, A.-B. and Skarstein, T.H. (2007). Separation of Norwegian coastal cod and Northeast Artic cod by outer otolith shape analysis. *Fisheires Research* .

Stransky, C., Garbe-Schonberg, C.-D. and Gunther, D. (2005). Geographic variation and juvenile migration in Atlantic redfish inferred from otolith microchemistry. *Marine and Freshwater Research* 56: 677-691.

Titus U., Mosher J.A and Williams B.K. (1984) Chance-corrected classification for use in discriminant analysis: ecological applications. *The American Midland Naturalist* 111, 1-7.

Torres, G.J., Gonzalez, J.A. and Morales-Nin, B. (2000). Variability of the sulcus acusticus in the sagittal otolith of the genus *Merluccius* (Merluciidae). *Fisheries Research* 46: 5-13.

Turan, C. (2000). Otolith shape and meristic analysis of herring *(Clupea harengus)* in the North-East Atlantic. *Arch. Fish. Mar. Res.*, 48: 213-225.

Turan, C. (2006). The use of otolith shape and chemistry to determine stock structure of mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). *Journal of Fish Biology* 69(SC): 165-180.

Tuset, V.M., Lombarte, A., Gonzalez, A.J., Pertusa, J.F. and Lorente, M.J. (2003). Comparative morphology of the sagittal otolith in *Serranus* spp. *Journal of Fish Biology* 63: 1491-1504.

Villegas-Hernández, H., González-Salas, C., Aguilar-Perera, A. and López-Gómez, M.J. (2008). Settlement dynamics of the coral reef fish *Stegastes partitus*, inferred from otolith shape and microstructure analysis. *Aquatic Biology* 1: 249-258.

Ye, Q., Noell, C. and McGlennon, D. (2002). Reproductive biology of sea garfish. In 'Fisheries biology and habitat ecology of southern garfish (*Hyporhamphus melanochir*) in southern Australian waters. (eds. Jones, G.K., Ye, Q., Ayvazian, S., Coutin, P.). FRDC Final Report 97/133.

5 ANALYSIS OF MICROSTRUCTURE AND SHAPE OF OTOLITHS FROM JUVENILE SOUTHERN GARFISH (*HYPORHAMPHUS MELANOCHIR*)

MA Steer, D Schmarr, and AJ Fowler

5.1 Introduction

Stock discrimination studies generally focus on adult populations as they typically express a broader range of physiological and biological attributes that can be used for stock identification purposes than is evident in earlier life history stages. Attributes such as age-related life-history characteristics, phenotypic expression, biogeochemistry and parasite loads may be more pronounced and offer greater distinction between potential population components. Furthermore, as these studies typically augment ongoing stock assessment, the collection of fisheries-dependent samples over large spatial scales is often more cost-effective than implementing an extensive fisheries-independent sampling regime. Investigating the earlier life history stages (e.g. eggs, larvae and 0+ juveniles) through structured surveys can also provide information which assists with stock identification, as the geographic extent of spawning and early dispersal may underpin the level of population structure and stock integrity (Pawson and Jennings 1996). For example, a species that spawns over an extensive area but has a relatively short planktonic duration may exhibit a greater level of population structure than a species that has an extended planktonic phase as a result of limited mixing (Riginos and Victor 2001).

Sampling the early life stages of fish is logistically challenging as it generally requires the use of specialised sampling equipment and methodology. Recent advances in laser ablation and micromilling techniques have allowed chemical analyses to target particular parts of adult otoliths that relate to particular life history stages (Campana et al. 1994; Gao et al. 2001; Ruttenberg et al. 2005). The natal origins and nursery areas for a variety of fish species have been retrospectively inferred from chemical analysis of the cores of adult otoliths (Patterson et al. 2004; Stransky et al. 2005). Some studies have also analysed the microstructure within the juvenile portion of sectioned otoliths to explore larval growth histories and have differentiated groups of fish on the basis the relative widths of growth increments (Brophy and King 2007). Although these retrospective studies can highlight differences in otolith chemistry and growth rates between populations of juvenile fish, they cannot necessarily determine discrete spawning aggregations because the exact back-calculated spawning date cannot be accurately ascertained. Ideally, comparisons should be made among groups of juvenile (0+) fish that share a common spawn date and have therefore grown through the same time-period. This alleviates the possibility of temporal variation confounding otolith

measurements. Analysis of otolith microstructure of similar-aged juveniles is a powerful tool for determining life-history trajectories and establishing population affiliation (Clausen et al. 2007). This is because otolith growth is chronological in nature and governed by environmental processes, therefore any measurable differences in otolith microstructure are most likely a result of spatial variation (Brophy and King 2007). Spatial differences in growth rates influence the overall shape of the otolith, providing another phenotypic measurement that can be used to differentiate between spatially distinct groups of fish (see Campana and Neilson 1985, this report). As yet, very few studies have collected juvenile fish to explore levels of population structure or delineate natal areas.

Multidisciplinary research investigating the population structure of garfish through chemical (i.e. trace elements and stable isotopes) and morphometric analyses of adult otoliths have provided evidence of fine-scale structuring within the fishery (Chapters 2, 3 and 4). However, there appeared to be some regional smearing of chemical signatures in the juvenile portion of the otoliths suggesting possible higher levels of mixing during the early life history stages (Chapters 2 and 3). Currently, the location of garfish spawning grounds and the extent of egg and larval dispersal are unknown, despite well documented information on reproductive timing, sexual maturation, physical characteristics of spawned eggs, and rates of embryonic and larval development (Ling 1958; Jordan et al. 1998; Jones et al. 2002; Noell 2005). There have been several egg and larval surveys with the principle aim of locating key spawning areas and habitats in State waters that have been unsuccessful (Noell 2005). Therefore, it is not known on what spatial scale the garfish fishery is replenished. This study aimed to explore whether natal areas could be delineated through otolith analysis by testing the hypothesis that juvenile garfish captured from different locations exhibited patterns of otolith growth that reflected distinct environmental conditions as a function of being spawned in different areas.

Teleost fish have three pairs of otoliths, i.e. the sagittae, lapilli and asterici, and each vary in location, function, size, shape, microstructure and time of formation (Secor et al. 1992). Lapilli are preferentially used in the analysis of otolith microstructure as their growth is characteristically conservative and therefore considered to reflect the early life history stages with greater accuracy (David et al. 1994; Hoff et al. 1997; Morales-Nin et al. 1999; Morioka and Machinandiarena 2001). Sagittae, on the other hand, are considered more appropriate for shape analysis as they are larger and more sculptured than lapilli which tend to be relatively featureless (Campana and Casselman 1993). The asterici are typically ignored as they are formed after hatching, rendering them inappropriate for most early life history studies (Hoff et al. 1997; Noell 2005). Therefore, in this study, spatial variation in the growth of otoliths of

juvenile garfish was assessed through analysis of the internal microstructure of the lapilli and the external morphology of the sagittae.

5.2 Materials and Methods

5.2.1 Comparison of sagittae and lapilli

A pilot investigation indicated that sections of sagittal otoliths collected from juvenile southern garfish were difficult to prepare and reliably interpret (Schmarr pers. com). It was, therefore, necessary to determine whether lapilli could be used as a feasible alternative in age-based investigations. An initial sample of 20 juvenile garfish, ranging in size from 33 to 54 mm total length (TL), was selected from a frozen stockpile that had been collected from Spencer Gulf in March 2007. This size range was selected because sagittae collected from larger garfish typically exhibit an extensive opaque area towards the otolith margin rendering them virtually impossible to obtain accurate daily age information (Schmarr pers. com). Sagittae from the smaller size classes were easier to interpret and more suited for this comparative study. All garfish were thawed and both the sagittae and lapilli were removed from each individual, cleaned of adhering tissue using a weak bleach solution, and air dried. Sagittae were embedded in epoxy resin (Struers epofix) and a transverse section of approximately 350 μm thickness was taken through the primordium using a slow speed gem saw with twin diamond blades (Stuers Accutom – 2). Each lapillus was whole mounted on a glass slide with crystalbond™ thermoplastic cement and dorso-ventrally ground down to the primordium, using progressively finer grades of lapping film.

Daily age estimates from both sagittae and lapilli were determined by counting alternating light and dark increments within the otolith microstructure under a high power microscope (100x), illuminated with transmitted light (Fig. 5.1). Each otolith was also assigned a readability score on a scale of 1 – 5, where 1 was excellent and 5 was unreadable. A second 'blind' count was made for each otolith without any reference to the first counts. If the two counts differed by >5% a third count was done. Mean estimated ages were determined from the lapillus and sagitta for each fish and a paired-sample, two-tailed t-test was used to determine whether these estimates were comparable.

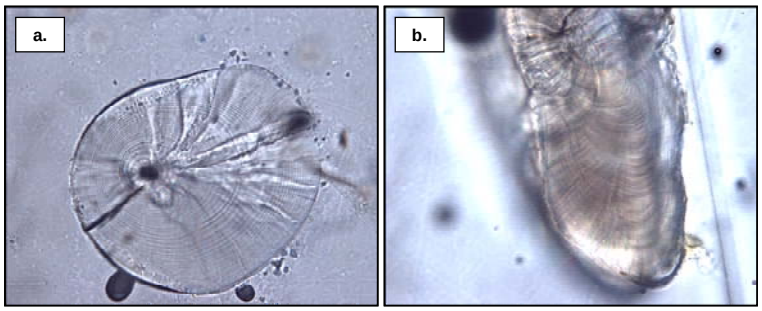

Figure 5.1. (a.) dorso-ventrally ground down lapillus, (b.) polished transverse section of a sagitta. Both otoliths were collected from the same fish. Note the clearer microstructure in the lapillus compared with the sagittae.

5.2.2 Analysis of lapilli microstructure

Additional samples of juvenile garfish were collected on 4th, 11th and 15th of February 2008 from three locations within Gulf St Vincent: Pelican Point (Port Adelaide), Port Wakefield and the Wirrina Cove marina, respectively (Fig. 5.2). These sites were selected as they are separated by ~120 km, experience different environmental regimes, are accessible, and known to support juvenile garfish. Up to 60 fish were dab-netted throughout the evening from shallow (<4 m) wind-protected areas. Garfish in the size range of 65 to 105 mm were targeted, to ensure that the same age cohort was sampled from the three locations and so had been spawned and grown through the same time period. Once captured, fish were place in labeled, sealed, plastic bags, and stored frozen until further processing.

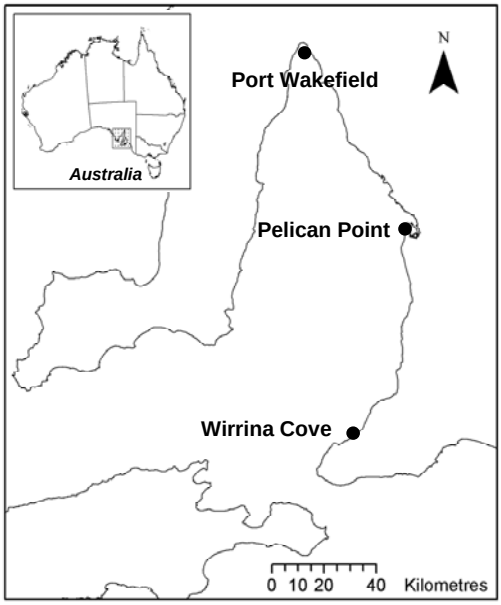

Figure 5.2. Map of Gulf St. Vincent identifying the three locations from which juvenile garfish were collected.

Lapilli were successfully collected and processed from 57 fish; 20 from Port Wakefield, 19 from Pelican Point and 18 from Wirrina Cove. The width of each increment was measured from digitized images captured through a high-resolution video camera mounted onto a Leica compound microscope at 100x magnification using Optimas (Version 6.5) image analysis software. The widths of the increments were measured along a standard transect, i.e. a curved axis comprising the longest route from the nucleus to the otolith margin. The measured increments for each otolith were subsequently divided into consecutive 10-increment sections for which a mean increment width was calculated as a measure of otolith growth. Data were screened for normality and homogeneity of variances and transformed where necessary. The mean increment widths were compared between sample locations using repeated-measures analysis of variance.

To further determine the relationships between sample locations, the 10-increment sections were analysed using discriminant function analysis. Before doing so, it was necessary to determine whether the increment sections were independent of each other. This was tested using fractal geometry, which analyses the spatial dependence of the variable in question (Palmer 1988). For example, if a variable consistently and uniformly increases (or decreases), as a function of distance along a transect, then there is strict spatial dependence. Alternatively, if there is complete spatial independence, then the value of the variable would be unpredictable. Spatial dependence was explored using a semivariogram, which summarizes the variance of the dependent variable as a function of scale (Palmer 1988). The semivariogram is the plot of the semivariance against distance (in this case, distance from the nucleus). The slope of the double logarithmic plot of semivariance against distance, m, is used to calculate the fractal dimension of the graph, D ($D = (4-m)/2$). If the dependent variable is a linear function of distance then the semivariogram will be parabolic, which would result in a slope of 2 in a double logarithmic plot and fractal dimension of 1. However, if the dependent variable is unrelated to distance then the slope of the semivariogram will be 0, which would result in a fractal dimension of 2. In this analysis the fractal dimension of the increment width data was 1.93. Therefore, the mean increment width for the 10-increment sections were considered spatially independent and were used as independent predictors in the discriminant function analysis. The resulting discriminant functions were plotted with 95% confidence intervals. Classification success was calculated using jackknifed cross-validation. The performance of discriminant functions was evaluated using Cohen's Kappa (κ) statistic, which provides an objective means of calculating the chance-corrected percentage of agreement between actual and predicted group memberships on a scale of 0 to 1, with 0 indicating no improvement over chance, and 1 indicating perfect agreement (Titus et al. 1984).

5.2.3 Sagittal shape analysis

Sagittae were also collected from the sampled juvenile garfish for shape and morphometric analysis; 20 from Port Wakefield, 20 from Pelican Point and 19 from Wirrina Cove. A series of morphometric indices and elliptical Fourier descriptors were calculated for each otolith using the same methodology described in Section 4.2.2. According to the Fourier power spectrum (see Equation 4.1), the first 13 harmonics explained 99.99% of the total shape variation, indicating that the overall shape of juvenile garfish sagittae can be adequately explained by 52 Fourier descriptors (13 harmonics x 4 coefficients). As each otolith was normalised by the 'Shape' program (see section 4.2.2), the first three coefficients (A_1, B_1, and C_1) were constant for all outlines, therefore reducing the number of Fourier descriptors used in the analysis to 49.

Morphometric indices and the Fourier descriptors were treated separately and combined for comparative analysis. The distribution of the data was log normal (Kolmogorov-Smirnov test of normality; $p > 0.05$), which was corrected using natural logarithmic transformations where required. Geographic variation in otolith shape was compared between sites using discriminant function analysis (DFA). Due to the large number of predictors (five otolith morphometrics and 49 Fourier descriptors in the combined analysis), which exceeded the number of samples in the smallest group, step-wise DFA was used to determine the most important predictors for the discriminant group membership. The entry of predictors to the analysis was determined by the statistical entry of Wilk's lambda (Λ) with a $p_{(entry)} = 0.05$ and $p_{(exit)} = 0.25$. The resulting discriminant functions were plotted with 95% confidence ellipses. Classification success was calculated using jack-knifed cross-validation and the performance of discriminant functions was evaluated using Cohen's Kappa (κ) statistic.

5.3 Results

5.3.1 Comparison of sagittae and lapilli

Age estimates were successfully determined from the sagittae and lapilli from fifteen garfish and were directly comparable ($n = 15$, $df = 1$, $p = 0.09$), indicating that the lapillus is a feasible alternative to the sagitta in age-related investigations for juvenile southern garfish (Fig. 5.3). Lapilli were also relatively simple to process and their microstructure was much easier to discern, with a better readability score of 2.3 compared to 3.3 for the sagittae.

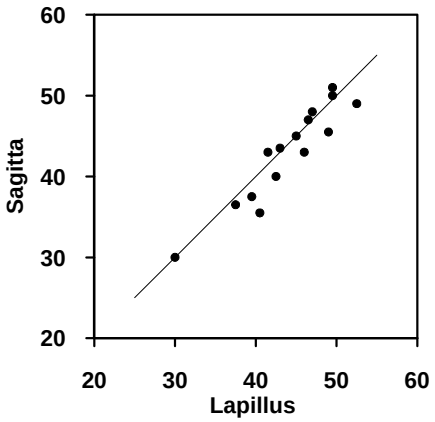

Figure 5.3. Comparison of increment counts from sagittae and lapilli.

5.3.2 Analysis of lapilli microstructure

Estimated ages of the sampled fish ranged from 69 to 109 days, indicating that garfish collected in early February 2008 were spawned from late October to early December 2007 (Fig. 5.4). The relative distribution of these back-calculated hatch dates was similar for each of the three regions (Kruskal-Wallis Test, $\chi^2 = 2.48$, df = 2, p = 0.29) and therefore the influence of any temporal variation on the regional comparisons was considered negligible. To maintain sufficient sample sizes, data analysis was restricted to the first eight 10-increment sections. A significant increment width and location interaction was detected ($F_{14, 371} = 5.33$, p <0.001; Table 5.1), indicating that the otoliths of juvenile garfish exhibited variable growth throughout the first three months of life, and the magnitude of this variation differed between locations. The otoliths from all garfish exhibited similar growth during the first 30 days (Table 5.2), after which the growth trajectories diverged depending on their location of capture (Fig. 5.5). From days 30 to 60, the otoliths from garfish collected from Port Wakefield exhibited the fastest growth, as their mean increment widths were approximately 10% wider than those collected from the other locations. This rate of growth was not maintained and by day 70 it had slowed dramatically to a rate similar to that of Pelican Point garfish otoliths. Growth rates remained the same for Pelican Point and Wirrina Cove garfish until they diverged at day 50 (Fig. 5.5). By day 60 there appeared to be a latitudinal gradient in otolith growth rate, with faster growth occurring in the north of Gulf St. Vincent and slower growth in the south, however, this was not maintained.

Table 5.1. Results of repeated measures ANOVA testing for spatial differences in the age-related increment width in juvenile garfish otoliths.

Source	df	MS	F
Within-Subjects			
Incr. Section	7	55.96	510.69***
Incr. Section*Location	14	0.58	5.33***
Error	371	0.11	
Between-Subjects			
Location	2	1.37	3.31*
Error	53	0.41	

Table 5.2. Results of a univariate ANOVA testing for differences between location for each of the 10-increment width sections.

Inc. No.	Df	MS	F
1 to 10	2	0.054	0.49^{ns}
11 to 20	2	0.145	0.878^{ns}
21 to 30	2	0.193	1.627^{ns}
31 to 40	2	0.898	6.688**
41 to 50	2	1.458	5.181**
51 to 60	2	2.352	7.742***
61 to 70	2	0.119	3.338*
71 to 80	2	0.24	7.962***

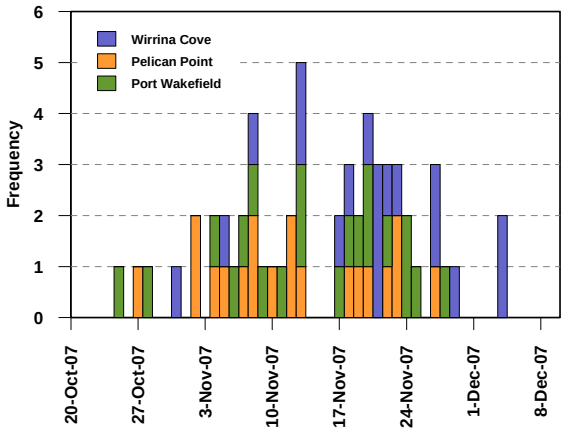

Figure 5.4. Distribution of hatch dates for juvenile garfish aged in this study.

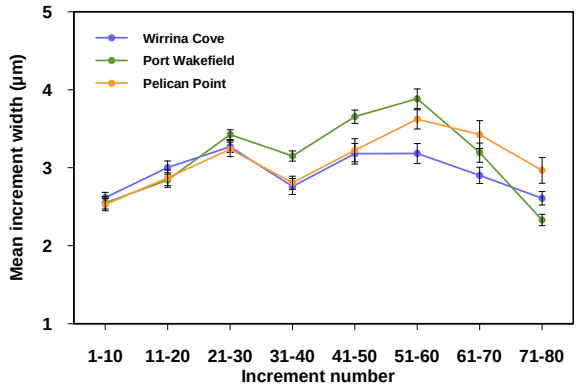

Figure 5.5. Mean increment width (± SE) by consecutive 10-increment section

Discriminant analysis based on independent estimates of otolith growth revealed clear spatial separation among juvenile garfish ($\Lambda = 0.353$, df = 16, $p < 0.001$) (Fig. 5.6). Two discriminant functions explained all of the between-group variability and 70% of all garfish were successfully classified to their site of capture, a success rate (κ) that was 60% better than chance. Separation along the first axis was predominantly driven by mean estimates of early otolith growth (days 30 to 50), whereas growth rates during the 60 to 80 day period contributed to the second discriminant function. Classification rates were highest for Port Wakefield garfish at 75%, followed by Pelican Point and Wirrina Cove at 72% and 61%, respectively.

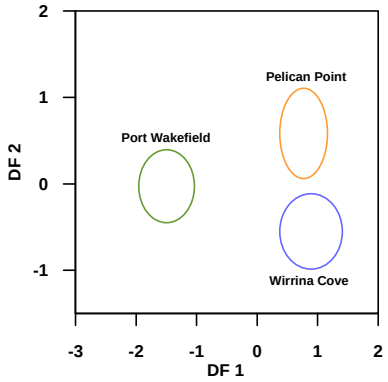

Figure 5.6. Discriminant function centroid and 95% confidence ellipse for juvenile garfish otoliths incorporating the mean increment widths of consecutive 10-increment sections.

5.3.3 Sagittal shape analysis

There was significant spatial variation in the shape of sagittae from juvenile garfish, with multivariate analyses identifying significant variation among locations for both the

morphometric data and elliptical Fourier descriptors (Tables 5.3 a, b). Coefficient of form and circularity were the only morphometric indices to significantly differ between locations, while otolith roundness, rectangularity and ellipticity did not exhibit any spatial variation (Table 5.3a). This indicated that juvenile garfish collected from Pelican Point had otoliths that were significantly more circular than those collected from the other two locations (Fig. 5.7). Only one Fourier descriptor (D_2,) displayed significant variation among locations (Table 5.3b).

Table 5.3. Results of the univariate analysis of variance testing for spatial differences in the shape of juvenile garfish otoliths for (a.) the five morphometric indices. (b.) the 49 Fourier descriptors (only significant results are presented). ns: not significant, * denotes significance for Bonferroni adjusted p values, $p < 0.01$ and $p < 0.001$ for morphometric and Fourier comparisons, respectively.

(a.)

Variable	MS	df	F
Coeff. Form	4.75E-03	2	7.33*
Roundness	4.24E-04	2	0.75ns
Ellipticity	1.19E-04	2	0.81ns
Rectangularity	3.06E-04	2	1.08ns
Circularity	9.39E-03	2	7.27*

(b.)

D2	9.61E-04	2	10.876*

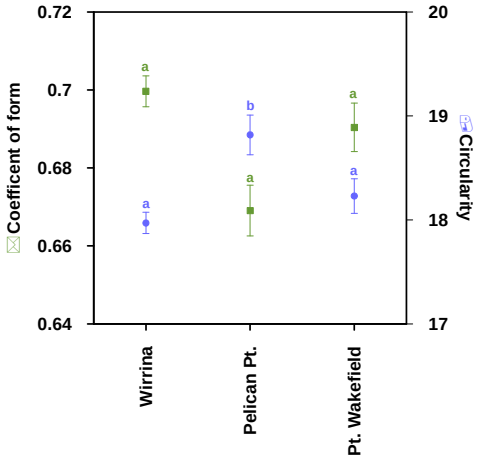

Figure 5.7. Spatial variation in otolith morphometrics. Lower case letters indicate results of *post hoc* comparison.

Discriminant function analysis performed with morphometric data detected significant variation in otolith shape between Port Wakefield and the other two locations ($\Lambda = 0.691$, $p = 0.011$) (Fig. 5.8). Two discriminant functions explained all of the between-group variability, and 58.6% of the individuals were successfully classified to their site of capture, a success rate (κ) that was 38% better than chance. Classification success was improved to 65.5% ($\kappa =$

0.48) when only the elliptical Fourier descriptors were analysed (Fig. 5.8). This analysis also had higher discriminant power ($\Lambda = 0.465$, $p < 0.001$) and was largely driven by low order Fourier descriptors (C_4, D_2, D_3) indicating that otoliths were separated on the basis of their overall elliptical shape, rather than by complex morphologies. Combining both the morphometric and elliptical Fourier datasets into a single step-wise analysis further improved the model's discriminant power ($\Lambda = 0.427$, $p < 0.001$) and classification success (69%, $\kappa = 0.53$) (Fig. 5.8). The low order Fourier descriptors (C_4, D_2 and D_3) and coefficient of form remained the main contributors responsible of the spatial separation. Classification rates were highest for Port Wakefield garfish at 80%, followed by Pelican Point and Wirrina Cove at 65% and 61%, respectively.

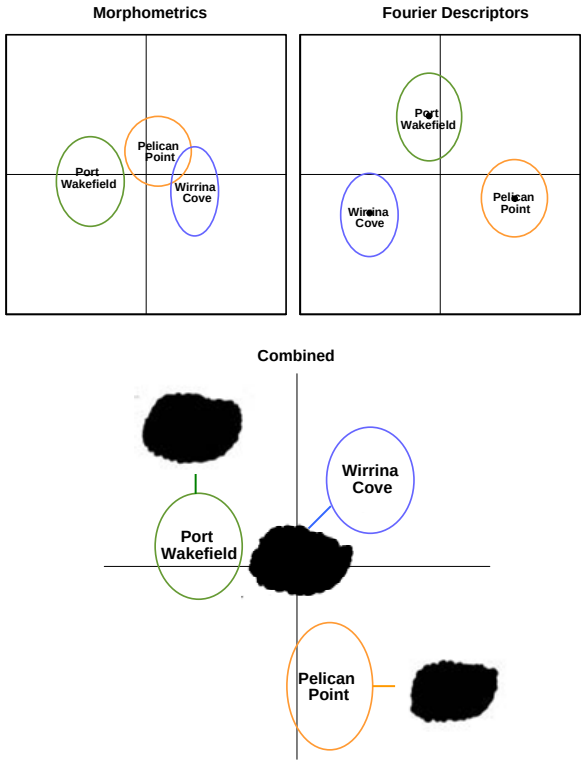

Figure 5.8. Discriminant function centroids with 95% confidence ellipses for juvenile southern garfish otoliths collected from three locations in South Australia, based on otolith morphometrics and elliptical Fourier descriptors separately and combined. Otolith silhouettes are representative of individuals at the extremities of each regional grouping.

5.4 Discussion

Lapilli were suitable for ageing juvenile southern garfish and considered more appropriate for microstructure analysis than sagittae as their growth increments were consistently clearer and easier to interpret. This finding was consistent with a variety of other otolith microstructure

studies (Hoff et al. 1997; Morales-Nin et al. 1999; Morioka and Machinandiarena 2001). Similar-aged juvenile garfish exhibited variable otolith growth throughout the first three months of life and the magnitude of this variation differed between locations. A single discriminant function analysis, which incorporated the entire increment width dataset, separated garfish to their location of capture with a high degree of confidence (70%). Similar spatial separation (69%) was also detected on the basis of the overall shape of sagittae. Although these two otoliths differ markedly in terms of their physical size, structure and rate of growth, their growth trajectories are intrinsically coupled to somatic growth (Gaudie and Nelson 1990; Jones 1992; Baumann et al. 2005). This coupling is particularly robust during the early life-history (Geffen 1995; Narimatsu et al. 2007). Therefore, it was not surprising that the results obtained from analysis of the microstructure of the lapilli concurred with the results of the shape analysis of the sagittae for juvenile garfish. Such mutual exclusiveness between the different measurements of the lapilli and sagittae, however, can only be applied to the life-time growth of the garfish as the growth increments within their sagittae were not clear and continuous enough to resolve any ontogenetic variation using shape analysis (see Burke et al. 2008).

Significant ontogenetic variation was detected among juvenile garfish via otolith increment width analysis. Lapilli from all garfish exhibited similar growth during the first 30 days, after which they diverged depending on their location of capture. The inability to detect spatial differences in growth within the first month of the garfish's life precludes any confident stock discrimination as their larval origin could not be accurately determined. It is possible that all garfish examined in this study had originated from a common area and dispersed to their location of capture in their second month. However, similarity in otolith growth pattern does not confirm that groups of fish are of the same larval origin, because geographically segregated groups of larvae may experience similar growing conditions (Brophy and King 2007). Growing conditions in the deeper gulf waters are more likely to be similar than shallower environments as they are not as affected by tidal temperature fluctuations (Petrusevics 2008). Offshore waters may, therefore, provide some refuge for juvenile garfish, although extensive larval sampling throughout the gulf indicated that the greatest densities were associated with shallow seagrass areas (Noell 2005). Through hydrodynamic modelling and the deployment of drift cards it has been demonstrated that the dispersal of propagules and larvae are typically advected from the centre of the gulf towards the western coast in summer and towards the metropolitan coastline in winter (Petrusevics 1991). Given the distances juvenile garfish would be required to cover in their second month to inhabit areas that exhibit distinctly different growing conditions and the counter water circulation pattern that operates within the gulf during summer; it is unlikely that all garfish examined in this

study have dispersed from a common origin. Alternatively, they may have originated from separate spawning areas and share a similar pattern of larval growth that was physiologically, rather than environmentally, determined; or have exhibited similar growth trajectories as a result of size-mediated selection pressures operating during the early life history stage (Gagliano et al. 2007).

Although the exact mechanism underlying the spatial divergence in otolith growth after the first month was not formally investigated it is likely to have been driven by temperature and food supply. Otolith growth from days 30 to 60 displayed a distinct latitudinal gradient, with garfish collected from Port Wakefield exhibiting the fastest growth as their mean increment widths were approximately 10% wider than those collected from the other locations. This spatial pattern of growth corresponds with the latitudinal temperature gradient that persists within Gulf St. Vincent throughout summer and autumn (Petrusevics 1993). Mean sea surface temperature during summer is approximately 3°C warmer in the northern part of the gulf than the southern part (Petrusevics 2008) and accelerated growth and increased productivity with increasing temperatures has been well documented (Campana and Hurley 1989; Brophy and Danilowicz 2002; Brophy and King 2007). Although garfish show considerable overlap in increment widths during their first month of growth, the overall analysis indicated that there was significant spatial variability in otolith growth to suggest that southern garfish do not form a single randomly mixing unit. Location-specific growth trajectories suggest that there are at least three sub-units within Gulf St. Vincent that have resulted from a single spawning season.

5.5 References

Baumann, H., Peck, M.A., and Herrmann, J.-P. (2005). Short-term decoupling of otolith and somatic growth induced by food level changes in postlarval Baltic sprat, *Sprattus sprattus. Marine and Freshwater Research.* 56: 539-548.

Brophy, D. and Danilowicz, B.S. (2002). Tracing populations of Atlantic herring (*Clupea harengus* L.) in the Irish and Celtic Seas using otolith microstructure. *ICES J. mar. Sci.* 59: 1305-1313.

Brophy, D. and King, P.A. (2007). Larval otolith growth histories show evidence of stock structure in Northeast Atlantic blue whiting (*Micromesistius poutassou*). *ICES J. mar. Sci.* 64: 1136-1144.

Burke, N., Brophy, D. and King, P.A. (2008). Shape analysis of otolith annuli in Atlantic herring (*Clupea harengus*): a new method for tracking fish populations. *Fisheries Research*.

Campana, S.E. and Casselman, J.M. (1993). Stock discrimination using otolith shape analysis. *Can. J. Fish. Aquat . Sci.* 50: 1062-1083.

Campana, S.E., Fowler, A.J. and Jones, C.M. (1994). Otolith elemental fingerprinting for stock identification of Atlantic Cod (*Gadus morhua*) using laser ablation ICPMS. *Can. J. Fish. Aquat . Sci.* 51: 1942-1950.

Campana, S.E., and Hurley, P.C.F. (1989). An age- and temperature-mediated growth model for cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) larvae in the Gulf of Maine. *Canadian Journal of fisheries and Aquatic Sciences.* 46: 1922-1929.

Campana, S.E. and Neilson, J.D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences.* 42: 1014-1032.

Clausen, L.A.W., Bekkevold, D., Hatfield, E.M.C. and Mosegaard, H. (2007). Application and validation of otolith microstructure as a stock identification method in mixed Atlantic herring (*clupea harengus*) stocks in the North Sea and western Baltic. *ICES Journal of Marine Science* 64: 377-385.

David A.W., Isely J.J., and Grimes C.B. (1994) Differences between the sagitta, lapillus, and asteriscus in estimating age and growth in juvenile red drum, *Sciaenops ocellatus*. *Fisheries Bulletin.* 92:509–515.

Gagliano, M., McCormick., M.I. and Meekan, M.G. (2007). Survival against the odds: ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. *Proceedings of the Royal Society, London B.* 274: 1575-1582.

Gao, Y., Joner, S.H. and Beargmann, G.G. (2001). Stable isotopic composition of otoliths in identification of spawning stocks of Pacific herring (*Clupea pallasi*) in Puget Sound. *Can. J. Fish. Aquat . Sci.* 58: 2113-2120.

Gauldie, R.W., and Nelson, D.G.A. (1990). Otolith growth in fishes. *Comparative Biochemistry and Physiology* 97: 119-135.

Geffen, A.J. (1995) Growth and otolith microstructure of cod (*Gadus morhua* L.) larvae *Journal of Plankton Research* 783-800

Hoff, G.R., Logan D.J., and Markle, D.F. (1997). Otolith morphology and increment validation in young Lost Liver and shortnose suckers. *Transacions of the American Fisheries. Society* 126: 488-494.

Jones GK., Ye Q., Ayvazian S. and Coutin PC (2002) Fisheries Biology and Habitat Ecology of Southern Sea Garfish (*Hyporhamphus melanochir*) in South Australian Waters. In 'Fisheries biology and habitat ecology of southern garfish (*Hyporhamphus melanochir*) in southern Australian waters. (eds. Jones, G.K., Ye, Q., Ayvazian, S., Coutin, P.). FRDC Final Report 97/133. 322pp.

Jordan A.R., Mills D.M., Ewing G. and Lyle J.M. (1998) Assessment of inshore habitats around Tasmania for life-history stages of commercial finfish species. Fisheries Research and Development Corporation (Project No. 94/037). Canberra, Australia.

Ling, J.K. (1958). The sea garfish, *Reporhamphus melanochir* (Cuvier and Valenciennes) (Hemiramphidae), in South Australia: breeding, age determination, and growth. *Aust. J. Mar. Freshw. Res.* 9: 60-110.

Morales-Nin,B., Stefano, M.D., Potoschi, A., Massutí, E., Rizzo, P., and Gancitano, S. (1999). Differences between the sagitta, lapillus and vertebra in estimating age and growth in juvenile Mediterranean dolphinfish (*Coryphaena hippurus*). *Scientia Marina* 63(3-4): 327-336

Morioka. S., and Machinandiarena, L. (2001) Comparison of daily increment formation pattern between sagittae and lapilli of ling (*Genypterus blacodess*) larvae and juveniles collected off Argentina. *New Zealand Journal of Marine and Freshwater Research.* 35: 111-119.

Narimatsu, Y., Hattori, T., Ueda, Y., Matsuzaka, H. and Shiogaki, M. (2007). Somatic growth and otolith microstructure of larval and juvenile Pacific cod *Gadus macrocephalus*. *Fisheries Science* 73: 1257-1264.

Noell C.J. (2005) Early life stages of the southern garfish *Hyporhamphus melanochir* (Valenciennes, 1846) and their association with seagrass beds. PhD Dissertation (University of Adelaide, Adelaide, South Australia).

Patterson, H.M., McBride, R.S. and Julien, N. (2004). Population structure of red drum (*Sciaenops ocellatus*) as determined by otolith chemistry. *Marine Biology* 144: 855-862.

Pawson, M.G. and Jennings, S. (1996). A critique of methods for stock identification in marine capture fisheries. *Fisheries Research* 25: 203-217.

Petrusevics, P.M. (1993). SST fronts in inverse estuaries, South Australia - Indicators of reduced gulf-shelf exchange. *Aust. J. Mar. Freshw. Res.* 44: 305-323.

Petrusevics, P.M. (2008). Satellite remote sensing of Gulf St. Vincent. *In.* Shepherd, S.A., Bryars, S., Kirkegaard, I., Harbison, P., and Jennings, J.T. Natural History of Gulf St. Vincent. Royal Society of South Australia, 496 pp.

Riginos, C. and Victor, B.C. (2001). Larval spatial distributions and other early life-history characterisitics predict genetic differentiation in eastern Pacific blennoid fishes. *Proceeding of the Royal Society, London B.* 268: 1931-1936.

Ruttenberg, B.I., Hamilton, S.L., Hickford, M.J.H., Paradis, G.L., Sheehy, M.S., Standish, J. D., Ben-Tzvi, O. and Warner, R.R. (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.

Secor, D.H., Dean, J.M., and Laban, E.H. (1992). Otolith removal and preparation for microstructural examination. *In* Stevenson, D.K. and Campana, S.E. (Eds) Otolith microstructure examination and analysis. *Canadian Special Publication Fisheries and Aquatic Sciences*. 117: 19-57.

Stransky, C., Garbe-Schonberg, C.-D. and Gunther, D. (2005). Geographic variation and juvenile migration in Atlantic redfish inferred from otolith microchemistry. *Marine and Freshwater Research* 56: 677-691.

Titus, K., Mosher, J.A. and Williams, B.K. (1984). Chance-corrected classification for use in discriminant analysis: ecological applications. *The American Midland Naturalist.* 111(1): 1-7.

6 GENERAL DISCUSSION

MA Steer, AJ Fowler and BM Gillanders

6.1 A combined approach to stock discrimination

In the last decade there has been an emphasis among fishery scientists to apply a suite of complementary techniques to stock discrimination studies (Begg and Waldman 1999). This 'holistic' approach has developed on the understanding that a fish 'stock' is not necessarily a genetic construct but can comprise multiple sub-populations, or components, that make up a stock-complex, with each group having some definable attribute that may be of interest to fishery managers (Stephenson 1999). For example, a homogeneous genetic population may comprise multiple, self-replenishing, components of which some may be susceptible to overfishing and localised depletion. The potential erosion of semi-discrete population components cannot be detected through genetic studies alone and may occur despite a level of overall management that was previously considered appropriate (Stephenson 1999). Integrating a variety of stock discrimination techniques that cover multiple aspects of the fish's biology is particularly pertinent in these complex situations and is likely to help disentangle the population structure with a greater level of confidence (Begg and Waldman 1999). Ideally, to achieve greater resolution and more meaningful comparisons two or more stock discrimination techniques should be applied to the same biological samples (Begg and Waldman 1999).

The genetic stock structure of the southern garfish, *Hyporhamphus melanochir*, has been described across its geographic range using mitochondrial DNA and two separate stocks have been identified in South Australia; one west of Eyre Peninsula and the other comprising the two gulfs (Donnellan et al. 2002). The results from the present study, through trace element analysis (Chapter 2), stable isotope analysis (Chapter 3) and morphometric analyses (Chapter 4) of adult garfish otoliths have independently indicated that the level of population structure within the State's waters is more complex than previously thought as it appears that stocks can be discriminated at a finer spatial scale. All of the separate and independent discriminatory techniques yielded consistent and corroborative results, however, given the importance of adopting a multi-disciplinary 'holistic' approach in delineating stock structure, it was considered more appropriate to integrate the results from each technique into a single computation and discuss the overall findings in this chapter.

6.1.1 Data integration and analyses

Two different types of temporal information were collected from each adult garfish sampled in this study. Chemical analyses of transverse sectioned otoliths, through laser ablation and standardised micromilling techniques, allowed the resultant data to be age-resolved against the otoliths' chronological internal structure. This same level of temporal resolution, however, could not be obtained from the morphometric analyses, which mathematically described the overall shape of the whole otolith, and therefore could only produce information that was integrated over the fish's entire life. Consequently, these two data sources were not compatible across the entire age range. As all sampled adult garfish were from the same age class (2+), it was acceptable, however, to combine the morphometric data with the chemical data averaged across the whole otolith into a single analysis as these measures related to the entire life-history of the garfish (Fig. 6.1). The combined, age-related, chemical and morphometric data were subjected to the same statistical analyses used in the previous chapters, where step-wise discriminant function analyses were carried out to assess whether each garfish could be reliably classified to its region of capture.

	Juvenile	Age 1	Age 2	Whole
Trace element	✓	✓	✓	✓
Stable isotope	✓	✓	✓	✓
Morphometrics	✗	✗	✗	✓

Figure 6.1. Schematic representation of the age-related, otolith data acquired from the three different stock discriminatory techniques used in this study and their relative compatibility.

6.1.2 The issue of spatial scale

Otolith chemical signatures can be considered powerful discriminators of groups of fish when differences exist, but are of negligible value when differences cannot be detected (Campana 1999). This inherently relies on the physio-chemical characteristics of regional waters being sufficiently different to produce distinctive chemical signatures (Fowler et al. 2004) and is likely to depend on the spatial scale of the investigation. For example, sites that are close to each other may share similar environmental conditions and water chemistry. It is possible that different fish populations inhabit adjacent sites but cannot be differentiated from each other on the basis of their similar chemical signatures. Similarly, sites that are separated by considerable distances but share similar geographic features and oceanography may also have comparable environments. In such situations, the distances between such sites may be large enough that it is highly unlikely that fish actively migrate between them. This seemed to be

the case for southern garfish in South Australia that inhabited geographically separated regions characterised by elevated temperatures and abnormally high salinities, specifically those from the West Coast, Northern Spencer Gulf and Northern Gulf St. Vincent. In many cases, discriminant function analysis misclassified garfish to such similar environments, which subsequently reduced the investigation's overall statistical power (Chapters 2 and 3). If garfish did actively migrate between these regions then the intermediate regions that bridge these geographic extremes would be expected to account for a proportion of the misclassified fish. This would be indicative of a large-scale movement gradient. However, for South Australian garfish there was no clear evidence that fish undertook large-scale migration between such regions, on the basis of the distribution pattern of misclassified fish. As such, garfish from these regions were considered to be independent of each other. In order to remove the confounding effect of these large-scale environmental similarities, the spatial scale of the current analyses were constrained to explore movement patterns at the broad-scale regional level within each gulf separately- WEST COAST.

6.2 Evidence of broad-scale regional discrimination

6.2.1 West Coast

Genetic studies have previously indicated that garfish from the West Coast constitute a distinct stock (Donnellan et al. 2002). Integration of all available otolith data from this current study indicated there was a finer level of population structure within the region. High rates of reclassification success indicated that there was very little mixing of fish between the three sites within the region, and this level of fine-scale separation remained temporally stable (Table 6.1). Consequently, the majority of West Coast garfish appeared to display a high degree of site-fidelity spending at least the first two years of their lives within these small bays. All three bays from where the garfish were collected are semi-enclosed, with very narrow mouths and extensive tidal sand bars at their entrances. It is, therefore, likely that the high site-fidelity of these garfish is a result of this constraining local geography.

Table 6.1. Classification success of the discriminant function analysis incorporating otolith trace element, stable isotope and morphometric data for garfish collected from the West Coast. The data presented are the percentage of otoliths from the site of origin (row) classified to each of the sites (columns).

JUVENILE	WC_SB	WC_BB	WC_VB
WC_SB	**87**	0	13
WC_BB	0	**63**	37
WC_VB	17	8	**75**

Age 1	WC_SB	WC_BB	WC_VB
WC_SB	**40**	0	60
WC_BB	10	**80**	10
WC_VB	0	17	**83**

Age 2	WC_SB	WC_BB	WC_VB
WC_SB	**100**	0	0
WC_BB	20	**80**	0
WC_VB	8	0	**92**

Whole	WC_SB	WC_BB	WC_VB
WC_SB	**100**	0	0
WC_BB	10	**90**	0
WC_VB	0	0	**100**

6.2.2 Spencer Gulf

Garfish within Spencer Gulf were clearly partitioned into northern and south western components and this separation was evident for the juvenile stage and throughout the first two years of their lives (Table 6.4). The combined characteristics of the otoliths clearly indicated that garfish inhabiting these regions experienced different environmental regimes, particularly on the basis of marked differences in mean concentration of strontium (Sr) and isotopic oxygen ($\delta^{18}O$) values. Strontium has been frequently used as a proxy for salinity and relied on to reconstruct migratory patterns for a range of diadromous species that move between freshwater, marine and estuarine habitats (Secor et al. 1995, Jessop et al. 2002, Milton and Chenery 2005). Similarly, oxygen isotopes have traditionally been excellent tracers of salinity in intermixing marine systems (Bigg and Rohling 2000). Region-specific salinity signatures in fish otoliths would be expected to be highly conspicuous within Spencer Gulf due to its exaggerated inverse estuarine environment (Petrusevics 1993). Given the clear environmental gradient within Spencer Gulf and corresponding differences in otolith chemistry, which were evident throughout the entire life of the fish, it is likely that these two regions within Spencer Gulf support semi-discrete garfish populations that are largely replenished by independent recruitment events and are relatively self-sustaining.

Table 6.2. Classification success of the discriminant function analysis incorporating otolith trace element, stable isotope and morphometric data for garfish collected from Spencer Gulf. The data presented are the percentage of otoliths from the region of origin (row) classified to each of the regions (columns).

Juvenile	NSG	SWSG
NSG	**81**	19
SWSG	13	**87**

Age 1	NSG	SWSG
NSG	**78**	22
SWSG	16	**84**

Age 2	NSG	SWSG
NSG	**85**	15
SWSG	18	**82**

Whole	NSG	SWSG
NSG	**82**	18
SWSG	11	**89**

6.2.3 Gulf St. Vincent

The regional population structure of Gulf St. Vincent garfish was not as distinct and temporally stable as that of Spencer Gulf. Moderate levels of classification success indicated that there were groups of garfish that had spent at least the last year of their lives (Age 2) in different environments and that there was some level of restriction that prevented complete

mixing among the regions (Table 6.5). However, this level of population structure was not evident during the juvenile stage. All adult garfish collected from Northern Gulf St. Vincent were retrospectively classified to have originated from either South West Gulf St. Vincent (86%) or Kangaroo Island (48%) (Table 6.5). At first glance, this may suggest that these two regions constitute significant nursery areas. However, results from extensive larval surveys do not support this finding, as the greatest abundances of < 30 day old larvae were generally concentrated in the upper northern and north-eastern parts of the gulf (Noell 2005). Similarly, high densities of juvenile garfish (< 109 days old) were found in the shallow (< 4 m), wind-protected areas of Port Wakefield (NGSV) (Steer pers. obs., Chapter 5). Both lines of evidence indicate that the northern-most region of the gulf is a significant nursery area for southern garfish and should contribute to population replenishment. The fact that juvenile garfish were not successfully reclassified to this highly productive region suggests that there may be underlying influences that smear any region-specific chemical signatures during the early life-history stage.

Although there is considerable information regarding the reproductive cycle, egg morphology, embryonic and larval development of southern garfish, the spawning locations and patterns of egg and larval dispersal remain key knowledge gaps. Garfish eggs possess chorionic filaments that enable them to adhere to vegetation (Jordan et al. 1998, Noell 2005) and reproductively mature females are generally associated with shallow seagrass meadows (Ling 1958, McGarvey et al. 2006). Therefore, it has been assumed that spawning garfish are associated with shallow areas of dense seagrass. However, there have been significant attempts to search for eggs in 'characteristic spawning habitat' that have proven unsuccessful (see Ling 1958, Noell 2005). If garfish eggs are indeed sessile and are laid in shallow environments, then it would be expected that the resultant larvae would exhibit distinct, regional chemical signatures in their otoliths. However, this was not observed, indicating that deeper water, where the water chemistry is more homogeneous as it is not as heavily influenced by coastal processes, may play a greater role during the early life-history stages than previously expected. There are a variety of scenarios where juvenile garfish can disperse into deeper waters. For example, larvae may either actively or passively disperse into deeper areas from shallow spawning grounds; or the eggs may attach to drift algae (Jordan et al. 1998), or on vegetation that becomes detached, providing an additional means of dispersion; or spawning may simply occur in deeper water. Given that Gulf St. Vincent is comparatively smaller than Spencer Gulf, and does not exhibit exaggerated latitudinal temperature and salinity fronts, any one of these localised dispersal scenarios may be sufficient to attenuate region-specific chemical signatures. The same 'deep-water' hypothesis can also reconcile

why differences in early growth were not detected in juvenile garfish collected from the northern and southern extremes of the gulf (Chapter 5).

Table 6.3. Classification success of the discriminant function analysis incorporating otolith trace element, stable isotope and morphometric data for garfish collected from Gulf St. Vincent. The data presented are the percentage of otoliths from the region of origin (row) classified to each of the regions (columns).

Juvenile	KI	NGSV	SWGSV
KI	**48**	0	52
NGSV	23	**0**	77
SWGSV	14	0	**86**

Age 1	KI	NGSV	SWGSV
KI	**10**	21	69
NGSV	3	**52**	45
SWGSV	13	23	**64**

Age 2	KI	NGSV	SWGSV
KI	**66**	0	35
NGSV	24	**41**	35
SWGSV	29	3	**68**

Whole	KI	SWGSV	NGSV
KI	**83**	17	0
SWGSV	14	**76**	10
NGSV	17	20	**63**

6.3 Conceptual 'stock structure' model

Integrated results from otolith trace element, stable isotope and morphometric analyses indicated that South Australia's southern garfish fishery consists of multiple sub-populations that exhibit various levels of intermixing. Although population components were more clearly defined at the regional spatial scale there was also strong evidence that a proportion of garfish within these regions were site attached. This suggests that garfish may exhibit two alternate migratory behaviours, one where individuals remain within a defined area and can be considered 'residents' and others that have a tendency to disperse more widely and can be considered 'migrants'. These two types of fish have been historically recognised by South Australian commercial garfish fishers and it has been suggested that they can be differentiated on the basis of their colour and external appearance (Florence pers. com.). Garfish that exhibit brown hues and are "dirty" in appearance are considered to be residential, whereas highly reflective, metallic blue coloured individuals are thought to have migrated from elsewhere. Similar divergent migratory behaviour has been documented in a range of fish including Western Australia pilchards (*Sardinops sagax*) (Edmonds and Fletcher 1997), Australian herring (*Arripis georgiana*) (Ayvazian et al. 2004), snapper (*Pagrus auratus*) (Fowler et al. 2004), and black bream (*Acanthopagrus butcheri*) (Elsdon and Gillanders 2005). With the technical advances in stock discrimination studies this behaviour is being

detected more frequently. Although the exact mechanism underlying this behaviour is not known it is thought to be an evolutionary strategy that minimises the risks associated with inhabiting unpredictable and variable environments (Secor 1999).

Clearly some level of inter-regional mixing occurs within the two gulfs based on genetic homogeneity (Donnellan et al. 2002). However, only a few migrants per generation are required to homogenise the genetic stock (Patterson et al. 2004). Given the essentially coastal distribution of garfish and their close association with seagrass habitats, it is likely that gene flow occurs via a one-dimensional 'stepping stone' model in which neighbouring sub-populations exchange genes (Kimura and Weiss 1964). The clear sub-division of Spencer Gulf into northern and south-western sub-populations and the fine-scale population structure in Gulf St. Vincent suggests that there may be additional inter-connecting sub-populations within the gulfs that contribute to homogenising the genetic stock which were not detected in this study.

6.4 Implications for management

Spatial differences in otolith chemistry and morphometrics indicated that there were several groups of garfish that had spent significant parts of their lives in different environments and that there was some level of restriction that prevented complete mixing among the regions. It has been argued that in such circumstances where multiple sub-units are detected within a population that each should be treated as discrete and conserved (Stephenson 1999). This is because there is a level of uncertainty regarding their role in preserving specific genes, the number of sub-units necessary to ensure stock viability in all conditions, and the effect of fishing on genetic resources and biodiversity (Stephenson 1999). At least five regional divisions were identified in this study, each exhibiting various levels of intermixing, but which can be considered semi-discrete (Fig. 6.2). Three of these were clearly defined as they exhibited negligible levels of inter-regional mixing; i.e. the West Coast, northern and south-western Spencer Gulf. The remaining two, however, were not as distinct from each other; i.e. northern Gulf St. Vincent and southern Gulf St. Vincent (which includes south western Gulf St. Vincent and Kangaroo Island), but demonstrated a level of population structuring that would regard them as separate under "precautionary principles". This level of population discrimination is sufficient to suggest that the current management framework of two discrete, gulf-specific stocks should be restructured to align with these smaller, semi-discrete, regional units. As such, the current computer-based fisheries model (McGarvey and Feenstra 2004) and stock assessment should be adjusted to cater for this new information.

The level of restructuring suggested above is necessary as high exploitation rates in any one of these regions could lead to localised depletion. Unlike a completely mixed stock, these regional population components have less capacity for being replenished by fish migrating from neighbouring areas (Turan 2006). Mismatching the size of spatial management units with the population structure can obscure localised depletion, as catches from nearby areas may counterbalance any fine-scale negative effects (Prince 2005). It is not until sub-populations become serially depleted that they can be detected over large spatial scales, by which time the sustainability of the fishery may be significantly compromised. Matching the scale of management with spatially defined population components reduces this risk and provides greater resolution in the detection of fine-scale perturbations in catch and effort data. Realigning the management units in South Australia's garfish fishery will not only increase the resolution of the stock assessment process but is also considered particularly pertinent for three main reasons. First, most commercial fishing effort is concentrated in the northern parts of the two gulfs, subjecting them to greater fishing pressure than anywhere else in the State. Secondly, there are already concerning indications that the garfish fishery has been impacted as a consequence of high exploitation rates. The age structure of the fish has been truncated to largely consist of only 1- and 2-year old fish (Fowler et al. 2008b). The maximum recorded age for garfish is ten years and lightly fished populations, like that of Baird Bay on the West Coast, are still include significant numbers of 4+ and 5+ age classes (Jones 1990). Finally, a recent stock status report indicated that the total commercial catch of garfish in the 2007/08 financial year was the lowest on record and that targeted haul net catch per unit effort had sharply declined over the past three years (Fowler et al. 2008a). This decline is more rapid than the previous decline of 2001 to 2003, which prompted management action (i.e. spatial net closures and net-buy-back scheme) and the development of this research project.

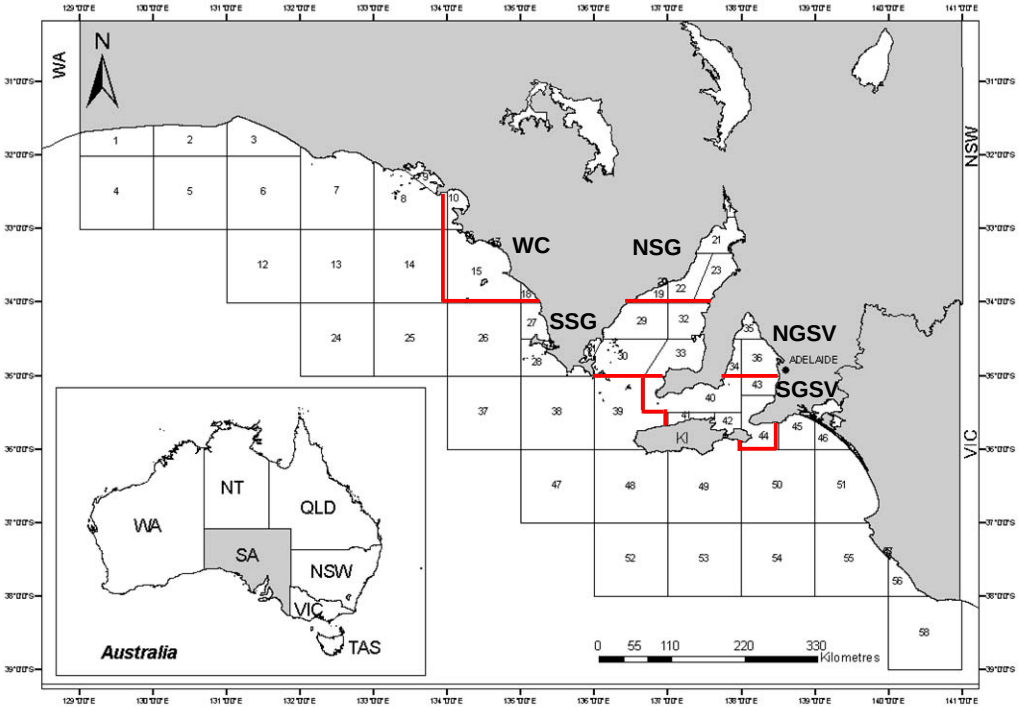

Figure 6.2. The Marine Fishing Areas (MFA's) of the South Australian Marine Scalefish Fishery. The bold red lines delineate potential spatial management units that the integrated results of the present study suggest are appropriate for the commercial garfish fishery.

6.5 Benefits and beneficiaries

This report has provided a basis for PIRSA Fisheries managers to amend the current management arrangements for South Australia's southern garfish fishery. PIRSA Fisheries have adopted the new spatial management units that were detailed in this report. SARDI's fisheries modelling team has also amended the GarEst model that was developed as part of FRDC project no. 1999/145 (McGarvey and Feenstra (2004)) to align with the smaller management units. In doing so, the parameter estimates have improved and we have a better understanding of the spatial scale over which population processes occur. This has lead to more accurate estimates of output parameters and to an improved assessment regarding the current status of the garfish stock and its future management.

6.6 Planned outcomes

The one planned outcome for this study that was identified in the original proposal was to determine the appropriate spatial scale of stock assessment for South Australia's southern garfish. Through the integration of multiple stock discriminatory techniques this outcome was achieved and five regional management units were identified.

6.7 Further development

It has been suggested that stock identification should be considered as a continuing process, evolving as management needs for stock assessment change, using the most up-to-date technologies where possible (Begg 1999). Rapidly developing technologies in the field of otolith chemistry, molecular genetics and electronic tagging may offer additional complementary techniques that further refine our understanding of stock structure in the future.

This project adopted a 'top-down' approach where inferences about the life-time movement and migration of garfish were retrospectively made from information retained within the otoliths of adult fish. Although sufficient information was obtained to clarify the population structure of South Australia's garfish fishery and to justify the restructure of the spatial management units, it is still not known where garfish spawn and whether there are key spawning areas throughout the State. Furthermore, the dispersive potential of the eggs and larvae is not known. Therefore, the level of mixing during the early life-history stages represents a key gap in our knowledge. It is fundamental to ascertain whether multiple spawning areas exist with management units as they critically underpin stock replenishment. Augmenting the findings of this project with 'bottom-up' early life history information will clarify some of the complexity that is currently evident in South Australia's garfish population structure.

Furthermore, this study only analysed one cohort of garfish and explored the age-resolved population structure for the first two years of the lives of the fish. Consequently, it does not investigate whether the observed patterns detected for this cohort persist over time, nor examine the extent of any subsequent population structure in older (> 2 years) fish. Repeating this study over a number of years will determine whether the level of population structure identified in this report is temporally stable. Currently, the South Australian garfish fishery appears to be age-truncated with the population dominated by 1 and 2 year-old fish (Fowler et al. 2008), and as such, it was difficult to sample fish from the older age classes. Garfish have been found to live up to 10 years (Jones 1990), however, the movement and migration capacity of these older fish is currently unknown. In the event of the fishery rebuilding to consist of a greater proportion of older fish, it may be necessary to re-run the otolith analyses across a broader range of fish ages. This will resolve patterns of movement and migration and determine the level of population structuring across a greater proportion of the life-history of garfish.

One manuscript has been published:

Steer, MA., Fowler, AJ and Gillanders, BM. (2009) Age-related movement patterns and population structuring in southern garfish (*Hyporhamphus melanochir*) inferred from otolith chemistry. *Fisheries Management and Ecology.* 16: 265-278. (Based on Chapter 2).

Another has been submitted:

Steer, MA., Halverson, GP., Fowler, AJ and Gillanders BM. (submitted) Stock discrimination of southern garfish (*Hyporhamphus melanochir*) by stable isotope ratio analysis of otolith carbonate. *Environmental Biology of Fishes.* (Based on Chapter 3)

6.8 Conclusion

This study examined regional, age-related, differences in the chemical composition of the otolith, in terms of their trace element (Chapter 2) and stable isotope (Chapter 3) concentrations, as well as exploring discrepancies in their overall shape (Chapters 4 and 5) and internal microstructure (Chapter 5). The combined results indicated that the population structuring of garfish is more complex than previously assumed and it seems that stocks can be discriminated at a much finer spatial scale. At least five regional divisions were identified, each exhibiting various levels of intermixing, but which can be considered semi-discrete. Three of these were clearly defined as they exhibited negligible levels of inter-regional mixing; i.e. the West Coast, northern and south-western Spencer Gulf. The remaining two, however, were not as distinct from each other; i.e. northern Gulf St. Vincent and southern Gulf St. Vincent (which includes south western Gulf St. Vincent and Kangaroo Island), but demonstrated a level of population structuring that would regard them as separate under "precautionary principles". Although population components were more clearly defined at the regional spatial scale there was also strong evidence that a proportion of garfish within these regions were site attached. This suggests that garfish may exhibit two alternate migratory behaviours, one where individuals remain within a defined area and can be considered 'residents' and others that have a tendency to disperse more widely and can be considered 'migrants'. The level of population discrimination, identified in this study is sufficient to suggest that the current management framework of two discrete, gulf-specific stocks should be restructured to align with these smaller, semi-discrete, regional units. The current computer-based garfish fishery model, which is integral to the assessment of the stock, should be adjusted to cater for this new information.

6.9 References

Ayvazian, S. G., Bastow, T. P., Edmonds, J. S., How, J. and Nowara, G. B. (2004). Stock structure of Australian herring (*Arripis georgiana*) in southwestern Australia. *Fisheries Research* 67: 39-53.

Begg, G. A. and Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fisheries Research* 43: 35-44.

Bigg, G. R. and Rohling, E. J. (2000). An oxygen isotope dataset for marine waters. *Journal of Geophysical Research* 105: 8527-8535.

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

Donnellan S., Haigh L., Elphinstone M., McGlennon D. and Ye, Q. (2002) Genetic discrimination between southern garfish (*Hyporhamphus melanochir*) stocks of Western Australia, South Australia, Victoria and Tasmania. In 'Fisheries biology and habitat ecology of southern garfish (*Hyporhamphus melanochir*) in southern Australian waters. (eds. Jones, G.K., Ye, Q., Ayvazian, S., Coutin, P.). FRDC Final Report 97/133.

Edmonds, J. S. and Fletcher, W. J. (1997). Stock discrimination of pilchards *Sardinops sagax* by stable isotope ratio analysis of otolith carbonate. *Marine Ecology Progress Series* 152: 241-247.

Elsdon, T. S. and Gillanders, B. M. (2005). Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. *Can. J. Fish. Aquat . Sci.* 62: 1143-1152.

Fowler, A.J., Gillanders B.M. and Hall K.C. (2004) Adult migration, population replenishment and geographic structure for snapper in South Australia. FRDC Final Report 2002/001, 152 pp.

Fowler, A.J., McGarvey, R., Steer, M.A., and Feenstra, J.E. (2008a). The South Australian Marine Scalefish Fishery Stock Status Report. SARDI Aquatic Sciences Publication No. F2007/000565-3, 29 pp.

Fowler, A.J., Steer, M.A., Jackson, W.B. and Lloyd, M.T. (2008b). Population characteristics of southern sea garfish (*Hyporhamphus melanochir*, Hemiramphidae) in South Australia. *Marine and Freshwater Research* 59: 429-443.

Jessop B.M., Shiao J.C., Iizuka Y. and Tzeng W.N. (2002) Migratory behaviour and habitat use by American eel *Anguilla rostrata* as revealed by otolith microchemistry. *Marine Ecology Progress Series* 233, 217-229.

Jordan A.R., Mills D.M., Ewing G. and Lyle J.M. (1998) Assessment of inshore habitats around Tasmania for life-history stages of commercial finfish species. Fisheries Research and Development Corporation (Project No. 94/037). Canberra, Australia.

Kimura M. and Weiss G.H. (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* 49, 561-576.

Ling, J. K. (1958). The sea garfish, *Reporhamphus melanochir* (Cuvier and Valenciennes) (Hemiramphidae), in South Australia: breeding, age determination, and growth. *Aust. J. Mar. Freshw. Res.* 9: 60-110.

Milton, D. A. and Chenery, S. R. (2005). Movement patterns of barramundi *Lates calcarifer*, inferred from 87Sr/86Sr and Sr/Ca ratios in otoliths, indicate non-participation spawning. *Marine Ecology Progress Series* 301: 279-291.

Noell C.J. (2005) Early life stages of the southern garfish *Hyporhamphus melanochir* (Valenciennes, 1846) and their association with seagrass beds. PhD Dissertation (University of Adelaide, Adelaide, South Australia).

Petrusevics, P. M. (1993). SST fronts in inverse estuaries, South Australia - Indicators of reduced gulf-shelf exchange. *Aust. J. Mar. Freshw. Res.* 44: 305-323.

Prince, J. (2005). Combating the tyranny of scale for Haliotids: micro-management for micro-stocks. *Bulletin of Marine Science.* 76(2): 557-577.

Secor, D. H. (1999). Specifying divergent migrations in the concept of stock: the contingent hypothesis. *Fisheries Research* 43: 13-34.

Secor D.H., Henderson-Arzapalo A. and Piccoli P.M. (1995) Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *Journal of Experimental Marine Biology and Ecology* 192, 15-33

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

Turan, C. (2006). The use of otolith shape and chemistry to determine stock structure of mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). *Journal of Fish Biology* 69(SC): 165-180.

APPENDIX 1:

Intellectual Property

There are no Intellectual Property issues associated with this project.

APPENDIX 2:

Staff involved

Dr Anthony Fowler	(SARDI)	Principal Investigator
A/Prof. Bronwyn Gillanders	(University of Adelaide)	Co-Investigator
Dr Mike Steer	(SARDI)	Research Scientist
David Schmarr	(SARDI)	Research Officer
Dr Galen Halverson	(University of Adelaide)	Research Scientist
William Jackson	(SARDI)	Technical Officer
Matt Lloyd	(SARDI)	Technical Officer