

# Fisheries



## Movement patterns and stock structure of Australian sardine (*Sardinops sagax*) off South Australia and the East Coast: implications for future stock assessment and management



Christopher Izzo<sup>1,2</sup>, Bronwyn M. Gillanders<sup>1</sup> and Tim M. Ward<sup>2</sup>

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PO Box 120 Henley Beach SA 5024

March 2012

Final Report to the  
Fisheries Research and Development Corporation



Government  
of South Australia



Australian Government  
Fisheries Research and  
Development Corporation

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## 1. NON TECHNICAL SUMMARY

**2009/021 Movement patterns and stock structure of Australian sardine (*Sardinops sagax*) off South Australia and the East Coast: implications for future stock assessment and management**

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### OBJECTIVES

1. To collate data held in jurisdictions to generate hypotheses regarding movement patterns and stock structure of Australian sardines throughout southern Australia.
2. To evaluate the use of otolith shape analyses for testing hypotheses regarding the movement patterns of sardine in southern Australia.
3. To evaluate the use of transect-based otolith microchemistry techniques (laser ablation ICPMS) for examining the movement patterns of sardines between gulf and shelf waters of South Australia and along the east coast of Australia.
4. To collate findings to determine: (i) whether or not additional spatial management (zones) would be needed to support a future increase in the TAC in the SA Sardine Fishery; and (ii) the degree to which estimates of spawning biomass from waters off northern New South Wales and southern Queensland reflect the size of the entire eastern Australian sardine stock.

### NON TECHNICAL SUMMARY

#### OUTCOMES ACHIEVED

The planned outcome for this study was to determine the stock structure and movement patterns of sardines off South Australia and the east coast of Australia and to provide advice to relevant stakeholders regarding the implications for stock assessment and fisheries management. This outcome was achieved through the application of three complementary techniques. Separate stocks were identified east and west of Bass Strait, with potential sub-structuring along the east coast and off South Australia.

The Australian population of sardine (*Sardinops sagax*) covers a larger geographical range but supports smaller catches, than other *Sardinops* populations. Locally significant commercial fisheries have been taken in five jurisdictions (Commonwealth, New South Wales, Victoria, South Australia and Western Australia).

Information on stock structure is needed to assess the level of co-ordination/sub-division that may be required for future stock assessment and fisheries management. This project used three complementary approaches to assess the level of sub-structuring of the Australian sardine population.

A semi-quantitative weight of evidence approach, using similarity matrices of existing information, was applied to infer patterns of stock structure. This approach suggested that there was a high degree of separation between the Western Australian, South Australian and the east coast (which includes Queensland, New South Wales and eastern Victoria) groups, with some sub-structuring apparent along the east coast.

Potential sub-structuring off south-eastern Australia was investigated using two otolith-based approaches: (i) Fourier analysis of otolith shape; and (ii) otolith chemistry.

Both techniques suggested the existence of separate east and south coast stocks, divided by Bass Strait. The findings of the shape analysis suggested the existence of separate South Australian, central Victorian (Port Phillip Bay) and east coast Australian groups. Otolith chemistry results suggested potentially separate northern and southern sub-groups off the east coast and potential spatial structuring off South Australia.

The combined results of this project indicated that the population structuring of the Australian sardine is complex. The two otolith-based techniques generally supported the population structure for south-eastern Australia suggested by the weight of evidence approach:

- Separate stocks off the south coast (South Australia, Port Phillip Bay), and east coast Australia (including Lakes Entrance and southern Queensland);
- Potential seasonal and age-related patterns of sub-structuring along the east coast;
- Inter-annual variation in sub-structuring off South Australia, most likely as the result of environmental fluctuations.

These findings provide a basis for evaluating the suitability of current spatial frameworks for assessment and management of sardine off south-eastern Australia. In particular, these findings suggest that there would be benefits in coordinated assessment and management of the east coast, which is currently managed separately by the Commonwealth, New South Wales and Victoria. Conversely, in South Australia, there is a need to assess options for finer scale spatial management.

**KEYWORDS:** Australian sardine; pilchard; *Sardinops sagax*; population structure; fishery management units; otolith; trace elements; Fourier analysis.

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## 3. BACKGROUND

### 3.1 STOCK DELINEATION OF SMALL PELAGIC TELEOSTS

Small pelagic fishes are keystone species in many marine ecosystems, providing a key food source for numerous predatory fishes, birds, and marine mammals, and acting as a link between plankton and predators (Louw et al., 1998; Cury et al., 2000; Ward et al., 2008b). Small pelagic fishes are also targeted in artisanal and industrial fisheries (Tacon and Metian, 2009). Approximately half of the world's annual fish harvest is comprised of small pelagic fishes (Fréon et al., 2005).

The global importance of small pelagic fishes makes the long-term sustainability of their fisheries critical for world food security. Due to their high mobility and capacity for mixing, most pelagic fish populations are regarded as a single homogeneous stock (Fréon et al., 2005). However, more recently stock sub-groups have been identified within the regional distributions of several species. For example, in northern Australia populations of Spanish mackerel (*Scomberomorus commerson*) and grey mackerel (*S. semifasciatus*) are comprised of multiple stock units (Buckworth et al., 2007; Welch et al., 2009).

Fisheries management boundaries have generally been established on the basis of fishing practices and jurisdictional responsibilities, with little biological basis for many spatial delineations (Hall, 2001). However, in the wake of the collapse of many pelagic stocks, the need to delineate management frameworks on the basis of stock units has been widely acknowledged as vital for effective management, monitoring, and assessment (e.g. Begg et al., 1999a; Secor, 2005; Welch et al., 2009). While many definitions for the term 'stock' are available (see review by Waldman, 2005), we adopt the working definition of a fish stock as "...an *intra-specific group of randomly mating individuals with temporal and spatial integrity* (Ihssen et al., 1981)". The delineation of the stock structure of pelagic fishes is difficult due to their extensive yet often patchy spatial distributions, extreme mobility, high capacity avoidance of capture, differential susceptibility to capture among cohorts and temporal variations in patterns of distribution and behaviour (McGurk, 1986; Ward et al., 1998). These species are also highly susceptible to environmental changes, which have the capacity to drive 'boom and bust' fluctuations in abundance, which may result in considerable habitat shifts (e.g. Lluch-Belda et al., 1991; Chavez et al., 2003; Takasuka et al., 2007). Furthermore, the trans-boundary migratory patterns of many pelagic species mean that management arrangements also need to account for resource sharing among regional or international neighbours (Cochrane et al., 1998; Caddy and Seijo, 2005).

There are many direct methods available for stock delineation (see Cadrin et al., 2005), including the use of genetic markers, otolith structure and chemical composition, morphometry, parasite diversity, and tagging experiments. Indirect evidence, such as regionally variable life history traits (i.e. growth, reproduction and distribution) and catch statistics have also been used as evidence of spatial stock structuring. More recently,

an emphasis has been directed towards multi-disciplinary approaches (e.g. the collation of findings from a broad spectrum of complementary techniques) to develop a holistic understanding of a species' biology, ecology and evolutionary processes (Begg and Waldman, 1999). This approach provides the greatest certainty for identifying stock units and defining stock boundaries (Waldman, 1999; Abaunza, 2008). This 'holistic' approach to assessing stock structure has been effective. As multiple temporal and spatial scales are addressed simultaneously, the power to detect stock units is increased (Begg and Waldman, 1999; Waldman, 1999; Abaunza et al., 2008a). For many pelagic fishes, biological and ecological data are obtained from only those components of the population(s) that occur on the seasonal fishing grounds (i.e. fishery dependent data) (Hammer and Zimmermann, 2005). While this strategy may provide adequate biological data for normal stock assessments, the spatial limitations of these studies can potentially be biased towards maintaining traditional stock management units.

While holistic approaches may be the most suitable option for the delineation of stock structure, combining the findings of multiple complementary approaches, each with varying spatial and temporal scales, into a single quantitative analysis is complex (Welch et al., 2009). Current methods for defining management units from these synthesised findings requires further development (Leonart and Maynou, 2003; Abaunza, 2008; Abaunza et al., 2008b). The semi-quantitative, comparative approach adopted in the present study provides a means of combining the results from a range of techniques for delineating stock structure (Abaunza et al., 2008b). In most cases, syntheses of the findings from a range of approaches to stock delineation are restricted to explicit time scales. However, for the purposes of defining management units, pooling all available information, regardless of time scale, may be the most appropriate option.

Synthesising information across a species' distribution may enable the formulation of a tangible hypothesis of stock structure. An example of a targeted pelagic species that has been extensively researched is the Australian sardine (*Sardinops sagax*). Much of the research has focused on targeted 'stocks' adjacent to major fishing ports. Insufficient work has been done to provide a robust view of the structure. Surprisingly, research conducted by Maurice Blackburn in the 1940-50s remains the most comprehensive analysis of the stock structure of this species to date. The ensuing body of sardine research requires consideration to provide a 'contemporary' understanding of the species' stock structure.

### **3.2 THE AUSTRALIAN SARDINE *Sardinops sagax* (Jenyns 1842)**

Sardines (pilchards) are distributed throughout the world's oceans (Cole and McGlade, 1998), with the largest populations centred on highly productive upwelling regions (Beckley and van der Lingen, 1999). These large populations support some of the world's largest fisheries and are the focus of extensive research (Schwartzlose et al., 1999; Stratoudakis et al., 2006). Major fisheries for this species occur in California, Peru, Chile, Japan and southern Africa (Beckley and van der Lingen, 1999).

Australia also has several fisheries that take sardine (ABARE, 2009). Small scale exploitation has occurred since the 1800s (Kailola et al., 1993), but combined national catches did not exceed 1000 t until the 1970s when several purse seine fisheries developed out of ports in southern and Western Australia (Fletcher, 1991a). Currently, the largest commercial fishery (total allowable commercial catch [TACC] in 2009 of 30 000 t) is located off South Australia (Ward et al., 2010).

The distribution of the Australian sardine includes coastal waters of the entire southern half of the continent, which is a total linear distance of 6700 km, making it the largest geographical distribution of all *Sardinops* populations (Fletcher et al., 1997). This population has not been characterised by the decadal fluctuations in abundance seen off southern Africa, Japan, South America and North America (Lluch-Belda et al., 1989, 1992), suggesting that Australia's marine environmental conditions may be relatively stable in comparison to those systems. In addition, fishing pressure in Australia has, in most cases, been constrained within sustainable limits and catches have generally been stable (Bernoth, 2002).

Despite the stability of environmental conditions and catches, there have been two mass mortality events that occurred across the entire Australian distribution (Gaut, 2000). These events, which occurred in 1995 and 1998/99 (Gaughan et al., 2000; Ward et al., 2001b), reduced the fishable biomass by up to 70% for some regions, and resulted in short-term depression in the commercial catches in most parts of Australia (Ward et al., 2001b). Currently, most regional stocks have recovered to pre-virus catch levels (Western Australia is the exception), suggesting that Australian sardine stocks are generally resilient to biomass depressions and have a strong capacity for rapid recovery (Bernoth, 2002).

Outside Australia, several spatially distinct sub-populations are recognised in *Sardinops* populations based on the presence of separate spawning grounds and differences in meristic, morphological and, or molecular characteristics (Felin, 1954; Parrish et al., 1989; Bowen and Grant, 1997). In Australia, a considerable amount of information has been generated to determine the population structure of the Australian sardine (Hall, 2001). However, there is little consensus among the findings of those studies (Fig. 3.1a). The most recently proposed composite image of the stock structure of the Australian sardine was by Whittington et al. (2008) based on a qualitative review of the available literature for the species (Fig. 3.1b).

Based on jurisdictional arrangements and current (varying) perceptions of the stock structure of Australian sardine, there are multiple independently-managed fisheries across southern Australia (Fig. 3.1). However, it has been suggested that there is large-scale mixing among regional sardine populations with temporal rather than spatial associations significantly influencing the stock structure, resulting in regional stocks being highly ephemeral (Yardin et al., 1998).

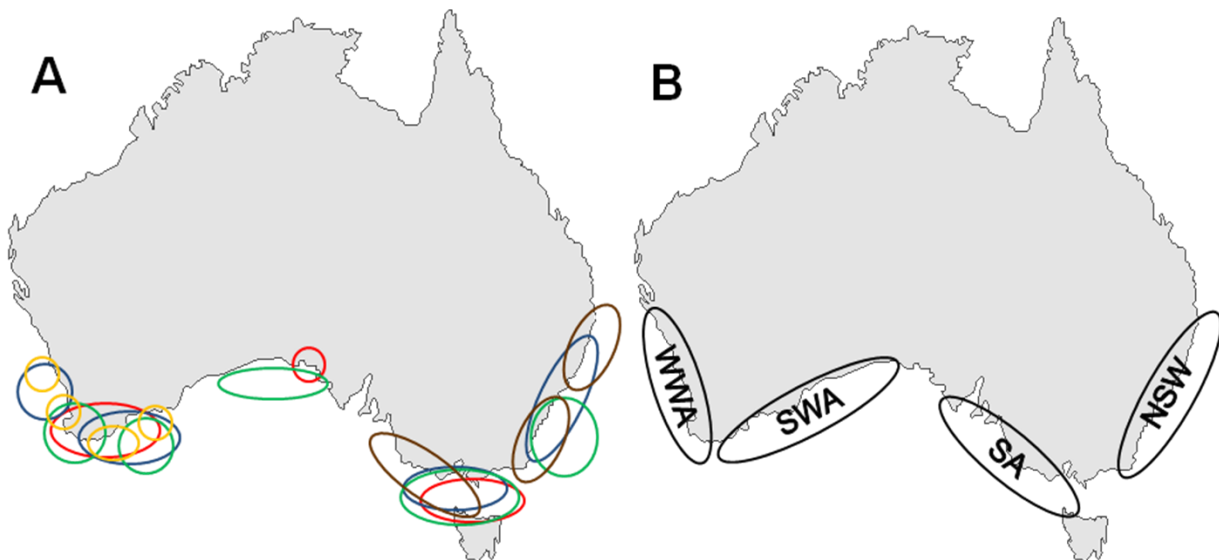


Figure 3.1 Proposed sub-populations of the Australian sardine throughout southern Australia from selected research, including: (A) red = Blackburn (1951); blue = Syahailatua (1992); green = Dixon et al. (1993); orange = Edmonds and Fletcher (1997); and brown = Yardin et al. 1998; and (B) adapted from Whittington et al. (2008) and NSW = south-eastern Queensland/northern New South Wales; SA = Victoria/South Australia; SWA = south coast Western Australia; WWA = west coast Western Australia.

Little current information is available on the stock structure of the sardine in New Zealand (Paul et al., 2001). The species is distributed around the North Island and the northern part of the South Island (Baker, 1972); however, catch statistics suggest that a larger biomass of sardines is found in the northernmost part of the North Island (Anonymous, 2009). The New Zealand sardine is considered separate from the Australian sardine population, on the basis of vertebral counts (Blackburn, 1951), size at maturity (Baker, 1972), and geographic isolation reflected in genetic differentiation (Yardin et al., 1998). As such, the New Zealand sardine population is not included in this report.

The stock structure of sardine in Western Australia has been determined over a series of studies (e.g. Fletcher and Tregonning, 1992; Fletcher, 1994; Fletcher et al., 1994; Edmonds and Fletcher, 1997; Gaughan et al., 2001a, 2001c, 2002). Based on spatial differences among biological parameters and annual catch statistics, it has been suggested that distinct breeding stocks occur off the west and south coasts of Western Australia (Fig. 3.1a). These populations have been shown to differ in life histories and show variable or no connectivity; hence, these populations are managed independently (Hall and MacDonald, 1986; Schwartzlose et al., 1999). In contrast, throughout the south-eastern Australian distribution of the species, there has been little consensus among studies of the stock structure of the sardine (Fig. 3.1a); and there have been few cooperative efforts that have sought to cover the entire distribution of the species (e.g. Ward et al., 1998).

For the Australian sardine, there is a fundamental need to better understand the stock structure and movement patterns of the species throughout its range. Two fisheries for Australian sardine have grown rapidly over the last decade. In 2009, the Total Allowable



Catch (TAC) and annual catch in the South Australian Sardine Fishery (SASF) were ~30K t, making it Australia's largest single-species fishery by weight (Ward et al. 2008). In 2009, the sardine catch in the NSW Ocean Haul Fishery was also at its highest historical level of ~2.0K t per annum (Ward and Rogers, 2008). In contrast, catches in the Western Australian Sardine Fishery declined from ~6-9K t during 1988-98 to ~1K t in recent years, apparently through combined effects of two mass mortality events, overfishing and, potentially, distributional changes associated with long-term environmental variability (Gaughan et al., 2004; Nardi et al., 2007).

Fishers off South Australia and NSW have expressed some interest in further increasing catch levels of sardine, and assessing if and how increased catches could be sustained. Interest in expanding these two fisheries has arisen because the global supply of sardines has recently contracted, largely due to quota reductions in the sardine fisheries off the west of North America (which have historically supplied sardines to the tuna farmers of Port Lincoln). Consultative groups for two State fisheries and one Commonwealth fishery have identified the need for information on the movement patterns and stock structure of Australian sardine as a high priority. These are the South Australian Sardine Fishery Research Sub-committee, NSW Ocean Haul Fishery Management Committee and Commonwealth Small Pelagic Fishery (SPF) Resource Advisory Group. Members of these consultative groups have participated in the development of this proposal and have provided letters of support. Industry representatives of the South Australian Sardine Fishery, NSW Ocean Haul Fishery and SPF have also provided supporting letters.

This study will address two key research questions.

- 1) Off South Australia, sardine fishing is conducted mainly in southern Spencer Gulf. However, the estimates of spawning biomass, which are the primary biological performance indicator for the fishery, are based on surveys that include all shelf waters off South Australia (e.g. Ward et al., 2007, 2008). This discrepancy has led to an agreement among industry representatives, fisheries managers, scientists and other stakeholders that any further increase in the TAC will need to be associated with the introduction of finer-scale spatial management (zoning), unless information is collected which shows that zoning is not required.

Currently, the TAC of 30K t is set at ~11% of the latest estimate of spawning biomass, which is a conservative exploitation rate by international standards (Ward et al., 2007, 2008). Hence, increasing the TAC is biologically feasible at a whole of stock level. However, establishing zones with separate TACs will increase the costs of fishing, so information on the patterns and rates of movement of sardines between southern Spencer Gulf and the eastern Great Australian Bight is needed to determine whether additional spatial management is needed to support an increase in the TAC.

- 2) Off eastern Australia, significant historical catches of sardine have been taken from waters between Victoria and southern Queensland. Currently, the most significant fishery off the east coast is located off southern NSW, around Eden. However,

estimates of spawning biomass for eastern Australia are available only for waters off northern NSW and southern Queensland (Staunton-Smith and Ward, 2000; Ward and Rogers, 2008). It has been hypothesised that a significant proportion of the east coast sardine stock migrates northwards into northern NSW and southern Queensland during late winter and early spring to spawn (Ward and Staunton-Smith, 2002; Ward et al., 2003). If this hypothesis is correct, then the estimate of spawning biomass from northern waters may largely reflect the size of the entire sardine stock off eastern Australia. However, if this hypothesis is incorrect, then the east coast population of sardines could be much larger than the current estimates of spawning biomass. Information on the patterns and rates of movement of sardines along the east coast is needed to enhance future stock assessment and to ensure that this resource is utilised and managed effectively.

Information on the patterns and rates of movement of sardines along the east coast is needed to enhance future stock assessment and to ensure that this resource is utilised and managed effectively. This study of the movement patterns and stock structure of sardine off southern and eastern Australia will be done within the context of a broad study of the entire Australian distribution. As a starting point existing life history and fishery information from all jurisdictions will be collated to develop hypotheses regarding movement patterns and stock structure. Large collections of otoliths that have been archived in all relevant Australian states (Western Australia, South Australia, Victoria, Tasmania, NSW and Queensland) will then be used to cost-effectively examine these hypotheses regarding movement patterns within and among jurisdictions. Some additional otolith samples may be required. The main focus of the study will be on the rates and patterns of movement of sardines (*i*) between shelf and gulf waters of South Australia and (*ii*) along the East Coast between Lakes Entrance, Victoria and waters off Fraser Island, southern Queensland.

## **4. NEED**

This project is needed to assess the potential for increasing catches of sardine in the fisheries off South Australia (i.e. SASF) and the East Coast (i.e. NSW Ocean Haul Fishery and SPF). For the SASF, information on the patterns and rates of movement of sardines between the Great Australian Bight (where the majority of the spawning biomass is located) and southern Spencer Gulf (where most fishing is conducted) is needed to determine whether (or not) future potential increases in the TAC should be accompanied by the establishment of zones within the fishery. For the NSW Ocean Haul Fishery and SPF, information on the patterns and rates of movement of sardines along the East Coast is needed to assess the extent to which estimates of spawning biomass from northern NSW and southern Queensland waters reflect the size of the entire sardine stock off eastern Australia.

## 5. OBJECTIVES

There are four overall project objectives, these are:

- To collate data held for jurisdictions to generate hypotheses regarding movement patterns and stock structure of Australian sardine throughout southern Australia.
- To evaluate the use of otolith shape analyses for testing hypotheses regarding the movement patterns of sardine in southern Australia.
- To evaluate the use of transect-based otolith microchemistry techniques (laser ablation ICPMS) for examining the movement patterns of sardine between gulf and shelf waters of South Australia and along the east coast of Australia.
- To collate findings to determine: (i) whether or not additional spatial management (zones) would be needed to support a future increase in the TAC in the South Australia Sardine Fishery; and (ii) the degree to which estimates of spawning biomass from waters off northern NSW and southern Queensland reflect the size of the entire eastern Australian sardine stock.

## 6. METHODS

In this study we incorporate the existing body of research (published papers, government reports, theses) to develop a contemporary understanding of stock structure. We do this using a weight of evidence approach and semi-quantitative method to identify patterns of connectivity, movement and stock boundaries of the Australian sardine. Potential incidents of population sub-structuring that relate to the south-eastern Australian sardine distribution will be tested using two otolith-based approaches to stock delineation: (i) normalised elliptical Fourier analysis of the outline shape of whole otoliths; and (ii) the analysis of the trace elemental composition of otoliths.

### 6.1 WEIGHT OF EVIDENCE APPROACH FOR DELINEATING THE STOCK STRUCTURE OF THE AUSTRALIAN SARDINE

Recently, Welch et al. (2009) described a means of integrating the findings of multiple approaches to stock delineation to define management units. Matrices were developed outlining significant differences/similarities among different regions based on the results of multiple complementary techniques to delineate stock structure in the grey mackerel (*Scomberomorus semifasciatus*). These matrices provide a weight of evidence approach, which is quantified using the mean number of tests carried out for each technique; thereby calculating a Stock Difference Index [SDI] (Equation 1) where DV is the difference value (i.e. different = 1, and not different = 0) and count is the number of tests.

$$SDI = \sum DV / COUNT (DV) \quad (\text{Equation 1})$$

By using the mean DV of the total number of techniques used to assess stock structure, this technique allows regions to be included that have limited sampling effort and information. The SDI approach provides a quantitative measure of the relative differences among regions (i.e. SDI = 1 maximum regional differences, SDI = 0 no regional difference). However, if no regional differences are detected among populations, this does not explicitly verify the existence of a single stock unit; it merely fails to falsify the null hypothesis of a single stock (Welch et al., 2009).

This study uses a weight of evidence approach to define manageable stock units; the development of multiple matrices in the present study provides a semi-quantitative analysis of differences/similarities among regional populations. Following Welch et al. (2009), differences were assessed among key geographic regions of the distribution of the Australian sardine (Fig. 6.1): wWA – west coast Western Australia, sWA – south coast Western Australia, SA – South Australia, Vic – Victoria, Tas – Tasmania, sNSW – southern New South Wales, and nNSW – northern New South Wales – southern Queensland. Differences in biological and ecological attributes of sardines among these regions were assessed from a total of 14 techniques for inferring patterns of stock structure and connectivity among regional Australian sardine populations (Table 6.1).

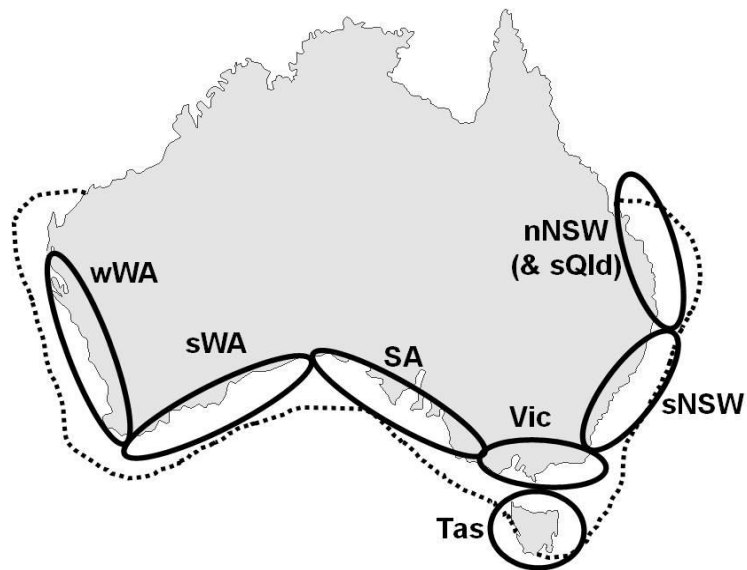


Figure 6.1 Australian distribution of the sardine (*Sardinops sagax*). The solid rings denote those regions examined by the integrated Stock Difference Index—weight of evidence approach.

Table 6.1 Techniques used to assess differences in biological and ecological attributes among regional Australian sardine populations.

<b>Attributes</b>	<b>Techniques</b>	<b>Number</b>
<i>Tagging and genetic approaches</i>	Tagging	<i>i</i>
	Mitochondrial DNA analysis (mtDNA)	<i>ii</i>
	Allozyme electrophoresis	<i>iii</i>
<i>Phenotypic variation</i>	Morphological and meristic analyses	<i>iv</i>
	Otolith shape analysis	<i>v</i>
	Otolith chemistry	<i>vi</i>
	Parasites	<i>vii</i>
<i>Patterns of commercial fishing</i>	Fishing seasonality	<i>viii</i>
	Regional catch compositions	<i>ix</i>
<i>Timing and seasonality of reproduction</i>	Size at maturity	<i>x</i>
	Timing of spawning	<i>xi</i>
	Distribution of larvae and eggs	<i>xii</i>
<i>Life history parameters</i>	Larval growth rates	<i>xiii</i>
	Adult and juvenile growth patterns	<i>xiv</i>

Relevant information was collated from published and unpublished reports and papers and the databases of participating research agencies and scientists. The bulk of the literature reviewed was produced by the respective regional fisheries agencies; hence, a large component of the references cited here comprises inter-governmental (i.e. regional) fisheries reports. Given that many of the references cited throughout the text consist of ‘grey literature’ and may not be readily available, all available reports were scanned and are available as PDFs from the authors upon request.

## 6.2 ARCHIVED COLLECTIONS OF SARDINE OTOLITHS

Otolith increment based ageing is a key component of the stock assessments of commercially targeted species of teleosts, with annual collections of otoliths being routinely made. Archived otoliths provide a time-series in which to answer questions about stock structure, movement patterns and to infer relative shifts in life history traits of commercial species (Rivers and Ardren, 1998).

The Australian sardine provides one such example of a targeted species that routinely undergoes wholesale sampling. As such, large collections of sardine otoliths have been archived in all relevant Australian state fishery agencies; including South Australia, Victoria, New South Wales and Queensland, potentially providing a cost-effective means of examining hypotheses regarding movement patterns within and among jurisdictions (refer to Supplementary Table A1). However, when collated, the available otolith collection for the Australian sardine varies in both spatial and temporal breadth, and a representative sample of sardine otoliths from throughout the species range over a single year is not currently available (refer to Supplementary Table A1).

Archived sardine otoliths were collated from regional fisheries agencies throughout south-eastern Australia. Otoliths were collected as part of annual sardine stock assessments (e.g. Ward et al., 1998, 2005b, 2007, 2008a, 2010; Ward and McLeay, 1999; Rogers and Ward, 2005; Stewart et al., 2010) (refer to Supplementary Table A1). Otolith collections for each of the years comprised samples that had been collected throughout the year (i.e. multiple months represented for a single year); thus, providing an otolith shape/chemical signal that represents multiple schools of fish (i.e. no one school of fish represents an entire sampling region).

In general, laboratory processing procedures are consistent throughout years and organisations. Each specimen was thawed and then weighed to the nearest gram and measured for fork length [FL] to the nearest millimetre. Sardines were separated into 10-mm FL size classes for analysis. The sex of the specimen was determined by visual assessment of the gonad. Specimens were assigned a state of sexual maturity (i.e. immature versus mature), based on published sex-specific sizes at maturity (Table 6.2).

Table 6.2 Published fork lengths (in mm) at maturity for male and female sardines from throughout Australia.

Region	Male	Female	Source
South Australia	Immature < 146 ≥ Mature	Immature < 150 ≥ Mature	Ward & Staunton-Smith 2002
Victoria	Immature < 129 ≥ Mature	Immature < 151 ≥ Mature	Kinloch et al. 1998
New South Wales	Immature < 136 ≥ Mature	Immature < 136 ≥ Mature	Stewart et al. 2010
Queensland	Immature < 145 ≥ Mature	Immature < 145 ≥ Mature	Ward & Staunton-Smith 2002

Sagittal otolith pairs were removed from the specimens and cleaned in ultrapure water. Otoliths were air dried and weighed to the nearest 0.01 mg prior to archival storage. Due to difficulties in interpreting the incremental structure of sardine otoliths (Fletcher,

1991b, 1995; Fletcher and Blight, 1996; Rogers and Ward, 2007), the ages of all sardines examined here are based on otolith weights. Estimates of age were calculated using region specific regression equations (Table 6.3). These regressions used only those otoliths that had a high level of reader confidence for age. Nevertheless, some uncertainty exists around the estimated ages of the sardines analysed.

Table 6.3 Region specific otolith weight (mg)—estimated age (years) linear equations for the Australian sardine.

Region	Otolith weight—Age linear relationship	Source
Southern Queensland	$Y = 0.270 X + 0.345$	Hall, 2001
Northern East Coast	$Y = 0.238 X + 1.263$	Stewart, unpublished data
Southern East Coast	$Y = 0.078 X + 1.099$	Stewart, unpublished data
Lakes Entrance, Victoria	$Y = 0.273 X + 0.504$	Hall, 2001
Port Phillip Bay, Victoria	$Y = 0.368 X + 0.310$	Hall, 2001
Spencer Gulf, South Australia	$Y = 1.950 X + 0.430$	Rogers and Ward, 2007
Coffin Bay, South Australia	$Y = 0.237 X + 0.868$	Hall, 2001

### 6.3 OTOLITH-BASED APPROACHES TO DELINEATING FISHABLE MANAGEMENT UNITS

Given the importance of correctly identifying fish stocks, there exists a diverse range of approaches to infer stock structure (see Cadrin et al., 2005). In spite of this methodological diversity, otolith-based approaches (i.e. otolith chemical composition, morphology and shape analysis) are considered the most appropriate for identifying significant fishable units as they reflect environmental and not genetic differences among fish populations (Campana and Neilson, 1985; Campana and Casselman, 1993; Begg and Waldman, 1999). From a practical fisheries management perspective, those sub-populations based on phenotypic otolith traits should be considered separate management units (Cadrin and Friedland, 1999).

Otoliths are naturally occurring chronometers of organismal growth and environmental history due to their metabolic stability and continual growth throughout the life of the fish (Campana and Neilson, 1985; Campana, 1999, 2005; Cardinale et al., 2004). These calcified structures (and their chemical composition) are formed in response to endogenous and exogenous factors that leave permanent, natural marks in the otoliths of individual fish (Campana, 2005).

As such, the elemental compositions of otoliths are routinely used to reconstruct the environmental histories of fish, including the stock structure and movement patterns (Gillanders, 2001; Elsdon and Gillanders, 2002). It is therefore possible to discriminate among groups of fish that have spent portions of their lives in different environments on the basis of the chemical composition of their otoliths (Gillanders and Kingsford, 2000).

The application of different elemental signatures (i.e. whole otoliths, edge and core regions, and profiles across the otoliths) provides a suite of information to assess various components of the life history of the fish. For example, spatial and temporal



differences are expected from otolith edge signatures as they reflect environmental conditions at the site of capture (Ashford et al., 2005). Conversely, analyses of whole otoliths or averaged concentrations across the otolith profile are better suited to identifying stock units, as these signatures reflect the environmental conditions experienced throughout the life history of the fish (e.g. Ashford and Jones, 2007). Furthermore, the application of these entire life history approaches is better suited to pelagic teleosts (e.g. the sardine), as they are less confounded by the intrinsic migratory nature of these fishes. Similarly, the core region of the otolith is better suited to identifying the stock structure of the species, as the chemistry of otolith nuclei, laid down during early life subsequent to spawning, records early spatial separation resulting from segregation during spawning (Ashford et al., 2006).

Analysis of otolith chemistry has been effective in identifying patterns of stock structure for sardines and other clupeid species (e.g. Brophy et al., 2003; Morales-Nin et al., 2005; Castro, 2007; Aldanondo et al., 2010). In Australia, the analysis of trace element and stable isotope signatures of whole sardine otoliths has helped elucidate patterns of large and fine scale sardine stock structuring, particularly in Western Australia (Edmonds et al., 1995; Edmonds and Fletcher, 1997; Gaughan et al., 2001a).

The external characteristics of the otolith, such as the shape and morphology, have become commonly used to assess differences between spatially separated populations of fish (Campana and Casselman, 1993; Turan, 2006; Hüsey, 2008). Variability in the gross morphology of the otolith results from environmental differences among conspecific groups of fish inhabiting different locations (Begg et al., 1999a; Abaunza et al., 2008a). In addition, otolith morphology may be influenced by specimen sex, age and size class, as well as stock and environment (Campana and Casselman, 1993; Bolles and Begg, 2000; Cardinale et al., 2004; Mérigot et al., 2007). The influence of these factors on otolith shape appears to be species specific (Hüsey, 2008). Hence, while otolith shape is exploited as a natural mark/tag to identify fish stocks and management units, potential factors that cause the differences in shape require consideration.

Morphometry of otoliths has been useful in stock discrimination studies, as otolith growth is highly correlated to somatic growth of the fish (Campana and Casselman, 1993). Several methods exist for quantitatively determining the shapes of otoliths (Cadrin and Friedland, 1999), including the otolith 'landmark' methods, based on points of reference of the otolith's structure (e.g. Turan, 2000; Tuset et al., 2003); which has been used to infer patterns of stock structure in Pacific sardines (*Sardinops sagax*) (Félix-Uraga et al., 2005). Alternative approaches consist of the more complicated otolith 'outline' methods (e.g. Burke et al., 2008a, b; Stransky et al., 2008b). Here, the outline of the otolith is numerically described using Fourier analysis. Fourier analysis has been used successfully in clupeoid stock discrimination studies (e.g. European anchovy, *Engraulis encrasicolus*) (Gonzales-Salas and Lenfant, 2007).

## 6.4 NORMALISED ELLIPTICAL FOURIER ANALYSIS OF OTOLITH SHAPE

Normalised elliptical Fourier analysis [EFA] was used to determine if differences existed among otoliths. This required the examination of (i) potential allometric effects on otolith shape and (ii) intra-annual differences in otolith shape. The methodology described below for EFA of the shapes of the outlines of sardine otoliths was applied throughout the course of the study.

High contrast otolith images were captured using a LEICA DC-300 digital camera mounted onto a LEICA stereo microscope at  $\times 10$ -magnification. Images were captured using IMAGE PRO-PLUS version 7.0. In order to maximise the contrast between the otolith and the black background, otolith images were taken under reflected light (Fig. 6.2a). Otoliths were orientated so that the sulcus was face up and the rostrum horizontally aligned. Otolith images were then imported into the SHAPE version 1.3 analytical software program (Iwata and Ukai, 2002: <http://lbm.ab.a.u-tokyo.ac.jp/~iwata>), and converted to binary images (Fig. 6.2b).

EFA was used to evaluate the numerical contours of the otolith outlines. EFA uses an orthogonal decomposition of a curve into a sum of harmonically related ellipses that when combined, approximate the original otolith shape (Kuhl and Giardina, 1982; Tracey et al., 2006). Thirty randomly selected otoliths were used to determine the number of harmonics required to sufficiently reconstruct the otolith outline. The first fourteen harmonics exceeded 99.99% of the mean cumulative power (Pothin et al., 2006) (Fig. 6.3). Each harmonic is composed of four coefficients ( $A$ ,  $B$ ,  $C$ ,  $D$ ) resulting in 56 elliptical Fourier coefficients [EFCs] per individual. However, due to the shape software normalising the EFCs for otolith size and orientation, the first three EFCs ( $A_1$ ,  $B_1$ ,  $C_1$ ) were constant among all otolith outlines, therefore, they were discarded from further analysis reducing the number of EFCs to 53.

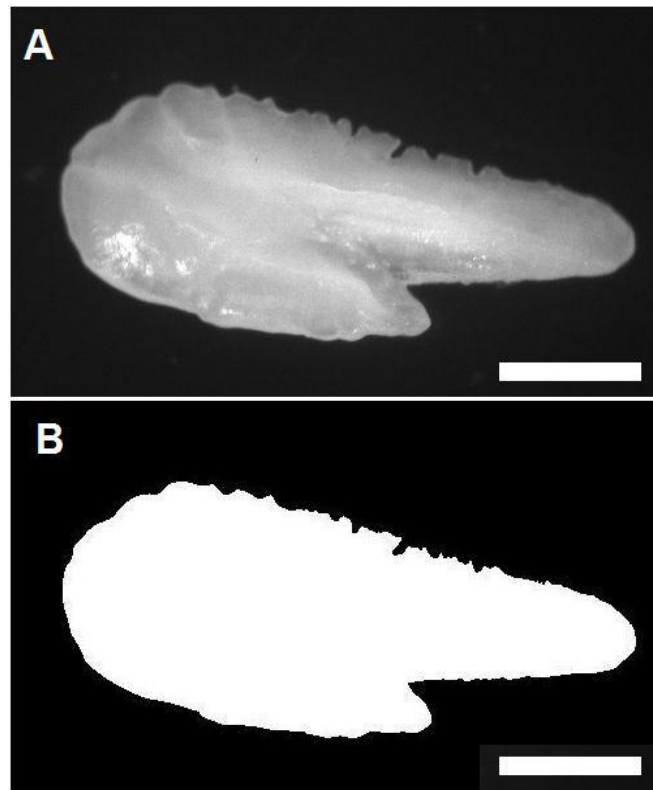


Figure 6.2 Representative digital image of the otolith of the Australian sardine: (A) original image; and (B) modified binary image. Scale bars = 0.5 mm.

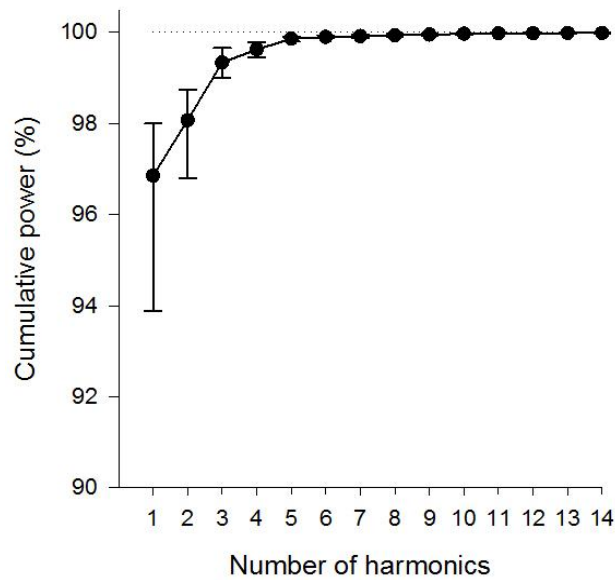


Figure 6.3 Variation in the mean cumulative power (%) of the mean Fourier power spectrum in relation to the number of harmonics required to sufficiently reconstruct the overall shape of the otolith of the Australian sardine. Vertical bar represent the maximum and minimum range of the cumulative power and the dotted line indicates the 99.99% cumulative power.

### 6.4.1 Allometric effects on otolith shapes

Seven archived sardine otolith collections were examined for the effects of specimen biology on otolith growth and shape (Table 6.4). The specific biological factors examined here included specimen sex, fork length (i.e. size class), estimated age (i.e. age cohort) and state of sexual maturity (i.e. immature vs. mature). Each otolith collection was caught within a one month period and consists of sardines of both sexes and states of sexual maturity (Table 6.4). Where available a range of age cohorts were represented (Table 6.5).

Table 6.4 Details of the archived sardine otolith data sets used in this study. Note, ( $\Omega$ ) samples from Jumping Pin consisted entirely of mature fish, and (\*) samples from Scotts Cove consisted entirely of immature fish.

Archived otolith dataset	Total			Male (n)	Female (n)	Size range (FL mm)	Otolith weight range (mg)
	(n)	Year	Month				
Port Phillip Bay, Victoria	67	1995	February	24	43	128 – 170	0.7 – 2.1
Jumping Pin, Queensland $\Omega$	77	1998	June	28	49	155 – 194	1.1 – 2.3
Scotts Cove, South Australia *	65	2003	February	34	31	126 – 144	0.7 – 1.1
Wedge Island, South Australia	118	2005	February	44	74	132 – 186	0.8 – 2.1
Greenly Island, South Australia	95	2006	November	26	69	123 – 210	0.7 – 2.3
Eden, New South Wales	134	2009	July	51	83	127 – 214	0.8 – 2.8
Coffin Bay, South Australia	94	2010	March	32	62	145 – 201	0.9 – 2.6

Table 6.5 Details of age cohort sample sizes for the archived sardine otolith data sets used in this study.

Archived otolith dataset	Total (n)	Age range (years)	Age cohorts (n)			
			2-year	3-year	4-year	5-year
Port Phillip Bay, Victoria	67	2 – 4	18	39	10	0
Jumping Pin, Queensland	77	3 – 5	0	33	32	12
Scotts Cove, South Australia	65	2	65	0	0	0
Wedge Island, South Australia	118	2 – 5	37	51	17	13
Greenly Island, South Australia	95	2 – 5	12	36	33	14
Eden, New South Wales	101	2 – 5	46	31	12	12
Coffin Bay, South Australia	94	2 – 5	13	33	32	16

Nonlinear regression analysis [NLR] was used to test relationships between otolith weight and somatic growth (measured here as fork length and body weight) and validate proportional otolith development. Single factor analysis of variance [ANOVA] was used to assess differences in otolith weight between the sexes (one factor analysis for each otolith dataset) and states of sexual maturity, as well as among the size classes and age cohorts.

EFCs of otolith shape were transformed to a Euclidean distance resemblance matrix using the PRIMER software package (version 6.0: Clarke and Gorley, 2006). Single factor

permutational multivariate analysis of variance [PERMANOVA] (Anderson, 2001) was used to assess differences in otolith shape (EFA) among biological parameters, i.e. specimen sex, size (FL-) class, age cohort, and maturity state designated; all factors were treated as fixed. For all PERMANOVA tests 4999 permutations of the raw data were used.

#### **6.4.2 Inter-annual variability in otolith shapes**

As a continuous otolith dataset does not exist for the Australian sardine, comparisons among regional populations sampled across multiple years may be necessary (refer to Supplementary Table A1) to examine patterns in spatial variability. Thus, potentially confounding effects of inter-annual variability of the otolith shape signal may obscure 'true' patterns of spatial variability. Therefore, prior to making comparisons of the shapes of otoliths among regional populations of sardines sampled from different years, it is essential to test the effects of inter-annual variability among samples collected from multiple years at a discrete sampling location.

The Spencer Gulf [SG] region of South Australia had the largest temporal breadth of archived sardine otoliths and spanned eight (non-sequential) years of sampling effort (Table 6.6) (refer to Supplementary Fig. A1). The samples from SG (n = 2222) were used to assess for temporal stability in otolith shape. Within this otolith collection the 2 (n = 715), 3 (n = 892), and 4 (n = 615) year age cohorts of sardines comprised the largest individual cohorts. These cohorts were analysed separately.

Given that the SG otolith collection consists of samples collected from multiple months of each sampling year, the data were further partitioned to prevent the confounding affects of fish movement within years (Table 6.6). Otoliths were divided into four seasonal collection periods that comprised three months: December to February [summer]; March to May [autumn]; June to August [winter]; and September to November [spring]. These three month sampling periods were analysed separately to test for seasonal inter-annual patterns of otolith shape variability.

Table 6.6 Sampling periodicity and sample sizes for Spencer Gulf sardine otoliths used in this study.

<b>Location</b>	<b>Sampling year</b>	<b>Sampling months</b>	<b>n</b>
Spencer Gulf	1995	March, April, and June to October	426
	1996	March	62
	2003	January to May, and August	435
	2004	January to June, and September to December	393
	2005	January to May, and December	135
	2006	January, March, April, June, November, and December	307
	2009	January to July	331
	2010	April, May, and June	133

EFCs of otolith shape were fit to a Euclidean distance resemblance matrix. For comparisons among the multiple sampling periods, a nested PERMANOVA design was used, with month of capture nested within the sampling year. For all PERMANOVA tests 4999 permutations of the raw data were used.

#### **6.4.3 Stock structure of sardine throughout south-eastern Australia, inferred from otolith shape**

This component of the study builds on the findings of the age and year class analysis to use EFA of the shapes of the otoliths to assess the population structure and movement patterns of the sardine throughout south-eastern Australia. Given that otolith shape reflects environmentally driven phenotypic traits, groups of fish with different shapes of the outlines of their otoliths can be considered separate management units (Cadrin and Friedland, 1999, 2005).

In total, 4740 sardine otoliths from across south-eastern Australia were examined (Table 6.7). Of these, 3130 were from fish caught throughout the austral autumn and winter periods. These fish were used in the subsequent analysis to facilitate comparisons of otolith shape (EFA) among samples collected at a similar time each year; limiting the confounding effects of intra-annual environmental fluctuations that may influence otolith shape (DeVries et al., 2002). Inter-annual variability in the shape of otoliths among the sardines caught in autumn/winter was tested using individuals caught from SG and among the entire autumn/winter otolith collection from south-eastern Australia.

Table 6.7 Regional fishing locations examined and referred to for otolith shape analysis in this report.

Location	Abbreviation used in text	Sampling years	Age cohorts (n)			Total (n)
			2-year	3-year	4-year	
Far West Coast of South Australia	FWC	2003, 2006	156	50	6	213
West Coast—Coffin Bay (SA)	WCCB	1995, 2003-06, 2010	84	227	279	619
Southern Spencer Gulf (SA)	SG	1995/96, 2003-06, 2009/10	715	892	615	2265
Kangaroo Island—Investigator Strait (SA)	KIIS	2003, 2006, 2009/10	142	66	21	232
Port Phillip Bay, Victoria	PPB	1995/96	153	98	8	292
Lakes Entrance, Victoria	LE	2011	7	104	65	179
Southern East Coast of Australia	SEC	2009	96	79	21	196
Northern East Coast of Australia	NEC	1998, 2009	85	12	1	98
Southern Queensland	QLD	1998	12	178	144	345

Within the autumn—winter otolith dataset the 2 (n = 1092), 3 (n = 1041), and 4 (n = 659) year old sardines comprised the largest individual age cohorts. Age cohorts were

analysed separately and in a combined dataset to assess variation across cohorts to get an understanding of age-related changes in stock structure.

EFCs were fit to a Euclidean distance resemblance matrix. Single factor PERMANOVA was used to assess differences in EFCs among regions and sampling years. When significant differences among regions were identified, pairwise analysis was conducted to determine differences among adjacent regional populations. For pairwise analysis the probability (p)-value was set at 0.05. For all PERMANOVA tests 4999 unrestricted permutations of the raw data were used.

## **6.5 ANALYSIS OF THE ELEMENTAL COMPOSITION OF SARDINE OTOLITHS**

The elemental composition of sardine otoliths was determined using laser ablation inductively coupled plasma mass spectrometry [LA-ICPMS].

### ***6.5.1 Otolith preparation***

One otolith per fish was prepared for LA-ICPMS. Each otolith was embedded in a resin spiked with indium chloride (approximately  $30 \mu\text{g}\cdot\text{g}^{-1}$ ) as a resin indicator. Transverse sections, approximately  $300 \mu\text{m}$  thick, were made using a lapidary saw. Otolith sections were polished with progressively finer grades of lapping film and air dried for 24 h. Otolith sections were then mounted onto microscope slides with an indium chloride spiked (approximately  $200 \mu\text{g}\cdot\text{g}^{-1}$ ) crystal bond thermoplastic cement. Each slide was then sonicated in ultrapure water and air dried for 24 h.

### ***6.5.2 Laser ablation inductively coupled plasma mass spectrometry***

Otolith sections were analysed using a NEW WAVE UP-213 high-performance ultraviolet laser ablation system connected to an AGILENT 7500CS ICP-MS. Laser ablation runs were programmed to follow a profile path from the otolith nucleus to the otolith edge (Fig. 6.3). All ablation runs were preceded by a pre-ablated run in order to score the profile path. Pre-ablation laser settings were:  $40 \mu\text{m}$  laser beam diameter, at a pulse rate of 4 Hz and a scan speed of  $20 \mu\text{m}\cdot\text{s}^{-1}$ . Ablation runs followed the pre-ablation path, with a laser setting reconfigured to:  $30 \mu\text{m}$  laser beam diameter, at a pulse rate of 4 Hz and a scan speed of  $3 \mu\text{m}\cdot\text{s}^{-1}$ .

Trace elements chosen for analysis were:  ${}^7\text{Li}$ ;  ${}^{23}\text{Na}$ ;  ${}^{24}\text{Mg}$ ;  ${}^{55}\text{Mn}$ ;  ${}^{88}\text{Sr}$ ;  ${}^{115}\text{In}$ ;  ${}^{138}\text{Ba}$ ; and  ${}^{43}\text{Ca}$  which was used as the internal standard. All elemental data were expressed as ratios to  ${}^{43}\text{Ca}$  in  $\mu\text{mol}\cdot\text{mol}^{-1}$ . Concentrations of elements were calibrated against the National Institute of Standards [NIST] 612 glass standard (Pearce et al., 1996). An EXCEL macro was used to reprocess elemental data and to subtract background measurements (Munro et al., 2008). The ICPMS was precise throughout the analysis period, as the coefficients of variation of repeated measures of the NIST standard did not exceed 5% (range: 0.7 - 4.1%) for any element; which was within accepted error thresholds (Campana et al., 1997).

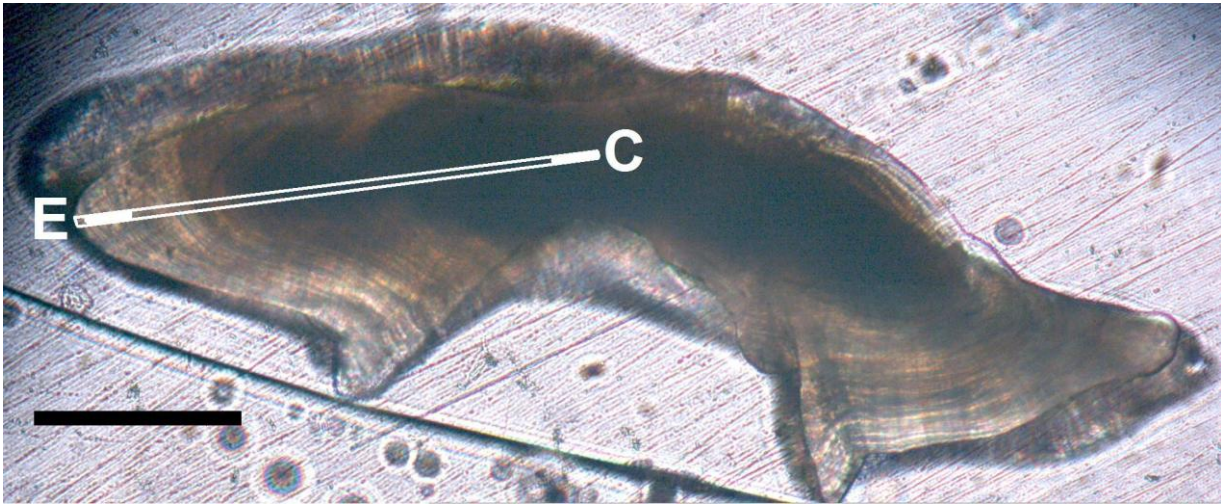


Figure 6.4 A transmitted light microscope image of a transversely sectioned sagittal otolith of an Australian sardine. The open line indicates the path of the laser transect. The solid sections of the profile indicate the 30  $\mu\text{m}$  otolith core (C) and edge (E) regions. Scale = 0.5 mm.

### 6.5.3 Relating element profile to otolith region

The partition of the transect element profile into 30  $\mu\text{m}$  core and edge regions replicated the spot ablation methodology employed by other otolith chemistry studies (e.g. Gillanders and Joyce, 2005), providing multiple elemental signatures of differing intrinsic timescales (Table 6.8) (Buckworth et al., 2007).

Table 6.8 Intrinsic timescales of the different otolith trace element signatures used in this study (adapted from Buckworth et al. 2007).

Otolith region analysed	Intrinsic timescale	Information provided
PROFILE (mean)	Organismal lifespan (~6-yrs)	Otolith chemistry can be used to characterise the life history of the specimens and provide a general image of the habitats occupied throughout their lifetime
EDGE	Days to months	Otolith chemistry can differentiate between the regions and be used to differentiate between the location of capture
CORE	Days to weeks	Otolith chemistry can be used to characterise the habitat of fish in early life and to separate spawning/hatching areas

In order to provide an otolith ‘edge’ elemental signature, the last 10  $\mu\text{m}$  of the transect profile was discounted and the preceding 30  $\mu\text{m}$  was averaged to provide a mean elemental signal for the otolith edge (Fig. 6.4). This edge elemental signature was assumed to accurately represent the environmental conditions at the site of capture (Table 6.8). Similarly, the first 30  $\mu\text{m}$  of the transect profile from the core (identified as a peak in the concentration of Mn:Ca: Brophy et al., 2004; Ruttenberg et al., 2005) was averaged to provide a juvenile elemental signature (Fig. 6.4, Table 6.8). Finally, the elemental values from across the entire profile, including the core and edge, were



averaged to provide a mean 'profile' signature, which is representative of the environment experienced throughout the life span of the fish (Fig. 6.4, Table 6.8).

#### **6.5.4 Analysis of the elemental data**

Analysis of the otolith trace element concentrations was consistent throughout the study. Elemental data were  $\log(x + 1)$  transformed prior to calculating Euclidean distances between each pair of measurements of element ratios. Canonical analysis of principal coordinates [CAP] (Anderson and Willis, 2003) was used to ordinate the matrices. To determine the classification success among sampling regions the 'leave-one-out' method was applied to variables in canonical space. Single factor PERMANOVA, which combines the concentrations of all six trace elements into a single matrix, was used to assess differences in otolith chemistry among age cohorts, months and regions of capture. When significant differences among regions were identified, pairwise analyses were conducted to determine if differences among adjacent regional populations were found. For pairwise analyses, the probability (p)-value was set at 0.05. For all CAP and PERMANOVA tests, 4999 permutations of the residual data under a reduced model were used. One-way ANOVA was performed on the six individual trace elements to test for spatial differences in otolith trace element composition.

### **6.6 STOCK STRUCTURE WITHIN REGIONS**

- This section meets the third project objective: *To evaluate the use of transect-based otolith microchemistry techniques (laser ablation ICPMS) for examining the movement patterns of sardine between gulf and shelf waters of South Australia and along the east coast of Australia.*

#### **6.6.1 New South Wales**

In total, 215 otoliths from New South Wales were collected as part of annual sardine stock assessments (Stewart et al., 2010). Fish were caught by commercial fishers under normal operating conditions from two key fishing regions: Eden (southern NSW:  $n = 111$ ); and Iluka (northern NSW:  $n = 104$ ) (refer to Supplementary Fig. A1). The bulk of the sardines from NSW were obtained between June and August 2009, with some additional samples also obtained in April and September 2009. As there was no evidence of differences in otolith trace elements at the edge region (i.e. reflecting the period immediately prior to capture) among sampling months (data not shown); element data were combined for month of capture in the subsequent analysis.

#### **6.6.2 South Australia**

Three South Australian otolith collections were utilised in this component of the study (refer to Supplementary Table A1). All samples were caught by commercial fishers under normal operating conditions, as part of annual sardine stock assessments (e.g. Ward et al., 2003, 2005b, 2008a, 2010). South Australian sardines were collected from four key fishing regions: Spencer Gulf [SG]; Kangaroo Island and the Investigator Strait

[KIIS]; the west coast and Coffin Bay [WCCB]; and the far west coast of South Australia [FWC] (refer to Supplementary Fig. A1). South Australian otoliths were caught from multiple, non-sequential years (2003, 2006 and 2009) and consisted of samples obtained from multiple months (Table 6.9).

Table 6.9 Locations and timing of sampling South Australian sardine otoliths used in this study.

<b>Year</b>	<b>Sampling months</b>	<b>SG (n)</b>	<b>KIIS (n)</b>	<b>WCCB (n)</b>	<b>FWC (n)</b>	<b>Total (n)</b>
2003	Jan to May, and July to Dec	416	61	81	65	623
2006	Jan, March, April, June, Nov, and Dec	183	19	149	92	443
2009	April to July, and Sept	146	90			236

The otolith edge trace elements of those samples obtained from South Australian in 2003 showed evidence of month-to-month variation (data not shown). Therefore, the data were split into two seasonal groups: ‘autumn—winter’ and ‘spring—summer’, to negate the confounding effect of intra-annual environmental fluctuations that may result in differences in the otolith elemental composition, while providing sufficiently robust sample sizes for analysis (Kalish, 1989, 1991). For the remaining otolith collections (2006 and 2009), trace element data were combined for month of capture in the subsequent analysis.

## 7. RESULTS

### 7.1 WEIGHT OF EVIDENCE APPROACH FOR DELINEATING THE STOCK STRUCTURE OF THE AUSTRALIAN SARDINE

- This section meets the first project objective: *To collate data held in jurisdictions to generate hypotheses regarding movement patterns and stock structure of Australian sardine throughout southern Australia.*

As the current review is a synthesis of research that has been undertaken over the last 60+ years, the sampling methodologies obviously differ considerably. Similarly, the degree of temporal and spatial coverage/effort among regions varies. Nevertheless, we strived to provide a comprehensive synthesis of the available data on key aspects of the ecology and biology of the Australian sardine with the aim of delineating the austral stock structure of this pelagic species. Readers are referred to Appendix 4 for an in-depth review of the available sardine literature. However, for the sake of brevity, key evidence for and against the stock structuring of the sardine are outlined in Table 7.1. Nine stock units across seven regions are proposed based on results from the weight of evidence approach (Table 7.1).

Table 7.1 Proposed stock units and evidence for and against these units based upon a weight of evidence approach to the delineation of stock units of the Australian sardine.

<b>Proposed stock units</b>	<b>Evidence for</b>	<b>Evidence against</b>
<b>Australia</b>		
<i>Multiple (3 to 4) units</i>	Spawning seasonality <sup>1,2</sup>  Morphology and meristics <sup>3,4</sup> Electrophoresis <sup>5,6</sup> Variable life history parameters Seasonal peaks in catches <sup>7</sup> Variable rates of recovery from PHV <sup>8,9,10,11,12,13</sup> Different rates of epidemic wave infection <sup>14,15</sup>	
<b>Australia</b>		
<i>Semi-continuous distribution</i>	Non-vector mediated transmission of pilchard herpes virus (PHV) <sup>14,15,16,17</sup> Mitochondrial DNA <sup>18</sup>	
<b>Western Australia</b>		
<i>2 to 4 units</i>	Otolith chemistry <sup>15,19,20,21</sup> Spatial variation in landings <sup>1,7,22</sup> Variable peaks in spawning: 1 vs. 2 <sup>1,23,24,25,26</sup> Morphology and meristics <sup>3,4</sup> Electrophoresis <sup>5</sup> Seasonal peaks in catches <sup>7,15</sup> Larvae/egg distribution: west vs. south <sup>15</sup> Self replenishing stocks <sup>1,15,22,24,27,28,29</sup> Larval growth rates <sup>29</sup>	Larvae/egg distribution: south coast <sup>1,23,24,30</sup> Inferred sardine migration <sup>1,31</sup> Parasite infection <sup>32</sup> Size at maturity <sup>15</sup>
<b>East coast (New South Wales)</b>		
<i>1 unit</i>	Seasonal landings <sup>33</sup> Size at maturity <sup>33,34,35</sup> Migration <sup>33</sup>	Otolith shape analysis <sup>33,36</sup> Morphology and meristics <sup>3,6</sup> Otolith chemistry <sup>6</sup> Electrophoresis <sup>6</sup> Anecdotal evidence of movement <sup>33,34,37,38</sup> Catch composition <sup>33,34,38,39</sup> Seasonal spawning <sup>3,33,34,39,40</sup> Larvae/egg distribution <sup>33,34,41,42,43</sup> Growth rates <sup>33,34,44</sup>

Table 7.1 cont.

Proposed stock units	Evidence for	Evidence against
<b>Victoria</b>		
1 unit	Electrophoresis <sup>6</sup> Larvae/egg distribution <sup>36, 45, 47, 48, 49, 50</sup> Otolith chemistry <sup>20</sup> Size at maturity <sup>2</sup> Seasonal spawning <sup>2</sup> Otolith shape: group <sup>36</sup> Growth rates: groups <sup>51</sup>	Morphology and meristics <sup>3, 6</sup> Catch composition <sup>7, 45, 46, 51</sup> Reproductive biology <sup>3, 47</sup> Otolith shape: sub-groups <sup>36</sup>
<b>South Australia</b>		
1 unit	Larvae/egg distribution <sup>8, 52, 53, 54</sup> Spatially restricted landings <sup>8</sup> Otolith chemistry <sup>6, 20</sup> Otolith shape: group <sup>36</sup> Size at maturity <sup>2, 34</sup> Seasonal spawning <sup>2</sup> Growth rates: groups <sup>51, 56</sup> Electrophoresis <sup>6</sup>	Otolith shape: sub-groups <sup>36</sup> Electrophoresis: sub-groups <sup>55</sup> Morphology and meristics <sup>3</sup> Catch composition <sup>2, 36, 50</sup> Reproductive biology <sup>3, 47</sup> Growth rates: sub-groups <sup>36</sup> Larval growth rates <sup>50, 57, 27</sup>
<b>Tasmania</b>		
1 unit	Morphology and meristics <sup>3</sup>	Larvae/egg distribution <sup>33</sup> Reproductive biology <sup>3, 47</sup> Growth rate (assumed) <sup>3</sup>

<sup>1</sup>(Gaughan et al., 2002); <sup>2</sup>(Kinloch et al., 1998); <sup>3</sup>(Blackburn, 1951); <sup>4</sup>(Syahailatua, 1992); <sup>5</sup>(Dixon et al., 1993); <sup>6</sup>(Yardin et al., 1998); <sup>7</sup>(Fletcher, 1991a); <sup>8</sup>(Ward et al., 2008a); <sup>9</sup>(Gaughan et al., 2008); <sup>10</sup>(Gaughan et al., 2000); <sup>11</sup>(Ward and McLeay, 1999); <sup>12</sup>(Ward et al., 2001a); <sup>13</sup>(Ward et al., 2001b); <sup>14</sup>(Murray et al., 2003); <sup>15</sup>(Fowler et al., 1997); <sup>16</sup>(Murray and Gaughan, 2003b); <sup>17</sup>(Whittington et al., 2008); <sup>19</sup>(Edmonds and Fletcher, 1997); <sup>18</sup>(Okazaki et al., 1996); <sup>20</sup>(Gaughan et al., 2001a); <sup>21</sup>(Edmonds et al., 1995); <sup>22</sup>(Cochrane, 1999); <sup>23</sup>(Fletcher et al., 1996); <sup>24</sup>(Fletcher et al., 1994); <sup>25</sup>(Fletcher and Tregonning, 1992); <sup>26</sup>(Blackburn, 1960); <sup>27</sup>(Fletcher and Sumner, 1999); <sup>28</sup>(Gaughan et al., 1990); <sup>29</sup>(Muhling et al., 2008); <sup>30</sup>(Gaughan and Fletcher, 1997); <sup>31</sup>(Fletcher et al., 1997); <sup>32</sup>(Langdon et al., 1992); <sup>33</sup>(Stewart et al., 2010); <sup>34</sup>(Ward and Staunton-Smith, 2002); <sup>35</sup>(Staunton-Smith, 1999); <sup>36</sup>(Hall, 2001); <sup>37</sup>(Dakin, 1937); <sup>38</sup>(Blackburn and Tubb, 1950); <sup>39</sup>(Joseph, 1981); <sup>40</sup>(Staunton-Smith and Ward, 2000); <sup>41</sup>(Ward et al., 2003); <sup>42</sup>(Gray and Miskiewicz, 2000); <sup>43</sup>(Uehara et al., 2005); <sup>44</sup>(Anonymous, 1998); <sup>45</sup>(Neira et al., 1999); <sup>46</sup>(Jackson et al., 1998); <sup>47</sup>(Blackburn, 1950); <sup>48</sup>(Jenkins, 1986); <sup>49</sup>(Neira et al., 1997); <sup>50</sup>(Neira et al., 1998); <sup>51</sup>(Morison and Hall, 1998); <sup>52</sup>(Ward et al., 2007); <sup>53</sup>(Ward et al., 2009); <sup>54</sup>(Rogers and Ward, 2005); <sup>55</sup>(Dredge, 1969); <sup>56</sup>(Rogers and Ward, 2007); <sup>57</sup>(Strong and Ward, 2009).

### 7.1.1 Integration of the available data: definition of Australian sardine management units

General patterns of sardine stock delineation were apparent when the multiple Stock Difference Index [SDI] matrices developed through the literature review were integrated (refer to Appendix 4 for the individual approach-based SDI matrices).

While a critical threshold SDI value was not explicitly defined by Welch et al. (2009), they suggested that  $SDI > 0$  is evidence for separate stocks. The SDI values indicate the level of corroboration among the different stock identification approaches, with higher values reflecting a greater level of corroboration (Welch et al., 2009). However, given the highly migratory nature of the sardine, we have assumed that among many adjacent regions there is the potential for mixing of stock units. Hence, we suggest that SDI values close to 0.5 may be representative of population/stock mixing in both time and space.

Table 7.2 Matrix of regional scale comparisons among 14 approaches reviewed in this paper. Pairwise SDI values were calculated as the number of approaches to indicate stock structure divided by the total number of approaches reviewed to assess structure among regional populations of sardines. Where, the fraction in parenthesis indicates the total number of significant differences observed from the total approaches for each pairwise group.

Regions	wWA	sWA	SA	Vic	Tas	sNSW	nNSW
wWA							
sWA	0.50 (5/10)						
SA	0.90 (9/10)	0.82 (9/11)					
Vic	0.90 (9/10)	0.90 (9/10)	0.73 (8/11)				
Tas	1.00 (3/3)	1.00 (3/3)	1.00 (3/3)	0.33 (1/3)			
sNSW	0.78 (7/9)	0.80 (8/10)	0.80 (8/10)	0.60 (6/10)	0.33 (1/3)		
nNSW	0.89 (8/9)	1.00 (9/9)	0.89 (8/9)	0.89 (8/9)	0.69 (2/3)	0.55 (6/11)	

Overall, the semi-quantitative SDI approach indicated that the stock structure of the Australian sardine consists of multiple stock units (Fig. 7.1, Table 7.2). The two Western Australian regions were clearly differentiated from all other regions as belonging to a separate stock. The cumulative pairwise SDI value (0.50) suggested that the west coast [wWA] and south coast [sWA] Western Australian regions have a moderate degree of connectivity.

The South Australian region [SA] was clearly defined as comprising a single stock unit. Victoria [Vic] was shown to be clearly separated from the regions to the west; however, spatially distinct boundaries between Victoria and Tasmania [Tas] were not identified, having the lowest pairwise SDI value (0.33). This infers a high degree of connectivity exists among these regions.

Tasmania shared an equally low pairwise SDI (0.33) value with southern New South Wales [sNSW]. A moderate pairwise SDI value (0.60) was identified between Victoria and southern New South Wales. These findings suggest that south eastern Australia

exhibits a considerable degree of population overlap, which may be the result of seasonally shifting stock boundaries (Yardin et al., 1998), facilitating a highly mixed stock.

The pairwise SDI values (0.55) indicate that two stocks could be present on the east coast of Australia, a southern and northern stock. The boundaries of the southern unit, however, appear to be variable and there appears to be a moderate level of connectivity with the northern unit.

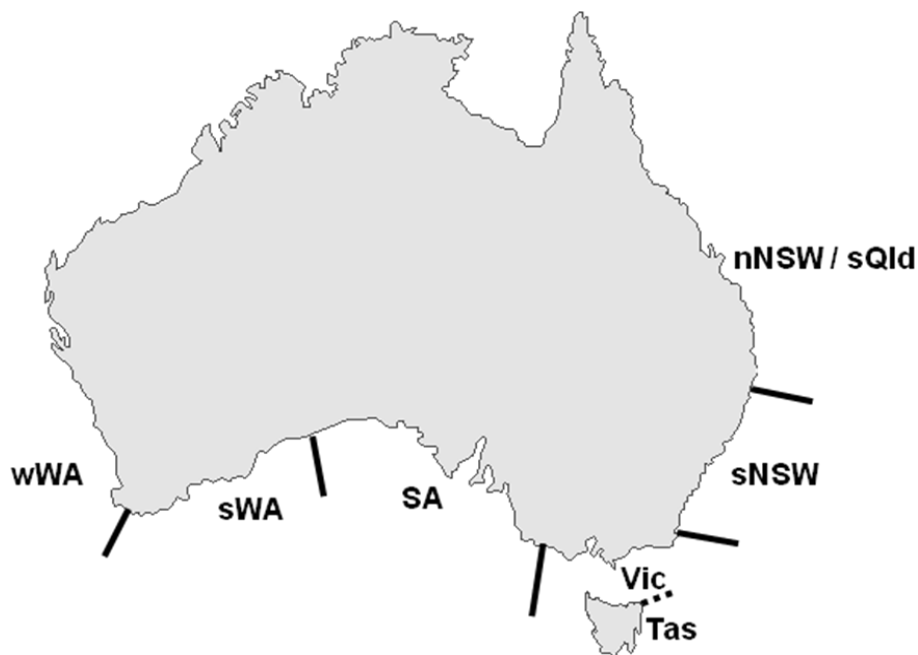


Figure 7.1 Map of Australia showing the approximate boundaries separating the stock units of the sardine. Boundaries are approximate due to limitations of collating literature that varies in both temporal and spatial sampling scales; which may also vary temporally. However, these boundaries conform to the major fishing regions of the species. The dotted line separating Tasmania and main land Australian indicates the uncertainty around the Tasmanian stocks.

## 7.2 ANALYSIS OF OTOLITH SHAPE

### 7.2.1 Allometric development of the Australian sardine otolith

There were no significant differences in otolith weights between the sexes for any of the otolith datasets (ANOVA:  $p > 0.05$ ); hence, data were combined for sex. Otoliths for each dataset showed a significant increase in weight in proportion to total body weight (NLR:  $p < 0.0001$ ) and fork length (NLR:  $p < 0.0001$ ) (Fig. 7.2 and 7.3).

Significant differences in otolith weight were identified between the immature and mature specimens for all otolith datasets (ANOVA:  $p < 0.001$ ). Similarly, there were significant differences in otolith weight among the size classes, with the exception of those samples from Jumping Pin and Scotts Cove (ANOVA:  $p > 0.05$ ).

The otolith of the sardine develops in proportion to the somatic growth (i.e. length and weight) of the individual. Sex, however did not influence the development of the sardine otolith, which is consistent with previous findings for the species (Blackburn, 1949; Hall, 2001). These findings confirm that sardine otoliths are suitable calcified structures for elucidating changes throughout the life history of individuals, i.e. through the analysis of otolith shape (EFA) and trace element composition.

### ***7.2.2 Allometric effects on otolith shape***

EFCs did not differ significantly between males and females for any of the otolith datasets; hence data for both sexes were combined for subsequent analysis (PERMANOVA:  $p > 0.05$ ). Similarly, for all datasets examined, single factor PERMANOVA tests did not identify significant differences in EFCs between size classes and age cohorts, nor between the stages of sexual maturity (PERMANOVA:  $p > 0.05$ ).

The biological factors that affected the development of the sardine otolith (refer to section 7.2.1 of this report) did not influence the shape of the sardine otolith (EFCs). These findings indicate that the shape of the otoliths of the Australian sardine is primarily influenced by the surrounding marine environment. Therefore, Australian sardine otoliths provide an environmentally sensitive bio-indicator that can be exploited to understand patterns of movement and stock structure.



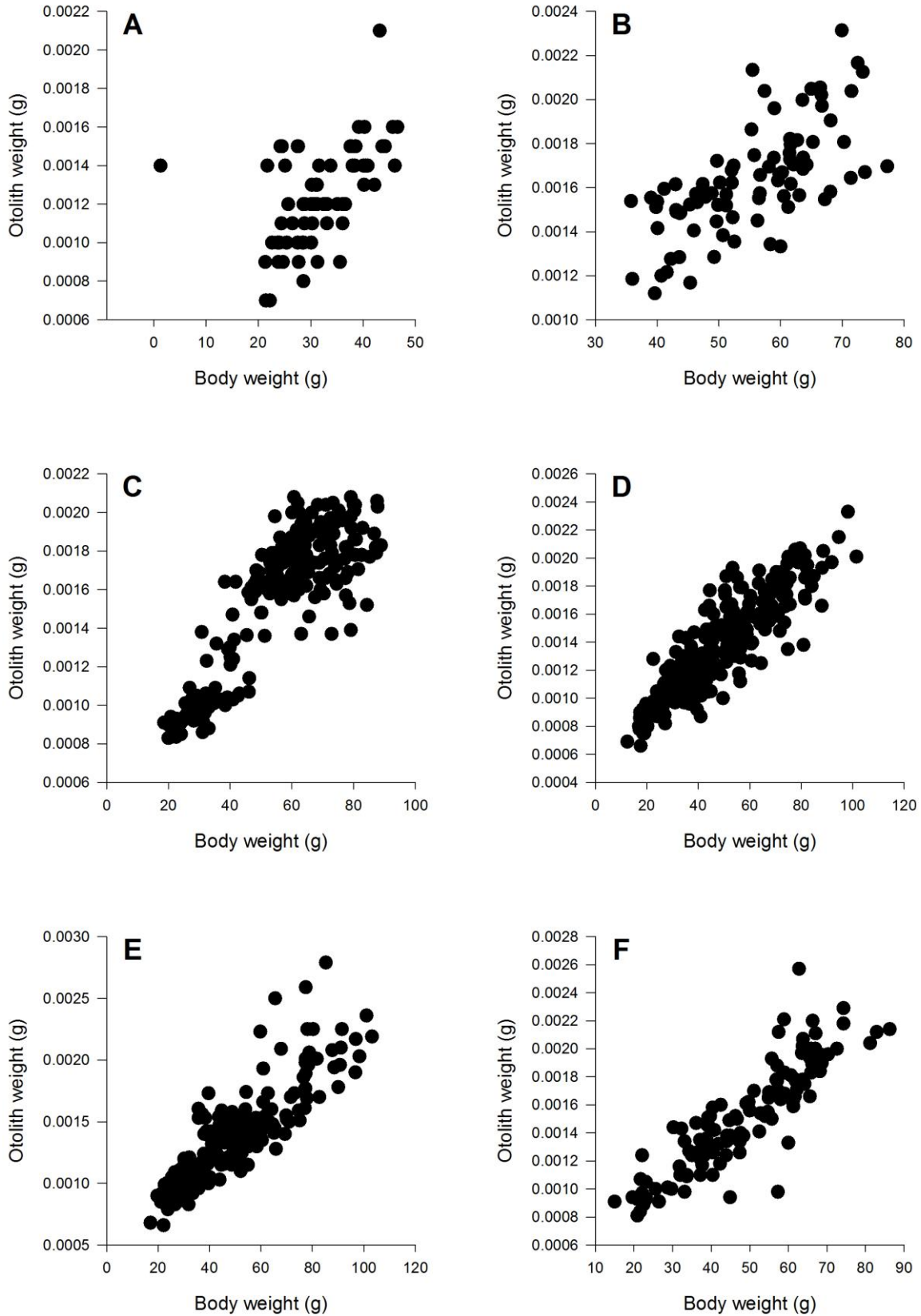


Figure 7.2 Allometric growth between otolith weight and body weight in six populations of sardines. Where: (A) Port Philip Bay; (B) Jumping Pin; (C) Wedge Island; (D) Greenly Island; (E) Eden; and (F) Coffin Bay. Note that the axis varies among panels.

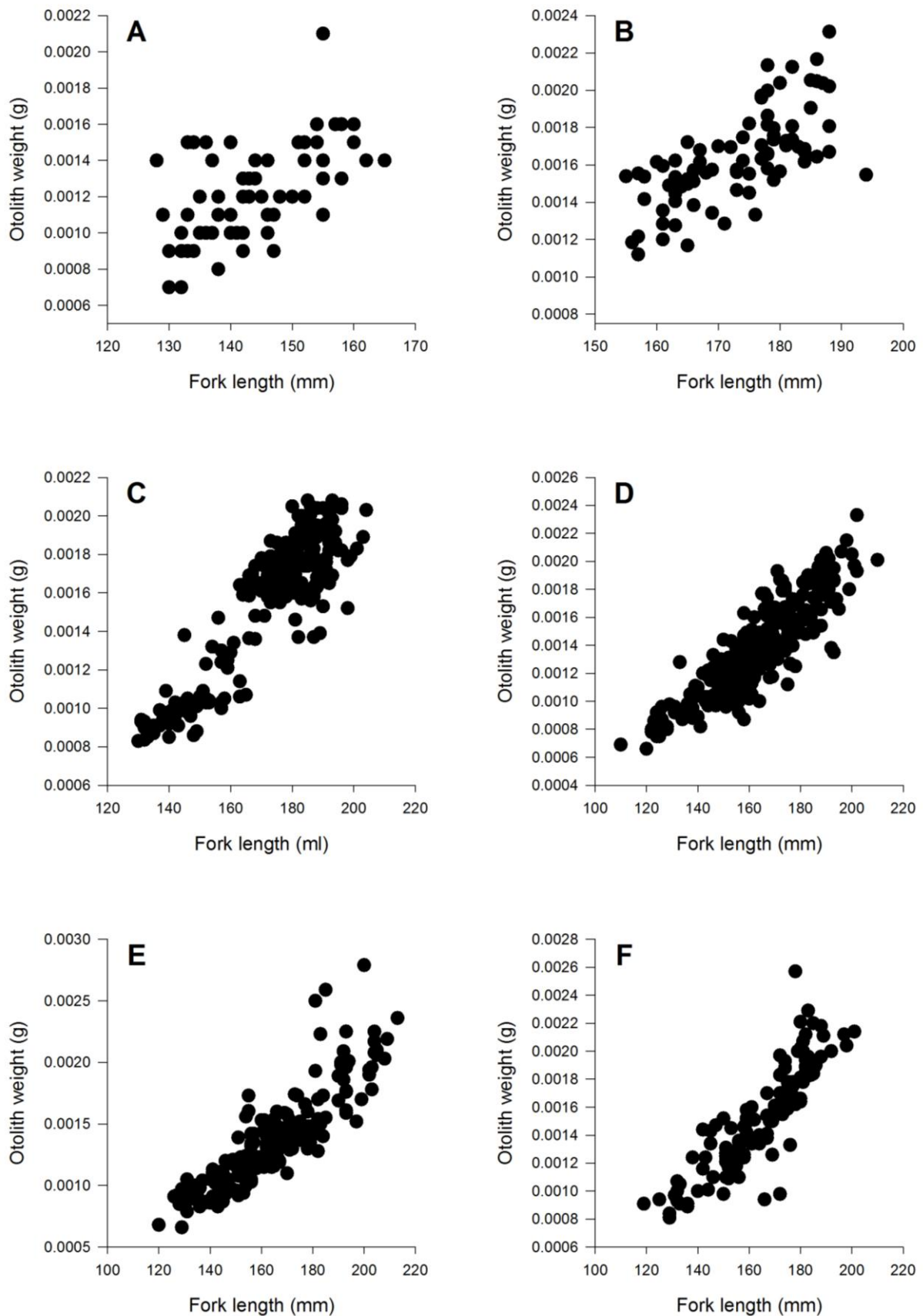


Figure 7.3 Allometric growth between otolith weight and fork length in six populations of sardines. Where: (A) Port Philip Bay; (B) Jumping Pin; (C) Wedge Island; (D) Greenly Island; (E) Eden; and (F) Coffin Bay. Note that the axis varies among panels.

### **7.2.3 Comparisons of otolith shape among non-sequential sampling years**

There was significant inter-annual variation in EFCs among the non-sequential sampling years in SG (Table 7.3). When the individual age cohorts were analysed separately, the 3-year cohort showed a significant difference among sampling years. Conversely, neither the 2 nor 4-year cohorts showed a difference among the sampling years (Table 7.3). Both the combined EFC dataset and the 2-year cohort displayed significant differences in EFCs among the sampling months (Table 7.3).

For seasonal comparisons of EFCs, no significant differences were found among the non-sequential sampling years when the age cohorts were analysed separately (Table 7.3); however, when the cohorts were combined significant temporal differences were identified in autumn and winter (Table 7.3). The 2-year cohort sampled in spring showed a significant difference among the sampling months (Table 7.3). These differences were also seen in the combined age cohort dataset for all seasons, with the exception of winter (Table 7.3).

### **7.2.4 Inter-annual variability in the shapes of the sardine otolith**

No inter-annual influence on the shapes of the otoliths for the individual or combined age cohorts were detected for the SG otoliths (Table 7.4). However, both the 2-year and combined age cohorts showed a significant difference among sampling months (Table 7.4).

When all sampling regions in south-eastern Australian were analysed, there was a significant year affect on EFCs (Table 7.4). When the individual age cohorts were analysed separately, sampling year did not influence otolith shape (EFCs) (Table 7.4). However, for all age cohorts, there was a significant (nested) month affect (Table 7.4). Given that no inter-annual affects on EFCs were detected, the subsequent analysis of spatial differences among regions sampled over a period of 16 years was limited to samples collected at a similar time each year. This was done under the assumption that monthly variability showed the same seasonal pattern annually.

Table 7.3 PERMANOVA results testing for temporal variability in otolith shape of Spencer Gulf sardines. P-values = > 0.05 (ns); ≤ 0.05 (\*); ≤ 0.01 (\*\*); and ≤ 0.001 (\*\*\*).

Source	2-year		3-year		4-year		All ages	
	df	MS	df	MS	df	MS	df	MS
<i>Seasons Combined</i>								
Year	7	0.012ns	7	0.020*	7	0.011ns	7	0.029*
Month (year)	32	0.012**	25	0.008ns	28	0.007ns	38	0.016**
Residual	675		859		579		2176	
<i>Summer</i>								
Year	4	0.007ns	4	0.013ns	3	0.011ns	4	0.008ns
Month (year)	5	0.006ns	3	0.007ns	6	0.008ns	7	0.011*
Residual	159		279		102		556	
<i>Autumn</i>								
Year	7	0.017ns	6	0.015ns	7	0.008ns	7	0.026*
Month (year)	10	0.009ns	7	0.008ns	7	0.007ns	11	0.011*
Residual	387		370		207		992	
<i>Winter</i>								
Year	4	0.017ns	2	0.013ns	3	0.007ns	5	0.033**
Month (year)	2	0.004ns	2	0.004ns	2	0.003ns	3	0.006ns
Residual	90		108		134		341	
<i>Spring</i>								
Year	2	0.010ns	2	0.012ns	1	0.017ns	2	0.014ns
Month (year)	2	0.017**	3	0.005ns	3	0.004ns	3	0.035***
Residual	39		102		136		292	

Table 7.4 PERMANOVA results testing for temporal stability of elliptical Fourier coefficients of sardine otolith shape throughout autumn and winter. P-values = > 0.05 (ns); ≤ 0.05 (\*); ≤ 0.01 (\*\*); and ≤ 0.001 (\*\*\*).

Source	2-year		3-year		4-year		All cohorts	
	df	MS	df	MS	df	MS	df	MS
<i>Spencer Gulf Region</i>								
Year	7	0.016ns	6	0.015ns	7	0.008ns	7	0.027ns
Month (year)	17	0.010**	12	0.007ns	13	0.008ns	20	0.013***
Residual	477		478		341		1333	
<i>All Regions</i>								
Year	9	0.028ns	8	0.028ns	9	0.037ns	9	0.088***
Month (year)	21	0.013***	22	0.019***	21	0.010**	29	0.026***
Residual	1005		1186		719		3091	

### **7.2.5 Stock structure of sardine throughout south-eastern Australia, inferred from otolith shape**

Otolith shape differed significantly among the regional populations for all age cohorts (PERMANOVA): 2-years: pseudo- $F_{8, 1083} = 10.170$ ,  $p = 0.001$ ; 3-year: pseudo- $F_{8, 1032} = 9.861$ ,  $p = 0.001$ ; and 4-year: pseudo- $F_{8, 650} = 7.953$ ,  $p = 0.001$ . Pairwise analysis was used to test for differences among adjacent regional sardine populations throughout south-eastern Australia (Table 7.5). Australian sardine population structure varied among age cohorts, suggesting the presence of age-based movement among the regions examined (Table 7.5, Fig. 7.4).

In South Australia, sardines from the 2-year age cohort showed a pattern of separation between the west coast of the state (FWC & WCCB) and the gulf bioregion (SG & KIIS) (Fig. 7.4a). EFA of the 3-year cohort indicated that SG and WCCB did not differ; however, EFCs for the SG and FWC samples remained significantly different (Fig. 7.4b). EFCs for SG and KIIS did not differ significantly among 3-year old sardines (Fig. 7.4b). Among the 4-year cohort the west coast (FWC & WCCB) and SG did not differ; however, KIIS appeared to be significantly isolated from the other regions in the state (Fig. 7.4c). For all age cohorts, the EFCs for the FWC and WCCB were not significantly different from one another (Fig. 7.4).

Table 7.5 Probability (p)-values among PERMANOVA pairwise groups of adjacent regional populations of the sardine in south-eastern Australia based on otolith shape. Refer to Table 6.7 for sampling location codes.

Adjacent population pairs	2-year	3-year	4-year
	p	p	p
WCCB — FWC	0.069	0.196	0.485
WCCB — KIIS	0.001	0.010	0.903
SG — WCCB	0.013	0.062	0.722
SG — KIIS	0.001	0.096	0.004
SG — FWC	0.061	0.021	0.416
PPB — SG	0.001	0.074	0.441
PPB — WCCB	0.001	0.005	0.287
PPB — KIIS	0.001	0.026	0.089
PPB — LE	0.004	0.001	0.006
LE — SEC	0.046	0.002	0.057
LE — NEC	0.018	0.034	0.012
NEC — SEC	0.052	0.063	0.595
QLD — SEC	0.040	0.001	0.087
QLD — NEC	0.011	0.094	0.363

There was significant separation between South Australia and Victoria for the 2-year age cohort (Fig. 7.4a). This degree of regional separation appears to become less distinct in the 3-year age cohort as SG and PPB did not significantly differ in EFCs (Fig. 7.4b). Nevertheless, PPB was significantly different from WCCB and KIIS (Fig. 7.4b). Conversely, the 4-year age cohort showed no significant difference between South Australia and Victoria (Fig. 7.4c).

For all age cohorts, PPB and LE remained significantly different from one another indicating a high degree of population separation (Fig. 7.4). Along the east coast of Australia, different patterns of sardine population structuring occurred. For the 2-year cohort of sardines, the east coast of Australia (SEC & NEC) including LE did not differ, forming a single meta-population (Fig. 7.4a). The 3-year age cohort showed a northward shift in the boundaries of this meta-population to be inclusive of southern Queensland (Fig. 7.4b); however, Lakes Entrance was isolated from those adjacent regions. Among the 4-year cohort the east coast of Australia appeared to form a large meta-population (Fig. 7.4c).

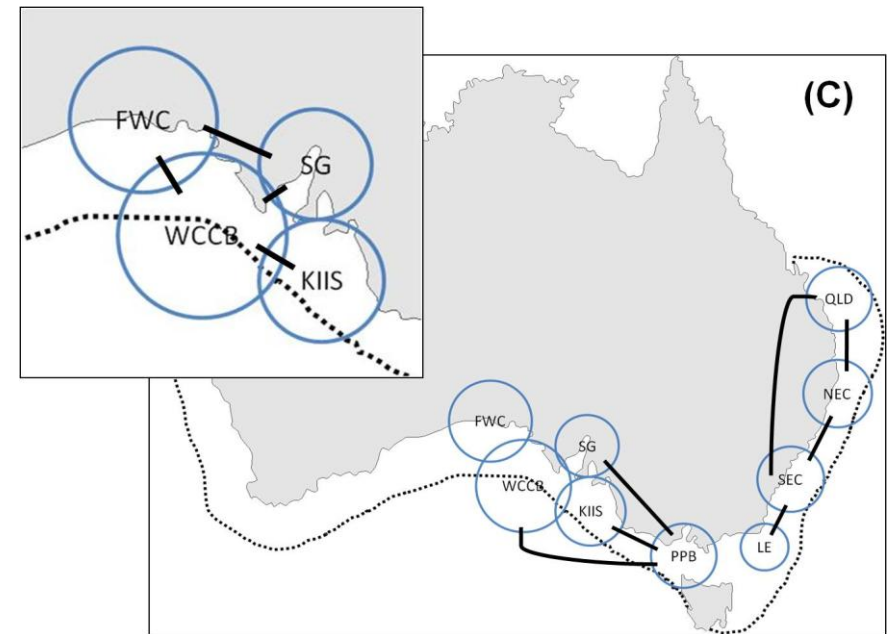
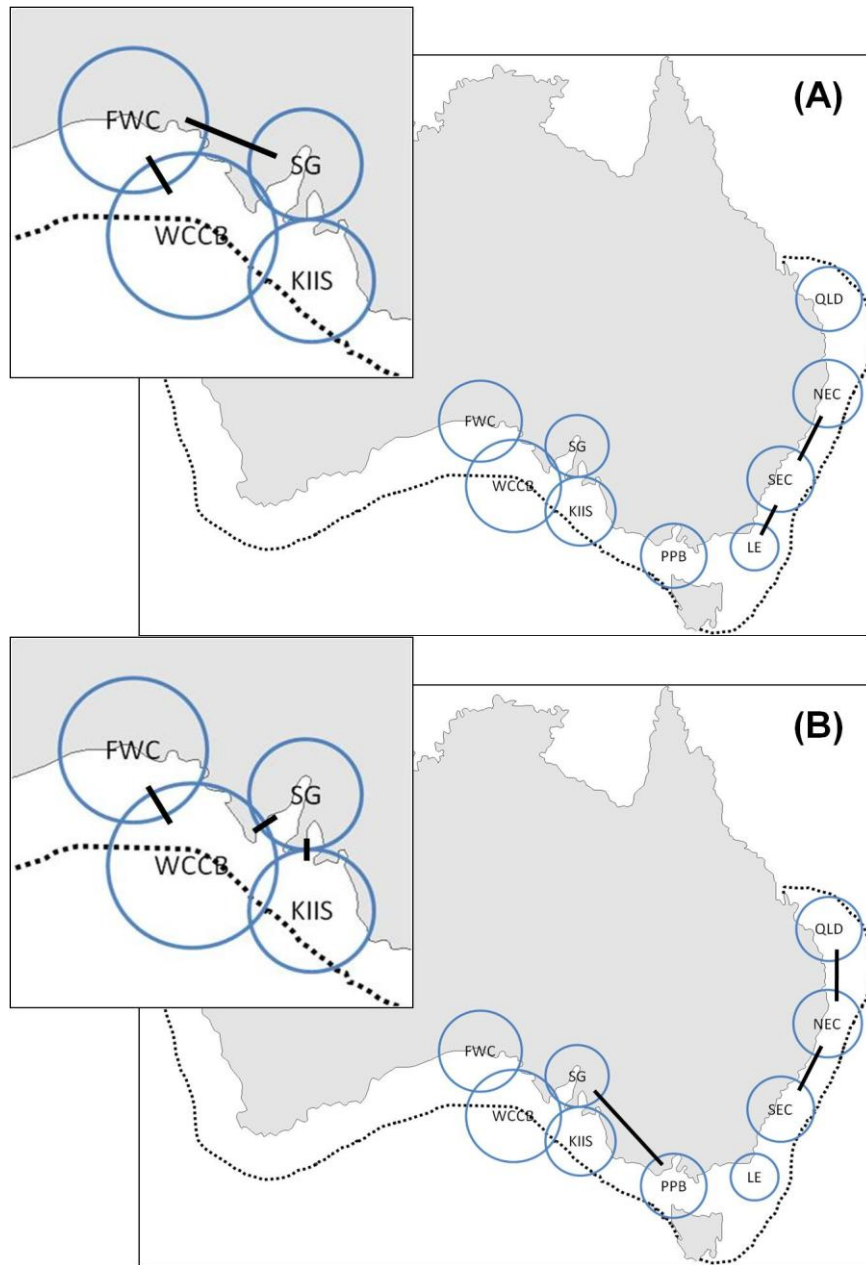


Figure 7.4 Patterns of age-based structuring among adjacent populations of the sardine in south-eastern Australia based on otolith shape: (A) 2-year cohort; (B) 3-year cohort; and (C) 4-year cohort. Inset is the expanded regions within South Australia to enhance reader clarity. The dotted black line indicates the Australian distribution of *Sardinops sagax*. Solid lines represent no significant differences among regional sardine populations (pairwise analysis:  $p > 0.05$ ). Refer to Table 6.7 for sampling location codes. See Table 7.5 for the corresponding probability ( $p$ )-values among pairwise groups.

## 7.3 ANALYSIS OF THE ELEMENTAL COMPOSITION OF SARDINE OTOLITHS

### 7.3.1 New South Wales

At the otolith edge, the 3-year cohort did not show a spatial difference in otolith multi-element composition (Table 7.6). Conversely, the 1 and 2-year cohorts showed significant differences in the otolith trace element composition between fishing regions (Table 7.6). Further analysis indicated that for both of these age cohorts, there were significant spatial differences among all six of the elements examined. The elemental signature at the otolith edge provided a relatively high level of discriminatory power between the locations of capture (Table 7.6).

Table 7.6 Multi-elemental otolith chemistry based discrimination among sardines from two areas in New South Wales. PERMANOVA p-values are indicated by the asterisks: > 0.05 (ns); ≤ 0.05 (\*); ≤ 0.01 (\*\*); and ≤ 0.001 (\*\*\*).

Otolith region	Age cohort	Allocation success (%)			df	MS
		Total	Eden	Iluka		
Profile (mean)	1-year	79.25	75.00	82.76	1, 105	0.298***
	2-year	81.93	75.61	88.10	1, 81	0.256***
	3-year	92.00	95.46	66.67	1, 23	0.129*
Edge	1-year	87.74	81.25	93.10	1, 105	0.715***
	2-year	85.54	87.81	83.33	1, 81	1.604***
	3-year	88.00	90.91	66.67	1, 23	0.059ns
Core	1-year	65.42	60.42	69.49	1, 105	0.343***
	2-year	68.68	63.42	73.81	1, 81	0.264**
	3-year	92.00	95.46	66.67	1, 23	0.206*

When the elemental profile data were averaged to provide an otolith profile signature, all three age cohorts showed significant differences between the two sampling regions (Table 7.6). ANOVA indicated that of the individual elements, Na:Ca and Mg:Ca were significantly different between locations for both the 1 and 3-year age cohorts. For the 2-year cohort, Li:Ca and Na:Ca were significantly different between locations. There was a moderate degree of classification success using the mean otolith profile (CAP > 79%: Table 7.6).

At the core region of the otolith, each age cohort represented a back-dated year class (i.e. 1-year = 2008; 2-year = 2007; and 3-year = 2006). All three year classes showed significant spatial differences in their element signatures at the otolith cores (Table 7.6). For the 1 and 2-year cohorts the individual elements, Na:Ca and Mg:Ca were significantly different between fishing locations, while Ba:Ca, Li:Ca and Na:Ca differed among the 3-year cohort (ANOVA:  $p < 0.05$ ). Among the age cohorts, there



was considerable variability in the allocation success of samples to their location of capture. With the exception of the 3-year cohort (CAP: 92%), there was a low level of correct classification (Table 7.6).

### 7.3.2 South Australia

In South Australia, significant age-related differences were detected at the otolith edge region for all years (PERMANOVA:  $p < 0.01$ ); hence, samples were separated into individual age cohorts for analysis.

Given that month-to-month variation in the elemental composition of otoliths was found for samples caught in 2003, the edge data for that year were split into two seasonal groups: autumn—winter and spring—summer. In spring and summer, the chemistry at the otolith edge did not differ among the fishing regions for any of the age cohorts (PERMANOVA:  $p > 0.05$ ). Conversely, during autumn—winter in 2003, the elemental composition at the otolith edge was significantly different among the fishing regions (Table 7.7). Significant spatial differences were identified in the older age cohorts from 2006 (Table 7.7). However, no spatial differences in the elemental composition at the otolith edge were found in the 2-year cohort from 2006 and the two age cohorts from 2009 (Table 7.7). At the otolith edge, element:calcium ratios varied among regions and this was consistent among age cohorts and sampling years (Table 7.7). Overall, pairwise analysis indicated an age-based pattern of population sub-structuring may be evident in South Australia for each of the sampling years (Fig. 7.5).

Table 7.7 Regional discrimination of sardines in South Australia based on elemental concentrations in otolith edges. PERMANOVA p-values are indicated by the asterisks:  $> 0.05$  (ns);  $\leq 0.05$  (\*);  $\leq 0.01$  (\*\*); and  $\leq 0.001$  (\*\*\*). Note, † denotes autumn and winter caught sardines. Individual elements shown indicate spatial differences in element:calcium ratios.

Year	Age cohort	Allocation success (%)					df	MS	Individual elements
		Total	SG	KIIS	WCCB	FWC			
2003 †	2-year	57.50	51.09		56.25	75.00	2, 153	0.059***	Ba, Li, Na, Sr
	3-year	66.85	65.10		66.67	78.26	2, 174	0.458***	Ba, Na, Sr
	4-year	42.98	38.20	57.90		66.67	2, 108	0.217*	Ba, Li, Na
2006	2-year	37.75	33.33	75.00	5.88	49.18	3, 150	0.090ns	
	3-year	48.15	59.72	46.67	43.66	32.26	3, 188	0.449***	Ba, Li, Mg, Na
	4-year	73.53	78.57		70.00		1, 101	0.378***	Ba, Sr
2009	2-year	67.71	69.57	66.00			1, 95	0.148ns	
	3-year	66.29	71.43	60.00			1, 88	0.096ns	

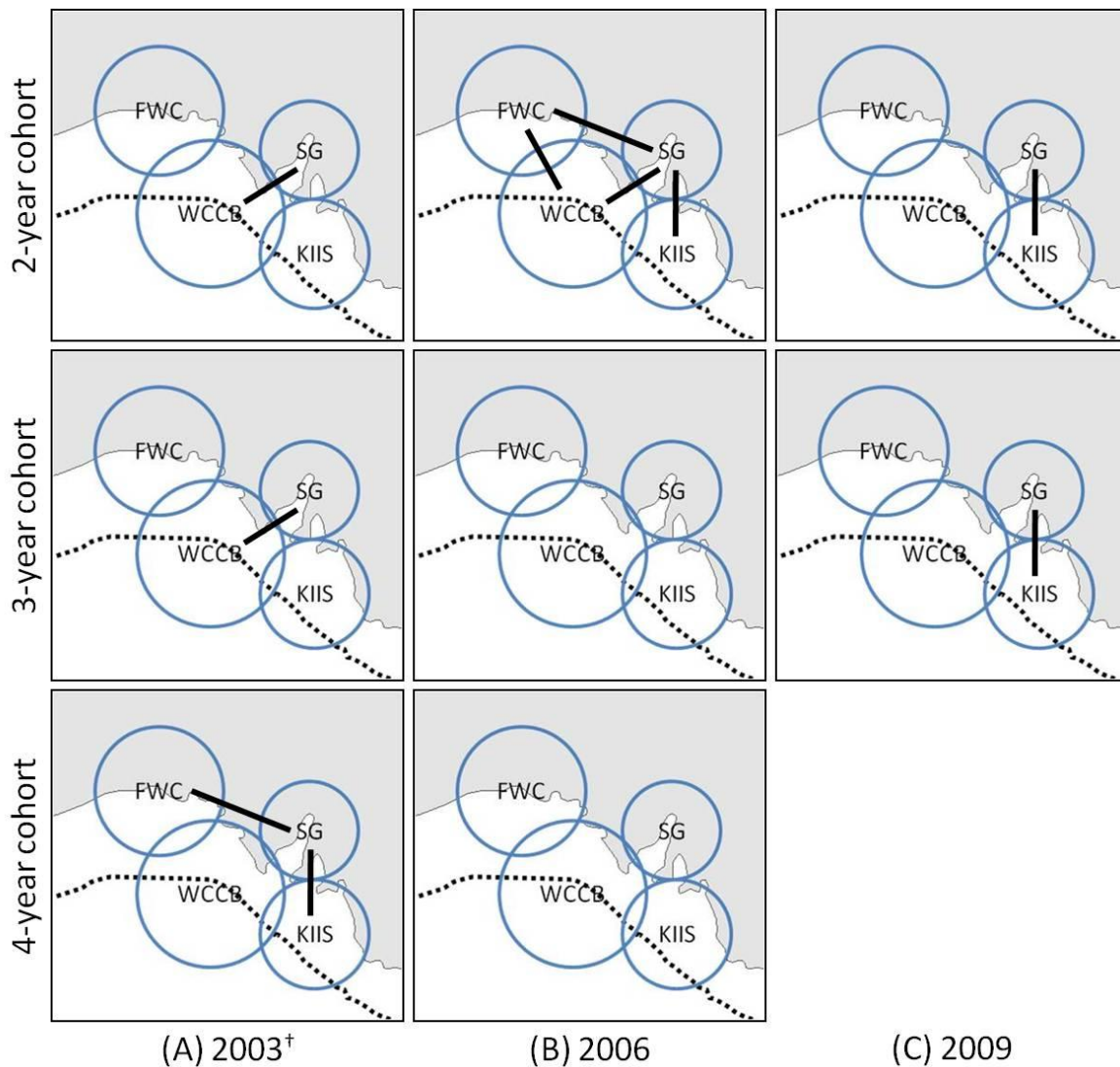


Figure 7.5 Age-related structuring of regional populations in South Australia based on otolith edge chemistry of sardines caught in three sampling years: (A) 2003; (B) 2006; and (C) 2009. The dotted black line indicates the Australian distribution of *Sardinops sagax*. Solid lines represent no significant difference among regional sardine populations (pairwise analysis:  $p > 0.05$ ). Note, † denotes autumn and winter caught sardines.

The mean elemental compositions of the otolith profiles showed significant differences among fishing regions, with the exception of the 2 and 3-year age cohorts, caught in 2006 and 2009, respectively (Table 7.7). Pairwise analysis indicated inter-annual patterns of population sub-structuring were evident (Fig. 7.6). Combinations of individual elements that showed significant spatial differences among regions were consistent among years and age cohorts (Table 7.8).

At the core region of the sardine otolith, significant spatial differences were detected for all of the age cohorts in 2003, and among the 3 and 2-year age cohorts caught in 2006 and 2009, respectively (Table 7.9). Pairwise analysis indicated that patterns of significant differences among adjacent fishing regions varied considerably with year class and sampling year (Fig. 7.7).

Table 7.8 Regional discrimination of sardines in South Australia based on elemental concentrations in otolith profiles. PERMANOVA p-values are indicated by the asterisks: > 0.05 (ns); ≤ 0.05 (\*); ≤ 0.01 (\*\*); and ≤ 0.001 (\*\*\*). Individual elements shown indicate spatial differences in element:calcium ratios.

Year	Age cohort	Allocation success (%)					df	MS	Individual elements
		Total	SG	KIIS	WCCB	FWC			
2003	2-year	53.49	53.70	53.57	72.09	30.56	3, 214	0.459***	All
	3-year	59.54	60.82	75.00	29.41	66.57	3, 214	1.137***	Li, Na
	4-year	40.74	32.43	51.85	61.11	83.33	3, 161	0.619***	Li, Na
2006	2-year	39.74	33.33	75.00	29.41	47.54	3, 150	0.397ns	
	3-year	46.03	66.67	33.33	36.62	25.81	3, 188	0.602***	Mn, Na, Sr
	4-year	65.69	66.67		65.00		1, 101	0.212**	Li, Na
2009	2-year	61.46	54.35	68.00			1, 95	0.168*	Mn, Na
	3-year	65.17	57.14	75.00			1, 88	0.062ns	

Table 7.9 Regional discrimination of sardines in South Australia based on elemental concentrations in otolith cores. PERMANOVA p-values are indicated by the asterisks: > 0.05 (ns); ≤ 0.05 (\*); ≤ 0.01 (\*\*); and ≤ 0.001 (\*\*\*). Individual elements shown indicate spatial differences in element:calcium ratios.

Year	Age cohort	Allocation success (%)					df	MS	Individual elements
		Total	SG	KIIS	WCCB	FWC			
2003	2-year	52.56	44.44	60.71	62.79	58.33	3, 214	0.755***	Mn, Ba, Li, Na, Sr
	3-year	45.12	45.03	50.00	17.65	65.22	3, 214	1.249***	Na, Sr
	4-year	33.95	24.32	62.96	33.33	83.33	3, 161	0.776***	Li, Na
2006	2-year	33.11	17.39	50.00	17.65	54.10	3, 150	0.261ns	
	3-year	47.09	73.61	13.33	42.25	12.90	3, 188	0.774***	Mn, Na
	4-year	62.75	68.33		54.76		1, 101	0.488ns	
2009	2-year	68.75	73.91	64.00			1, 95	0.356**	Mg, Na
	3-year	59.55	63.27	55.00			1, 88	0.118ns	

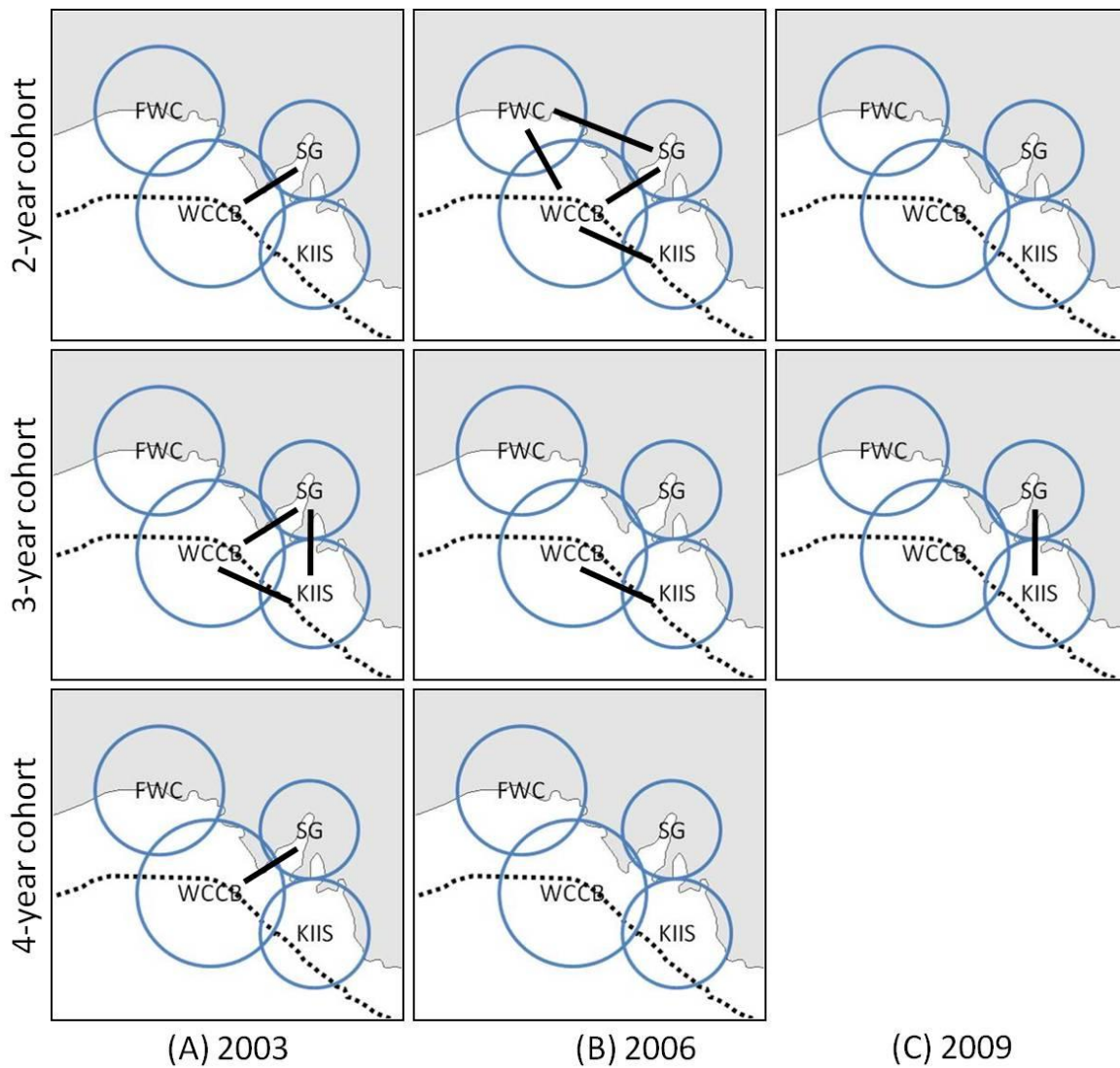


Figure 7.6 Age-related structuring of regional populations in South Australia based on the mean otolith profile chemistry of sardines caught in three sampling years: (A) 2003; (B) 2006; and (C) 2009. The dotted black line indicates the Australian distribution of *Sardinops sagax*. Solid lines represent no significant difference among regional sardine populations (pairwise analysis:  $p > 0.05$ ).

In general, the trace element signature of the South Australian sardine otolith provided a relatively poor level of spatial discriminatory power among the regions of capture, in spite of significant spatial differences in otolith element concentrations. This trend was consistent among the three elemental signatures (edge, core, and profile) and among the individual age cohorts and sampling years (Table 7.7, 7.8 and 7.9).

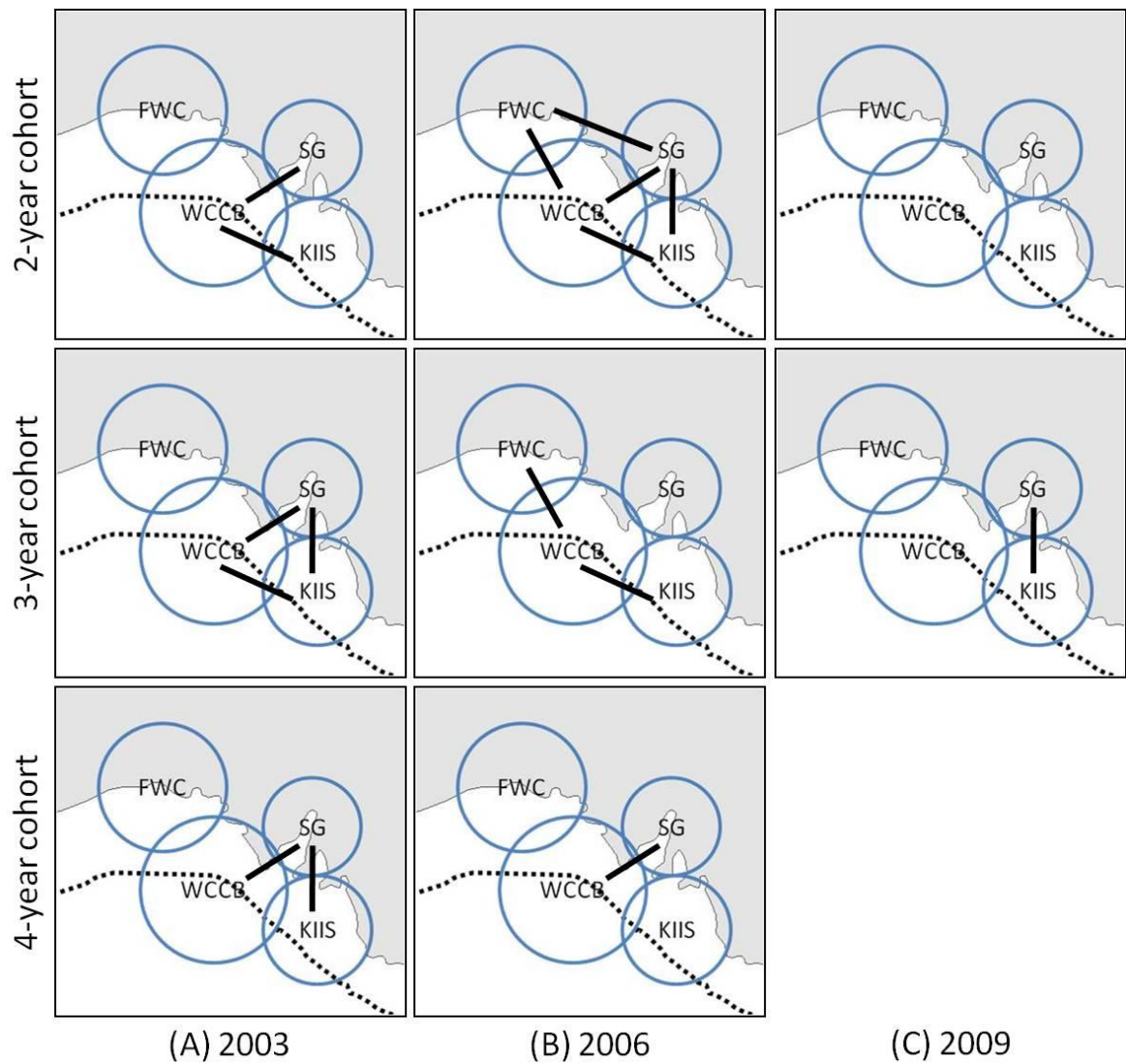


Figure 7.7 Age-related structuring of regional populations in South Australia based on the otolith core chemistry of sardines caught in three sampling years: (A) 2003; (B) 2006; and (C) 2009. The dotted black line indicates the Australian distribution of *Sardinops sagax*. Solid lines represent no significant difference among regional sardine populations (pairwise analysis:  $p > 0.05$ ).

## 8. DISCUSSION

### 8.1 WEIGHT OF EVIDENCE APPROACH FOR DELINEATING THE STOCK STRUCTURE OF THE AUSTRALIAN SARDINE

#### *8.1.1 Weight of evidence approach for delineating stock structure*

Previously multiple approaches to the delineation of fish stock structure have integrated complementary findings using a qualitative comparative discussion approach (e.g. Abaunza et al., 2008b). The literature review was conducted using the Stock Difference Index [SDI] outlined by Welch et al. (2009) to better identify regional differences among sardine populations and to make semi-quantitative judgements of stock divisions. Furthermore, the compilation of SDI values from multiple approaches for elucidating stock structure provides a measure of confidence in these judgements (Welch et al., 2009).

The data synthesised in the present review were from 14 approaches that either directly or indirectly sought to delineate the stock structure of the Australian sardine. For several regional populations which are data deficient (i.e. Tasmania), the final SDI value was determined from < 5 approaches. Nevertheless, the collation of a wide range of sources of biological, ecological and fisheries data in this study has facilitated the identification of multiple stocks that are consistent with the conventional line of thinking.

The broad spatial scale adopted here for comparisons among populations potentially failed to resolve finer spatial-scale differences among sardine populations. While this study separated Western Australia into two nominal biogeographic regions (i.e. south coast Western Australia and west coast Western Australia); the sardine population of southern Western Australia is recognised as being comprised of multiple sub-units (e.g. Fowler et al., 1997). Furthermore, the SDI scoring system may mask potential seasonal patterns of connectivity among adjacent sardine populations by only recognising differences at the regional scale thus increasing the mean SDI value, i.e. the sardine spawning migration on the east coast of Australian (Ward and Staunton-Smith, 2002; Stewart et al., 2010). However, among those adjacent regions where seasonal stock overlap is suggested (e.g. northern and southern New South Wales, south and west coast Western Australia, as well as Tasmania and south-eastern Australia) the cumulative pairwise SDI values were approximately 0.50 (Table 7.2). This study suggests that SDI values in this range represent fronts of population/stock mixing in both time and space. This thinking seems appropriate as it provides a degree of flexibility to account for the highly migratory, trans-boundary nature of the sardine and may better reflect the seasonality of large scale biomass movement patterns that could otherwise be masked by this analysis.

Drawing data together from over 60 years and from multiple research organisations, ranging in both the breadth of spatial and temporal sampling, lends itself to creating a high degree of uncertainty in defining the positions of stock boundaries, especially where there appears to be seasonal boundaries among some populations. Nevertheless, the integrated weight of evidence approach adopted by this study has facilitated the identification of biologically tangible stock divisions of the Australian sardine, on which future management actions can be based.

## **8.2 ANALYSIS OF OTOLITH SHAPE**

### ***8.2.1 Inter-annual variability in the shapes of the sardine otolith***

The Australian sardine otoliths showed variable patterns of temporal variability. When samples collected over a 15 year period were analysed for temporal differences in otolith shape (EFCs), partitioning into multiple age cohorts reduced the level of variability among the multiple non-sequential sampling periods.

Comparisons among populations consisting of mixed age cohorts may be limited to fish sampled within the same year. Alternatively, over relatively short time periods (i.e. 2 to 8 years/seasons) otolith shape indices have been shown to remain stable over time (e.g. DeVries et al., 2002; Jónsdóttir et al., 2006; Stransky et al., 2008a). However, even for periods when sampling effects were limited (e.g. Gonzales-Salas and Lenfant, 2007; Mérigot et al., 2007), significant inter-annual variation has been observed among descriptors of otolith shape. While inter-annual variability in otolith shape descriptors (EFCs) may be species specific, over considerable sampling periods (i.e. 15+ years), these inter-annual differences may become highly significant among temporally separated populations. To date, this study appears to utilise the longest time period of archived otolith sampling for comparisons of otolith shapes.

Inter-annual fluctuations in environmental conditions are the most likely source that is influencing the degree of temporal instability of the shapes of teleosts otoliths, as rates of growth (somatic growth and otolith development) within a population will correspondingly fluctuate among years (Campana and Casselman, 1993; Gonzales-Salas and Lenfant, 2007). This study demonstrated that by restricting inter-annual comparisons of the shapes of the otoliths of the Australian sardines to discrete three to six month periods, intra-annual environmental fluctuations can be negated, producing a 'seasonal' otolith shape (EFC) signal that is largely stable through time (i.e. among multiple non-sequential sampling years). Using this 'seasonal' approach, an 8-year pooled otolith dataset of King mackerel (*Scomberomorus cavalla*) otoliths provided a greater degree of spatial classification success than an annual dataset (DeVries et al., 2002). By limiting further inter-annual analyses of archived sardine otolith shape (EFCs) to narrowly defined 'seasons', when mixing of populations can be assumed as minimal, it may be considered valid to make comparisons among geographically separated populations of sardines sampled across years.

These findings indicate that for individual age cohorts of Australian sardine it is valid to make comparisons of otolith shape (EFCs) among otoliths collected from geographically separated regions across multiple years. Furthermore, observed patterns of temporal variability were further dampened by partitioning the EFC data into discrete six and three month periods. The findings here indicate that by restricting comparisons of EFCs among multiple years to an individual 'season' (e.g. three to six month periods), inferences about stock structure may be possible.

### ***8.2.2 Stock structure of sardine throughout south-eastern Australia, inferred from otolith shape***

Analysis of the outlines of sardine otolith shape (EFA) suggests that the stock structure of the species throughout south-eastern Australia is variable among age cohorts. In general, distinct sardine populations appear to exist both between and within jurisdictional boundaries. There were three to four semi-independent sub-populations distributed throughout south-eastern Australia: a South Australian unit; a separate unit in Port Phillip Bay and; one or two overlapping units in New South Wales, which are inclusive of Lakes Entrance and southern Queensland. Identifying areas of population mixing across state boundaries (i.e. straddling stocks) was complicated; however, these findings indicated that at the boundaries of many of the locations examined, sardine populations overlapped.

The identification of multiple regional sardine populations is consistent with the current line of thinking and existing body of research. It is not inconceivable that population structuring at both the inter- and intra-state levels exists; as this is the widely accepted scenario for the sardine populations in Western Australian (e.g. Fletcher and Tregonning, 1992; Dixon et al., 1993; Fletcher et al., 1994; Edmonds and Fletcher, 1997; Fowler et al., 1997; Gaughan et al., 2001a, 2001c, 2002). While the findings of the pairwise analysis are not definitive, they do provide a better understanding of patterns of population structuring.

In South Australia, the pattern from otolith shape analysis (EFA) was largely consistent with the current understanding of the population dynamics of the species. Spawning occurs in two key bioregions, the west coast/Coffin Bay and in southern Spencer Gulf (Ward and McLeay, 1999; Ward and Staunton-Smith, 2002; Ward et al., 2006). These regions appear to retain juvenile fish for the first one to two years of life, with maturing sardines moving from southern Spencer Gulf to the west coast/Coffin Bay region later in life (Rogers and Ward, 2007), effectively forming a single meta-population. These age-related patterns of population structuring were evident from the otolith shape analysis. Currently, the South Australian sardine resource is managed as a single fishable biomass under a total allowable commercial catch (TACC) system (Ward et al., 2010). However, given the structuring observed, there is potential to investigate finer scale spatial divisions of current management regions in South Australia, as is done in south coast Western Australia (Cochrane, 1999).



Previous examination of sardine otolith shape (EFA) found that the populations sampled from Spencer Gulf, Coffin Bay, Port Phillip Bay and Lake Entrance all had morphologically distinct otoliths (Hall, 2001). Similarly, elemental concentrations of otoliths also supports the basis for separate stocks in South Australia and Port Phillip Bay (Yardin et al., 1998; Gaughan et al., 2001a). However, these findings indicate that among the older cohorts, there population structuring between South Australia and Port Phillip Bay are not immediately evident. The findings of this study are more closely aligned with those electrophoretic studies, which show South Australia and Victoria (Port Phillip Bay) as overlapping, yet separate management units (Dixon et al., 1993; Yardin et al., 1998).

The separation of stocks between Port Phillip Bay and Lakes Entrance was consistent with the thinking that two sardine management units exist in Victorian waters, mirroring past EFA findings (Hall, 2001). Fish from these regions differ considerably in life history parameters and spawning periodicity (Kinloch et al., 1998; Morison and Hall, 1998), as well as catch compositions (Jackson et al., 1998); and the current Victorian sardine resource is managed based on these separate stocks (Hall, 2001). These bioregions are most likely isolated from one another by the Bassian Isthmus that is a significant biogeographic boundary that prevents mixing between the two Victorian sardine stocks (e.g. Waters, 2008).

Age-related trends of population connectivity on the east coast of Australia were found, with a high degree of population mixing throughout the south east (southern New South Wales and Lakes Entrance). There appears to be sub-grouping among the younger cohorts and a single meta-population of mature fish (i.e. 4-year olds). Among the older age cohorts the entire east coast, including Lakes Entrance appeared to comprise a single stock unit. These findings are consistent with the northward spawning migratory behaviour has been proposed for the east coast distribution of the Australian sardine (Ward and Staunton-Smith, 2002; Stewart et al., 2010). This observed pattern of population overlap may necessitate the requirement for a coordinated approach to stock assessment and management arrangements among these adjacent regional sardine stakeholders.

Using archived otoliths collated from multiple non-sequential sampling years required that the EFC data were partitioned such that inter-annual variations did not confound spatial differences. Limiting the EFA to samples caught throughout autumn and winter provided a temporally stable 'autumn—winter snapshot' of the stock structure of the Australian sardine. This autumn—winter snapshot for analysis largely coincided with the periods of peak sardine spawning throughout Australia (with the expectation of northern NSW and Queensland: Table 8.1); and was assumed to cover a period of time when the mixing of stocks would be minimal (DeVries et al., 2002). However, temperature-driven sardine spawning has resulted in asynchronous spawning events throughout the species range (Table 8.1) and regional differences in the spawning patterns have previously been used as evidence of partitioning among populations (e.g. Gaughan et al., 2001c).

Furthermore, sardine migrations are thought to be largely linked to spawning, as is the case for *Sardinops* populations in California (Hammann et al., 1988), New Zealand (Baker, 1972) and South Africa (van der Lingen and Huggett, 2003). However, due to the autumn—winter sampling window, seasonal patterns of sardine movement may not be identifiable. As there were insufficient samples collected throughout summer and spring this could not be adequately assessed here.

Table 8.1 Regional spawning seasons of the Australian sardine (adapted from Hall 2001).

Region	Spawning season	Source
Far West Coast of South Australia	Summer/Autumn	Ward & Staunton-Smith 2002; Ward et al. 2009
Coffin Bay, South Australia	Summer/Autumn	Kinloch et al. 1998; Ward & Staunton-Smith 2002; Ward et al. 2006
Spencer Gulf, South Australia	Summer/Autumn	Kinloch et al. 1998; Ward & Staunton-Smith 2002; Ward et al. 2006
Port Phillip Bay, Victoria	Spring/Summer	Hoedt & Dimmlich 1995; Kinloch et al. 1998; Neira et al. 1999
Lakes Entrance, Victoria	Spring/Summer	Blackburn 1950
Southern New South Wales	Summer/Autumn	Blackburn 1949; Joseph 1981; Stewart et al. 2010
Northern New South Wales	Winter/Spring	Blackburn 1950; Ward & Staunton-Smith 2002; Stewart et al. 2010
Southern Queensland	Winter/Spring	Blackburn 1950; Ward & Staunton-Smith 2002; Stewart et al. 2010

Analysis of the shape of otoliths suggested that separate stocks exist in three bioregions, in line with current thinking. Management of the Australian sardine resource at the regional scale for South Australia and Victoria would seem appropriate. However, these otolith shape analyses suggest some overlap of stocks at state boundaries (e.g. between Lakes Entrance (Victoria) and Eden (NSW)). Hence, collaborative management and assessment approaches need to be considered. In addition, finer scale management units may be required off South Australia.

## 8.3 ANALYSIS OF THE ELEMENTAL COMPOSITION OF SARDINE OTOLITHS

### 8.3.1 New South Wales

Consistent patterns among the three elemental signatures (edge, core, profile) were observed from otoliths from northern and southern NSW, suggesting significant sub-grouping between the two. Those spatial differences were similar for the three age cohorts examined, with the exception of the edge signal of the 3-year old mature fish.

Elemental chemistry of otolith edges for the 3-year age cohort suggests these fish inhabit a common environment and may indicate mixing among mature fish along the east coast. However, the otolith edge still provided a high level of allocation success, indicating sub-grouping (DeVries et al., 2002).

Our otolith chemistry findings are consistent with previous studies of stock structure of sardine in NSW. Spatial differences in the life history parameters (Blackburn, 1951), morphometric and meristic traits (Blackburn, 1951; Yardin et al., 1998) and otolith shape analysis (Hall, 2001) have highlighted the possible existence of northern and southern sub-groups of sardines. In addition, a third central NSW sub-group, based around Gosford, has been suggested (Yardin et al., 1998). However, this could not be tested here due to lack of samples from this region.

The exclusion of samples from off the central NSW coast may have removed the opportunity to test for the potential existence of a mixing zone on the East Coast. The findings of the otolith shape analysis (EFA) presented here (refer to Section 7.2.5), indicates that there is a high degree of sardine connectivity along the East Coast (Table 7.5, Fig. 7.4).

Along the east coast of Australia, the spawning period of the sardine varies with latitude (Table 8.1) (Stewart et al., 2010). In southern NSW, the peak in spawning occurs during the austral summer and is followed by a southern Queensland/northern NSW winter/spring spawning period (Blackburn, 1950; Ward and Staunton-Smith, 2002; Stewart et al., 2010). At the core region of the otolith, significant spatial differences were detected for all year classes.

Most of the otoliths for this analysis were collected throughout the austral winter months, therefore our findings fail to fully elucidate patterns of population structuring on the east coast of Australia. Spawning in northern NSW occurs in winter and the results from recent fat staging and gonad examinations indicate that sardines from Iluka and Eden maintain different reproductive/physiological conditions during winter (Stewart et al., 2010). In order to better understand the movement patterns of the species, sampling across the breadth of the east coast from throughout the entire year is required.

### **8.3.2 South Australia**

The observed population structuring in the waters of South Australia showed age-related patterns for all years analysed and for all otolith analysis methods, but these differed among years. These inter-annual, age-related patterns yielded a complex picture of population structuring with significant differences within age cohorts driven by pairwise differences among one or more of the adjacent fishing regions.

Inter-annual patterns of population structuring may be the result of environmentally induced shifts in the range of the sardine. The most likely candidate to drive range shifts is the strength of the local northern boundary upwelling system: the Flinders

Current (Middleton and Cirano, 2002). While upwelling occurs annually off the South Australian coast during summer and winter, the Flinders Current shows inter-annual fluctuations in strength that affect the distribution of sardine egg and larval biomass in the region (Ward et al., 2006).

Previous studies have indicated that fine scale population structuring was evident in South Australia (e.g. Dredge, 1969). Analysis of the outlines of the shapes of sardine otoliths (Hall, 2001) and growth data (Morison and Hall, 1998) indicated that the Port Lincoln (Spencer Gulf region) and Coffin Bay populations differed significantly. Similarly, Blackburn (1951) suggested that the reproductive patterns of the Coffin Bay fish and Spencer Gulf fish differed sufficiently to constitute separate population groups. However, pairwise analysis did not support this pattern of sub-structuring, instead indicating that the far west coast may consist of a sub-group from the rest of the state.

Overall there appeared to be low degrees of population structuring between the waters of Spencer Gulf and the adjacent shelf regions. This absence of significant structuring was largely evident among all of the otolith elemental signatures examined for each sampling year. Continuity among the fishing regions showed annual and seasonal patterns. Nevertheless, the generalised pattern seen here indicated that the gulf and shelf waters in South Australia constitute a meta-population, with the potential for finer scale structuring. This was especially evident as the trace element signature at the otolith edge provided a relatively poor level of spatial discriminatory power among the regions of capture in spite of spatial differences in otolith element concentrations.

Our findings are consistent with the assumed movement patterns of the sardine in South Australia (Rogers and Ward, 2005), where juvenile sardines are thought to utilise the semi-protected waters of the southern Spencer Gulf and the near shore islands as a nursery habitat. The interface at the gulf and shelf waters has the potential to facilitate alongshore movement of juvenile fish between gulf and shelf groups. This is supported by the generally continuous egg and larval distribution seen throughout the shelf and gulf waters of South Australia (Ward and Staunton-Smith, 2002; Ward et al., 2006). However, patterns of distribution vary in response to inter-annual fluctuations in the local marine environment, altering the spawning range and, or distribution of juvenile fish (Bruce and Short, 1990; Ward et al., 2006). Findings for the otolith core region provide evidence to support patterns of environmental driven inter-annual variability in population structuring, as two alternative patterns of population groupings were evident among the year classes analysed. The first pattern was characterised by a lack of structuring, whereby a common spawning environment was evident in the gulf and shelf water facilitating the mixing of eggs and larvae in the waters of South Australia. The second pattern was characterised by sub-grouping, with variable patterns of fine scale structuring among adjacent fishing regions, in particular the far west coast region being separated from the rest of the state.

### ***8.3.3 Fine scale sardine structuring inferred from otolith chemistry***

Analysis of trace element composition in otoliths of Australian sardines suggested the potential separate northern and southern sub-groups off New South Wales (NSW). In contrast, the South Australian sardine population consists of a meta-population, which experiences spatial and temporal patterns of fine-scale structuring. Options for finer scale spatial management in both states need exploring to prevent potential localised reductions in biomass. Identification of fine scale management units is consistent with the current understanding for the species' distribution in Western Australia (e.g. Edmonds and Fletcher, 1997; Gaughan et al., 2001a). The NSW results provide a temporal (winter) snapshot of the sardine structuring. This proposed pattern of population sub-grouping requires further investigation of broader seasonal/annual patterns to ascertain whether the two stocks identified here occur throughout the year, and whether there is annual variation potentially linked to changes in the East Australian current.

## 9. BENEFITS AND ADOPTION

The beneficiaries of this research are the fisheries management agencies (PIRSA, NSW DPI, Victorian DPI and AFMA) and stakeholders associated with the South Australian Sardine Fishery, New South Wales Ocean Haul Fishery, Victorian Marine and Estuarine Scale Fish Fishery and Commonwealth Small Pelagic Fishery.

Adoption of these results is enhanced by the presence of at least one member of the project steering committee on the committees for the South Australian, Commonwealth (Tim Ward, SARDI Aquatic Sciences) and NSW (John Stewart, NSW DPI) fisheries.

The improved understanding of Australian sardine stock structure and movement patterns in south-eastern Australia that this project provides suggest there is a basis for assessing (i) the benefits of coordinated assessment and management of sardine off the east coast and (ii) options for finer scale spatial management of sardines off South Australia.

The results of the project were extended to PIRSA and other members of the South Australian Sardine Fishery Research and Management Committee at formal meetings in Port Lincoln in October 2011 and February 2012. These findings are being incorporated into the review of the Harvest Strategy that is currently underway.

Findings were also presented to AFMA and members of the Commonwealth Small Pelagic Fishery Resource Assessment Group (RAG) at formal meetings in November 2011 and February 2012. Based on these findings the RAG has advised AFMA that coordinated assessment and management is required for the east coast stock.

A presentation was given to scientists, fisheries managers and commercial fishers associated with the New South Wales Ocean Haul Fishery in February 2012. A similar presentation will be given to Victorian DPI and stakeholders in the Victorian Marine and Estuarine Scale Fish Fishery April 2012.

Presentations on this project and related studies were presented at the Tri-national Sardine Forum and Californian Cooperative Oceanic Fisheries Investigations (CalCofi) Conference in San Diego, California in December 2011.

## 10. FURTHER DEVELOPMENT

Research and other activities that should be undertaken to further develop sardine research and management in Australia include the following:

***Disseminate research outputs:*** The findings of this study will be used to revise the Harvest Strategy for the South Australian Sardine Fishery. Findings will also be used as a basis for establishing processes for coordinated assessment and management of the east coast stock. Papers are being prepared for submission and publication in international peer-reviewed journals. The Principal Investigator presented these findings at the CalCOFI Conference in San Diego in December 2011.

***Increased collaboration among regional stakeholders:*** Future studies would be improved by involvement of researchers from all relevant jurisdictions.

***The application of the Stock Difference Index:*** The Stock Difference Index [SDI] approach to delineating stock structure does not deal effectively with spatial and temporal variability. This issue may limit the application of the SDI—weight of evidence approach in pelagic species, as this group is characterised by extensive migrations and environmentally driven inter-annual fluctuations in distribution and abundance. The future application of this approach to assessing the stock structure of pelagic teleosts should take these issues into consideration.

***Important gaps in the data:*** Our review of the available literature for sardines highlighted that large spatial knowledge gaps exist. Research has focused primarily on regions of high fishing pressure; there is a paucity of basic information for regions where little targeted fishing is conducted (e.g. Tasmania, Queensland, and south-eastern South Australia). These spatial knowledge gaps hamper efforts to fully elucidate the stock structure and the degree of population connectivity of the Australian sardine.

***Future research:*** This study has identified that there are few (contemporary) examples of studies that encompass the entire Australian distribution of the sardine. For those studies that have sought to delineate the stock structure of the Australian sardine, restricted spatial sampling has limited the identification of stock units to either extremely fine or coarse spatial units. While these findings are informative, differences in the spatial and temporal scales of the approaches utilised in different studies may impede integrated analysis.

***Data management after project:*** Sardine otoliths were collated from regional jurisdictions and agencies. Where appropriate samples will be returned to their source collection, or housed at SARDI. The resultant data is stored as Excel files, which are located on the UoA network directory. Network backup is run daily. For future research use, access must be granted by the relevant agencies.

## **11. PLANNED OUTCOMES**

The planned outcome for this study was to provide stakeholders with advice regarding the implications for stock assessment and fisheries management of the stock structure and movement patterns of sardine off South Australia and east coast of Australia.

The key finding was that separate east and south stocks could be identified, with some sub-structuring likely along the east coast and off South Australia.

It is likely that these findings will influence stock assessment and fisheries management arrangements for sardine off south-eastern Australia.



## 12. CONCLUSION

This project has collated relevant published and unpublished data from across all jurisdictions to generate hypotheses regarding the movement patterns and stock structure of the Australian sardine throughout southern Australia (Objective 1). Those hypotheses generated relating to broad and fine scale movement patterns of sardine in southern Australia were then subsequently tested using otolith shape analyses (Objective 2) and transect-based otolith microchemistry techniques (Objective 3).

Integrated results from otolith trace element and otolith shape analysis indicated that the Australian distribution of the sardine consists of multiple sub-populations that exhibit various levels of overlap (Objective 4). Overall, the two otolith-based techniques generally supported the population structure for south-eastern Australia suggested by the semi-quantitative weight of evidence approach. At least three major regional sardine groups were identified, each exhibiting various levels of overlap, but which can be considered semi-independent. These included South Australia, central Victoria (Port Phillip Bay), and east coast Australia (which includes Queensland, New South Wales, and eastern Lakes Entrance). Although population components were defined at the regional scale, the combined results of the otolith-approaches provided strong evidence that two of these regional groups, South Australia and the east coast, demonstrated a level of population sub-structuring.

The combined results of this project indicated that the population structuring of the Australian sardine is highly complex, as the species shows both age-related and inter-annual/seasonal patterns of sub-structuring, which impede the delineation of discrete population boundaries. This complexity in the population structure of the sardine is consistent with the findings of previous studies on the species (e.g. Dixon et al. 1993; Ward et al. 1998).

The discrimination of these major regional sardine groups provides a basis for determining the spatial scales of future stock assessment and management. On the east coast Australia, the finding suggests that there may be a need for coordinated assessment and management of fisheries under the jurisdiction of the Commonwealth (SPF), New South Wales (Ocean Haul) and Victoria (Lakes Entrance). In South Australia, findings suggest that there may be a need to explore options for finer scale spatial management.

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## **14. APPENDIX 1: INTELLECTUAL PROPERTY**

There are no Intellectual Property issues associated with this project.

## 15. APPENDIX 2: STAFF

**Principal Investigator:**

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**16. APPENDIX 3: SUPPLEMENTARY INFORMATION ON ARCHIVED OTOLITH COLLECTION**

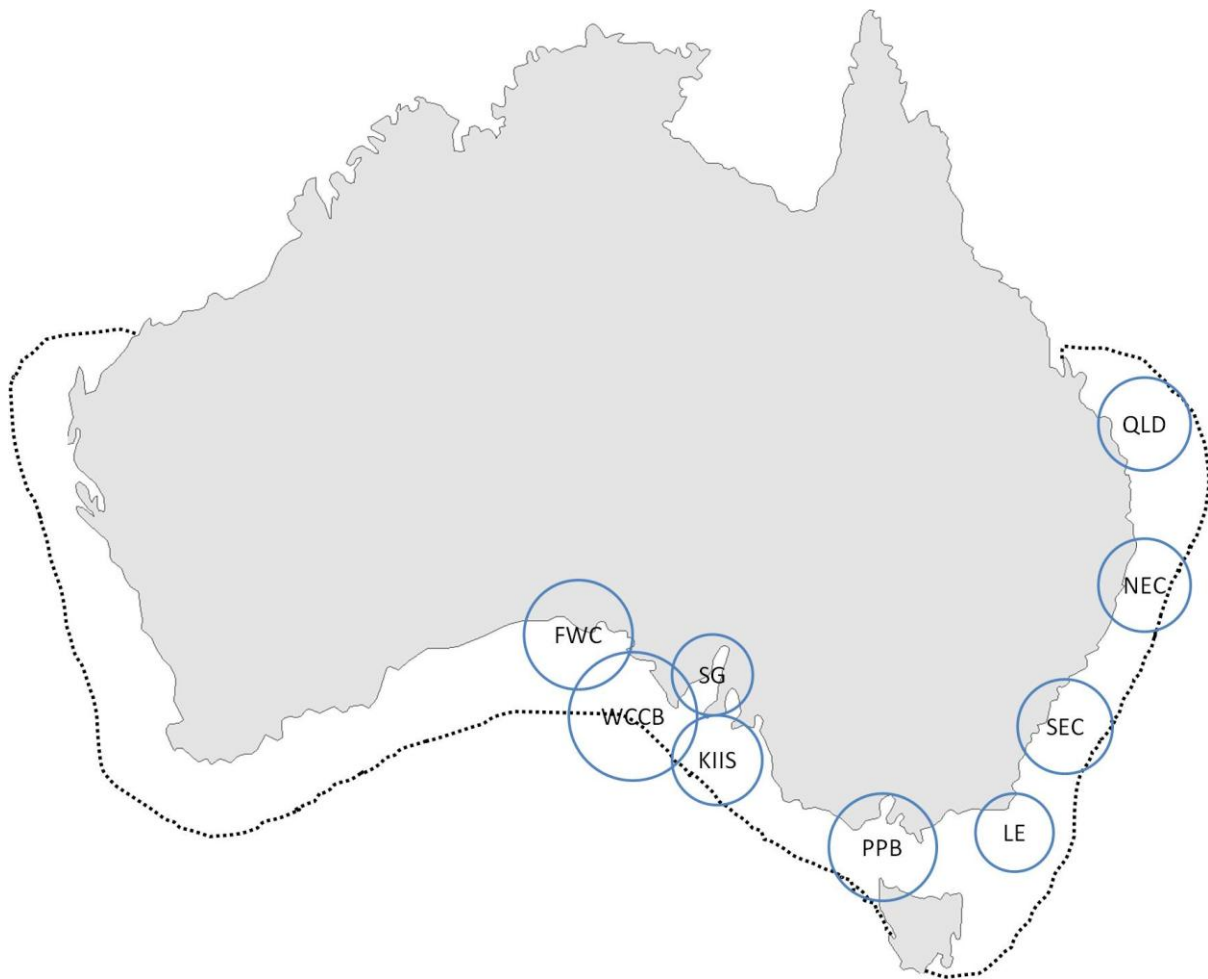


Figure A1 Approximate locations of the geographic 'regions' used to distinguish south-eastern Australian sardine populations throughout this study. FWC – far west coast; WCCB – West Coast/Coffin Bay; SG – Spencer Gulf; KIIS – Kangaroo Island; PPB – Port Philip Bay; LE – Lakes Entrance; SEC – Southern East Coast; NEC – Northern East Coast ; and QLD – Queensland. The dotted black line indicates the Australian distribution of *Sardinops sagax*.



Table A1 Age-partitioned sample sizes of the available archived Australian sardines photographed for shape analysis. Numbers in parenthesis indicate sample sizes for trace element analysis.

Year		Regions								
		Spencer Gulf	Kangaroo Is	Coffin Bay	Far Wt Coast	Pt Philip By	Lk Entrance	Sth NSW	Nth NSW	Qld
1995	2-yrs	99		3		118				
	3-yrs	203		76		98				
	4-yrs	124		89		10				
1996	2-yrs	49				33				
	3-yrs	7								
	4-yrs	6								
1998	2-yrs								14	12
	3-yrs									178
	4-yrs									144
2003	2-yrs	133 (108)	61 (28)	50 (43)	27 (36)					
	3-yrs	164 (177)	2 (4)	8 (17)	20 (23)					
	4-yrs	138 (111)	21 (27)	16 (18)	6 (6)					
2004	2-yrs	132		13						
	3-yrs	131		6						
	4-yrs	130								
2005	2-yrs	49								
	3-yrs	1		28						
	4-yrs	85		80						
2006	2-yrs	87 (69)	4 (4)	15 (17)	129 (61)					
	3-yrs	180 (72)	13 (15)	66 (71)	30 (31)					
	4-yrs	40 (42)		54 (60)						
2009	2-yrs	119 (46)	52 (50)					96 (41)	71 (42)	
	3-yrs	138 (49)	39 (40)					79 (15)	12 (10)	
	4-yrs	74 (51)						21	1	
2010	2-yrs	47	25	13						
	3-yrs	68	11	33						
	4-yrs	18		40						
2011	2-yrs						7			
	3-yrs						104			
	4-yrs						65			
Total	2-yrs	715 (223)	142 (84)	94 (60)	156 (97)	151	7	96 (41)	71 (42)	12
	3-yrs	892 (298)	65 (59)	217 (88)	50 (54)	98	104	79 (15)	12 (10)	178
	4-yrs	615 (204)	21 (27)	279 (78)	6 (6)	10	65	21	1	144

## 17. APPENDIX 4: WEIGHT OF EVIDENCE SUPPORTING MATERIAL

This study collated the available literature (e.g. published papers, government reports, theses) in order to develop a biologically tangible understanding of the stock structure of the Australian sardine. Using a weight of evidence approach, this paper aimed to semi-quantitatively define/approximate patterns of connectivity, movement and stock boundaries throughout the distribution of the Australian sardine.

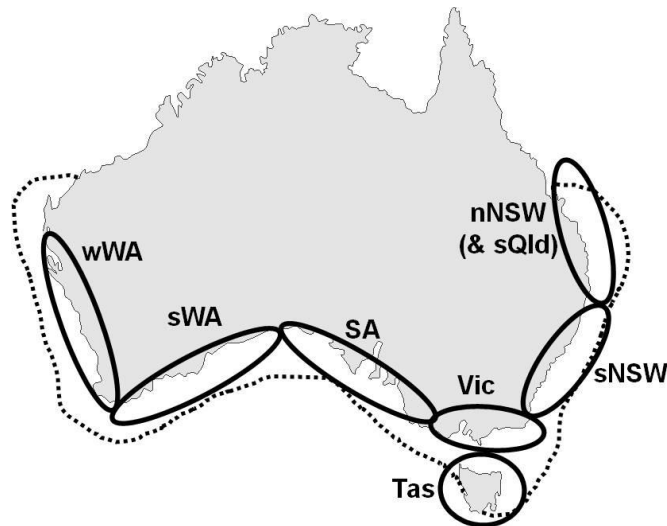


Figure A2 The Australian distribution of the sardine (*Sardinops sagax*). The solid rings denote those regions examined by the integrated Stock Difference Index—weight of evidence approach.

This study uses a weight of evidence approach to defining manageable stock units; the development of multiple matrices in the present study provides a semi-quantitative analysis of differences/similarities among regional populations. Following Welch et al. (2009), differences were assessed among key geographic regions of the distribution of the Australian sardine (Fig. A2): wWA – west coast Western Australia, sWA – south coast Western Australia, SA – South Australia, Vic – Victoria, Tas – Tasmania, sNSW – southern New South Wales, and nNSW – northern New South Wales—southern Queensland. Differences in biological and ecological attributes of sardines among these regions were assessed from a total of 14 direct and indirect techniques for inferring patterns of stock structure and connectivity among regional Australian sardine populations (Table A2).

Table A2 Techniques used to assess differences in biological and ecological attributes among regional Australian sardine populations.

<b>Attributes</b>	<b>Techniques</b>	<b>Number</b>
<i>Tagging and genetic approaches</i>	Tagging	<i>i</i>
	Mitochondrial DNA analysis (mtDNA)	<i>ii</i>
	Allozyme electrophoresis	<i>iii</i>
<i>Phenotypic variation</i>	Morphological and meristic analyses	<i>iv</i>
	Otolith shape analysis	<i>v</i>
	Otolith chemistry	<i>vi</i>
	Parasites	<i>vii</i>
<i>Patterns of commercial fishing</i>	Fishing seasonality	<i>viii</i>
	Regional catch compositions	<i>ix</i>
<i>Timing and seasonality of reproduction</i>	Size at maturity	<i>x</i>
	Timing of spawning	<i>xi</i>
	Distribution of larvae and eggs	<i>xii</i>
<i>Life history parameters</i>	Larval growth rates	<i>xiii</i>
	Adult and juvenile growth patterns	<i>xiv</i>

Nine stock units across seven regions are proposed based on results from the weight of evidence approach (refer to Table 7.1).

## **TECHNIQUES FOR ASSESSING THE STOCK STRUCTURE OF THE AUSTRALIAN SARDINE**

### ***Tagging and genetic approaches***

#### ***i. Tagging***

Due to the logistical difficulties in tagging this small bodied species (Janssen, 1939), no conventional tag and recapture studies have been undertaken on the Australian sardine (Fletcher, 1990; Gaughan et al., 2001a), impeding the ability to assess the connectivity of geographically separated regions. The information that is available on the movements of sardines in Australia is largely limited to anecdotal evidence provided by fishers and researchers. For example, along the New South Wales coast line a winter and spring northward movement of sardines is reported (Stewart et al., 2010), while there are reports of an east to west migration during autumn along the south coast of Western Australia (Fletcher et al., 1997).

However, as sardines are capable of extensive migrations, this will greatly affect the geographic ranges of populations and may also limit the availability of sardines in some areas to specific seasons (Fletcher, 1990). What is known of migrations is thought to be largely linked to spawning (Ward and Staunton Smith 2002), as is the case for sardines off the waters of California (Hamman et al., 1988; Beamish et al., 2005), New Zealand

(Baker, 1972), Japan (Tameishi et al., 1996), South Africa (van der Lingen and Huggett, 2003), and the Mediterranean (Carrera and Porteiro, 2003).

Given the paucity of tag—recapture information; this approach to the stock delineation of the Australian sardine was omitted from the weight of evidence (SDI) analysis.

### **ii. Electrophoresis**

The available scientific information on the degree of connectivity among populations of sardines in Australia is largely based on molecular analysis. There appears to be a high level of genetic heterogeneity with the Australian populations of sardine, but there is limited evidence of spatially consistent stock structure (Yardin et al., 1998).

The findings of the allozyme electrophoretic analyses are relatively consistent, indicating that multiple (three to four) large stocks are present across the distribution of the species (Dixon et al., 1993; Yardin et al., 1998). In general, these separate stocks fit pre-defined geographic boundaries, with distinct stocks identified in Western Australia, South Australia, and south-eastern Australia (Victoria), in addition to the east coast (New South Wales) of Australia.

Allozyme analysis also enabled further sub-division of these larger stocks units. In Western Australia, two separate stocks were identifiable: a west coast and a south coast stock (Dixon et al., 1993). Similarly, within New South Wales, two or three separate stocks may be present, adhering to a broad north—south sub-division (Yardin et al., 1998). Gene frequencies (polymorphic esterase) identified fine scale differences between multiple sites in South Australia (Coffin Bay, Cape Jervis and Port Adelaide), indicating that sub-structuring may exist (Dredge, 1969).

Allozyme studies indicate that despite there being molecular heterogeneity among stocks, each is comprised of a series of contiguous quasi-independent sardine sub-populations, with population differences being variable among years at a single location (Dixon et al., 1993; Yardin et al., 1998). Populations display overlapping boundaries, making the distinction of discrete stock unit's complex. Boundaries may shift in response to environmental conditions and the migratory schooling behaviour of the sardine (Dixon et al., 1993; Yardin et al., 1998).

### **iii. Mitochondrial DNA analysis**

The designation of loose stock boundaries is supported by mitochondrial (mt)DNA analysis. A survey of *Sardinops* mtDNA polymorphism indicated that there was considerable genetic similarity within the Australian sardine stock (Okazaki et al., 1996). However, the sampling regime of the study was limited, as a single representative sample was obtained from three broadly separated regions in Australia: Jervis Bay (New South Wales), Boston Bay (South Australia), and Two Peoples Bay (Western Australia).

## Summary

When combined, the available molecular data suggests that a complex stock structure is present throughout the distribution of the Australian sardine. While separate stocks are identifiable, these stocks are ephemeral and seasonal patterns of connectivity among these units appears to exist (Table A3).

Table A3 Matrix of regional comparisons among approaches to assess the distribution of the Australian sardine. Where, 1 = differences, 0 = no differences among regions for each approach, and (-) = no data available. Note: 'E' refers to the electrophoretic findings; and 'mt' refers to mitochondrial DNA analysis.

Regions	wWA		sWA		SA		Vic		Tas		sNSW		nNSW	
	E	mt	E	mt	E	mt	E	mt	E	mt	E	mt	E	mt
wWA														
sWA	1	-												
SA	1	-	1	0										
Vic	1	-	1	-	1	-								
Tas	-	-	-	-	-	-	-	-						
sNSW	1	-	1	0	1	0	1	-	-	-				
nNSW	1	-	1	-	1	-	1	-	-	-	1	-		

## Patterns of phenotypic variation

### iv. Morphological and meristic findings

Phenotypic characters have long been used for examining the stock structure of the Australian sardine (e.g. Blackburn, 1951). Initial analysis of the morphological and meristic characteristics of samples from throughout the distribution of the Australian sardine suggested that three distinct stocks of sardines were present in Australian waters: an eastern group (New South Wales), a south-eastern group (Victoria and South Australia), and a south-western group (Western Australia) (Blackburn, 1951). Subsequent analysis was largely consistent with this stock structure; however, the Western Australian stock was further separated into west coast and south coast populations (Syahailatua, 1992).

While these four stocks are generally agreed, there remains some uncertainty regarding the structure of the sardine stock along the east coast of Australia. Vertebral counts suggest that the New South Wales population can be divided into a southern region and a northern region that includes southern Queensland (Blackburn, 1951).

Morphological analysis of populations from Queensland, New South Wales, Victoria and South Australia, sampled over a four-year period, showed geographical grouping, with the possible existence of two or more major stocks consisting of several overlapping temporal groups (Yardin et al., 1998). In particular, morphological similarities suggest a degree of connectivity exists between the south-eastern Victorian (i.e. Lakes Entrance) and southern New South Wales sardine populations (Yardin et al., 1998).

#### ***v. Otolith shape analysis***

Fourier analysis of the outline shapes of sardine sagittal otoliths indicated that fish from Spencer Gulf, Coffin Bay, Port Phillip Bay and Lakes Entrance have morphologically distinct otoliths (Hall, 2001). These differences showed patterns of temporal variability within sites and between age cohorts (Hall, 2001). Overall, these differences in otolith shape descriptors reflect differences in habitat conditions experienced by the different regional sardine populations (Campana and Casselman, 1993) and mirror differences in rates of growth among populations (Hall, 2001). Similarly, preliminary analysis of Fourier descriptors of otolith shape between northern and southern New South Wales sardine populations suggests a size (length) dependent separation of these populations (Stewart et al., 2010).

#### ***vi. Otolith chemistry***

The chemical composition of Australian sardine otoliths has been examined throughout the distribution of the species. To date, populations from the western and eastern ranges of the species have been examined.

Analyses of the stable isotope composition of otoliths from multiple fishing regions in Western Australia identified fine scale patterns of spatial separation through time and have since been used to discriminate management units (Edmonds et al., 1995; Edmonds and Fletcher, 1997; Gaughan et al., 2001a). Otolith isotope analysis separated the Western Australian sardine stock into three stock units: Fremantle as a west coast stock; Esperance as an eastern stock; and a unique south western stock unit comprised of Albany and Bremer Bay (Edmonds et al., 1995; Edmonds and Fletcher, 1997; Gaughan et al., 2001a). Analysis of otolith isotopes was also able to separate Western Australian regional sardine populations (both the south and west coast) from Victoria and South Australia (Gaughan et al., 2001a).

Trace element analysis was consistent with the isotopic findings, showing a distinct south and west separation among adult fish, with further differentiation among sites on the south coast (Edmonds et al., 1995; Fowler et al., 1997). However, there was an equally high degree of temporal as well as spatial variation (Fletcher et al., 1997).

Fine-scale spatial separation of the south-eastern Australian sardine populations was similarly determined through otolith chemical analysis. Analysis of a suite of elements detected a distinct Australian east coast (New South Wales) stock that could be further seasonally separated into northern and southern units (Yardin et al., 1998). The southern range of the species was shown to be divisible into southern (South Australia) and south-eastern (Victoria) overlapping populations, with a high degree of mixing evident (Yardin et al., 1998).

#### ***vii. Parasites***

Parasite diversity has been given increasing attention as a novel approach to natural tagging of wild fish populations for assessing population connectivity and the identification of stock units (MacKenzie, 2002; MacKenzie and Abaunza, 2005). To

date, parasite diversity has not been directly assessed as a natural tag for stock delineation of the Australian sardine. Host infection rates of the myxosporean parasite *Kudoa thyrssites* were shared between regions on the south and west coasts of Western Australia (Langdon et al., 1992). Whether these findings are indicative of connectivity among regions that are generally considered biogeographically separated is unknown. Due to the paucity of parasite information, this approach to identifying Australian sardine stocks was omitted from the weight of evidence (SDI) analysis.

### Summary

Phenotypic approaches to determining stocks of the Australian sardine are consistent in their findings, indicating that major stock units conform to current geographic (state) boundaries (Table A4). Hence, these data suggest that four unique stock units are identifiable. Further patterns of (seasonal) overlap/connectivity are inferred by the data, potentially facilitating the transfer of fish among adjacent regional populations and confounding the ability to define stock boundaries in both time and space.

Table A4 Matrix of regional comparisons among approaches to assess phenotypic variation of the Australian sardine. Where, 1 = differences, 0 = no differences among regions for each approach, and (-) = no data available. Note: 'M' refers to the findings of the morphological and meristic studies; 'S' refers to the findings of the otolith shape analysis; and 'C' refers to the otolith chemistry findings.

Regions	wWA			sWA			SA			Vic			Tas			sNSW			nNSW		
	M	S	C	M	S	C	M	S	C	M	S	C	M	S	C	M	S	C	M	S	C
wWA																					
sWA	1	-	1																		
SA	1	-	1	1	-	1															
Vic	1	-	1	1	-	1	1	1	1												
Tas	1	-	-	1	-	-	1	-	-	1	-	-									
sNSW	1	-	-	1	-	-	1	-	1	1	-	1	1	-	-						
nNSW	1	-	-	1	-	-	1	-	1	1	-	1	1	-	-	1	1	1			

## Commercial fishing patterns for the Australian sardine

### viii. Fishing seasonality

Sardine catches are generally taken throughout the year in all regions of Australia where they are targeted (Fletcher, 1991a). However, regionally commercial catches of sardines show inter-annual peak over a 3 to 4 month period. For all regions where sardines are taken, peak catches occur throughout March and June, with the exception of southern Queensland—northern New South Wales where catches peak during August and November.

### ix. Regional catch compositions

Spatial patterns of sardine landings vary among fishing regions, even in adjacent populations, suggesting the presence of multiple centres of sardine abundance.

However, for some regions sardine abundances may undergo considerable inter-annual fluctuations, which may in turn result in inter-annual differences in catch composition (Fletcher, 1991a; Hall, 2001). Nevertheless, there exist general patterns in regional sardine catch compositions (i.e. age cohort composition).

In South Australia peak sardine catches are concentrated in southern Spencer Gulf throughout March and June (autumn/winter), following peaks in upwelling in the area (Middleton and Bye, 2007; Ward et al., 2008a, 2010). The southern Spencer Gulf region is also the centre of peak sardine egg and larval abundances (Ward et al., 2007, 2009). These peak abundances are reflected in the catch compositions during the peak periods of fishing, which are largely comprised of small adult fish several months (summer/spring) after the subsequent spawning period (Ward et al., 2008a).

Those large mature sardines taken in South Australia are generally caught in Coffin Bay and along the far west coast (Kinloch et al., 1998; Hall, 2001); suggesting mature/spent sardines migrate offshore west out of the Spencer Gulf region into the west coast of South Australia. There is evidence of an ontogenetic shift in distribution with larger, older fish most commonly found in shelf waters and smaller, younger fish mainly found in embayments including Spencer Gulf (Rogers and Ward, 2005). However, there exists little/no information about the movement patterns of sardines in South Australia and this hypothesis requires further testing.

Pelagic teleost surveys in the Great Australian Bight suggest that annual fluctuations in biomass and catch rates of pelagic fish (e.g. sardines) may have deterred sardine fishing in this region (Stevens et al., 1984; Fletcher, 1991a). Commercial fishing activity in the Great Australian Bight occurs in the eastern (South Australian) region (Ward et al., 2005a). Sporadic pulses of migration may provide connectivity between the south coast of Western Australia and the far west coast of South Australia.

In Victoria, targeted sardine fishing was historically focused in Port Phillip Bay, with peak catches being taken between March and June (summer/autumn) (Fletcher, 1991a; Jackson et al., 1998). In the adjacent Bass Strait, influxes of small sardines were evident in October, with these fish remaining in the region throughout November (Hobday, 1988). The Port Phillip Bay sardines and those of adjacent open sea Victorian waters are thought to consist of a single population, based on large fluctuations in commercial catches and the absence of significant spawning in Port Phillip Bay (Neira et al., 1997, 1998).

Commercial catches of sardines taken from Port Phillip Bay are dominated by 0+ and 1+ juvenile fish, with a small proportion of fish in the 2 to 5-year cohorts (Morison and Hall, 1998; Neira et al., 1999). Fish taken (in smaller quantities) from Lakes Entrance and are typically large, mature sardines (Hall, 2001) and are caught sporadically, possibly reflecting inter-annual variability in fish recruitment (Jackson et al., 1998).

In southern Queensland—northern New South Wales, sardines are seasonally abundant during winter/spring, predominately as mature/spawning fish (Joseph, 1981;



Ward and Staunton-Smith, 2002). This seasonal abundance is reflected in the catch rates for the region, which peak between August and November and are at their lowest between December and June (Ward and Staunton-Smith, 2002; Stewart et al., 2010).

The spawning fish from northern New South Wales—southern Queensland are thought to migrate southward in New South Wales following the favourable oceanographic conditions to continue spawning (Ward and Staunton-Smith, 2002; Stewart et al., 2010). This migratory pattern is supported by catch data from the southern region where large, mature sardines are caught in large quantities throughout March to June (autumn/winter), as well as by anecdotal reports of large-scale fish movement along the east coast (Dakin, 1937; Stewart et al., 2010). The extent of this southern migration is not well known (Ward and Staunton-Smith, 2002), and there have been no investigations as to whether these fish also contribute to the biomass of north eastern Victoria (i.e. Lakes Entrance).

In general, the bulk of the catch for Western Australia is taken between March and June (autumn/winter) (Fletcher, 1991a). This peak in regional catch coincides with the peak in spawning season in Western Australia, which is reflected in the commercial catches that consist of mature fish (Fletcher, 1991a). Multiple regional fishing areas are distributed along the Western Australian coast, with the delineation of two main fishing regions: the south coast and the west coast (Fowler et al., 1997). These regions operate at different capacities, recording significantly different catches and hence are managed independently (Cochrane, 1999; Gaughan et al., 2002). Across the south coast range of the species catches show a clear longitudinal gradient in total landings resulting in the sub-division of the southern management units into three independent management zones (Fletcher, 1991a; Gaughan et al., 2002).

### **Summary**

These data indicate that peak catches of sardines throughout Australia occur between March and June (Table A5). Spatial differences in the catches of sardines between and within fishing regions suggest the presence of multiple fishable sardine stocks. While there may be evidence for some connectivity among major fishing regions (i.e. along the east coast), these appear to be seasonal and may complicate assessment of stock units.

Table A5 Matrix of regional comparisons of patterns of commercial fishing for the Australian sardine. Where, 1 = differences, 0 = no differences among regions for each approaches, and (-) = no data available. Note: 'S' refers to fishing seasonality; and 'C' refers to regional catch compositions, including annual landings.

Regions	wWA		sWA		SA		Vic		Tas		sNSW		nNSW	
	S	C	S	C	S	C	S	C	S	C	S	C	S	C
wWA														
sWA	0	0												
SA	0	1	0	1										
Vic	0	1	0	1	0	0								
Tas	-	1	-	1	-	1	-	0						
sNSW	0	1	0	1	0	1	0	1	-	0				
nNSW	1	1	1	1	1	1	1	1	-	0	1	0		

### **Patterns of spawning and egg/larval abundance/density**

#### **x. Size at maturity**

In general, the size at sexual maturity for the Australian sardine varies considerably among populations in Australia (Table A6). The sizes at maturity were comparable between southern Queensland and southern New South Wales (Staunton-Smith, 1999; Ward and Staunton-Smith, 2002; Stewart et al., 2010), adding weight to the hypothesis that fish from a single stock unit migrate north to spawn in winter then return south to continue spawning throughout the austral summer/spring.

Table A6 Regional comparisons of estimated sizes at maturity of the Australian sardine. Where, subscript letters refer to estimates for males and females; and na when data is not available.

Region	Mat <sub>M</sub> (mm)	Mat <sub>F</sub> (mm)	Reference
southern Qld—northern NSW	~145	~145	Staunton-Smith 1999; Ward & Staunton-Smith 2002
southern NSW	124 - 136	131 - 136	Joseph 1981; Stewart et al. 2010
Victoria	123 - 129	123 - 151	Kinloch et al. 1998
Tasmania	na	na	
South Australia	142 - 146	148 - 150	Kinloch et al. 1998; Ward & Staunton-Smith 2002
Great Australian Bight	>120	>120	Stevens et al. 1984
Western Australia	~120	~120 - 130	Fletcher 1990; Fowler et al. 1997

The estimated sizes at maturity for New South Welsh sardine are comparable to those for southern Australia; a comparative analysis indicates that South Australian sardine mature at larger sizes than their Victorian counterparts (Kinloch et al., 1998; Ward and Staunton-Smith, 2002). The recently estimated sizes at maturity far exceeded the original estimates (70 – 105 mm Body Length) (Blackburn, 1950, 1951), suggesting that both the South Australian and Victorian populations could be sub-divided based on the respective reproductive biology of each population. Blackburn (1951) suggested that

the South Australian sardine population could potentially consist of a gulf waters and a west coast stock, while the Victoria population may consist of a Port Phillip Bay and Lakes Entrance stock. The updated sizes at maturity presented in Table A6 were calculated from fish collected throughout South Australia and Victoria, masking any potential sub-populations in each state.

In Western Australia, the mature and immature fish form spatially independent cohorts (Fletcher, 1990). The mature cohort generally inhabits the continental shelf waters and comprises the bulk of the targeted fishing biomass (Fowler et al., 1997). Commercial catches typically consist of maturing and mature schooling fish longer than 120 mm Fork Length providing an approximate size at maturity (Fowler et al., 1997). The approximate Western Australian size at maturity is the smallest in Australia..

Differences in the onset of sexual maturity are primarily the result of differing rates of growth among the populations, suggesting considerable differences in the respective population's life histories. The little data available for the Tasmanian sardine population suggests they share a similar reproductive life history to their Victorian counterparts (Blackburn, 1950, 1951).

#### ***xi. Timing of spawning***

There is considerable information available for the timing of sardine spawning across the Australian distribution of the species. The initiation of sardine spawning via environmental cues has resulted in asynchronous spawning events throughout the sardines' range (Fig. A3). Changes in the distribution of spawning adult sardines is thought to be primarily related to water temperature, in Australia and overseas (Blackburn, 1960; Fletcher, 1990; Lluch-Belda et al., 1991, 1992; Fletcher and Tregonning, 1992). However, additional factors such as food availability (Ward and Staunton-Smith, 2002) and the strength of prevailing oceanographic conditions (i.e. upwelling strength) (Staunton-Smith and Ward, 2000) have also been shown to coincide with the timing of sardine spawning in Australia, potentially resulting in spatial and temporal variability in spawning (Stewart et al., 2010). Variable spawning patterns throughout the range of the Australian sardine has the potential to inhibit inter-breeding among spatially separated populations of sardines (Blackburn, 1951) and regional differences in the spawning patterns of the sardine have previously been used as evidence of partitioning among populations (e.g. Gaughan et al., 2001c; Silva et al., 2006).

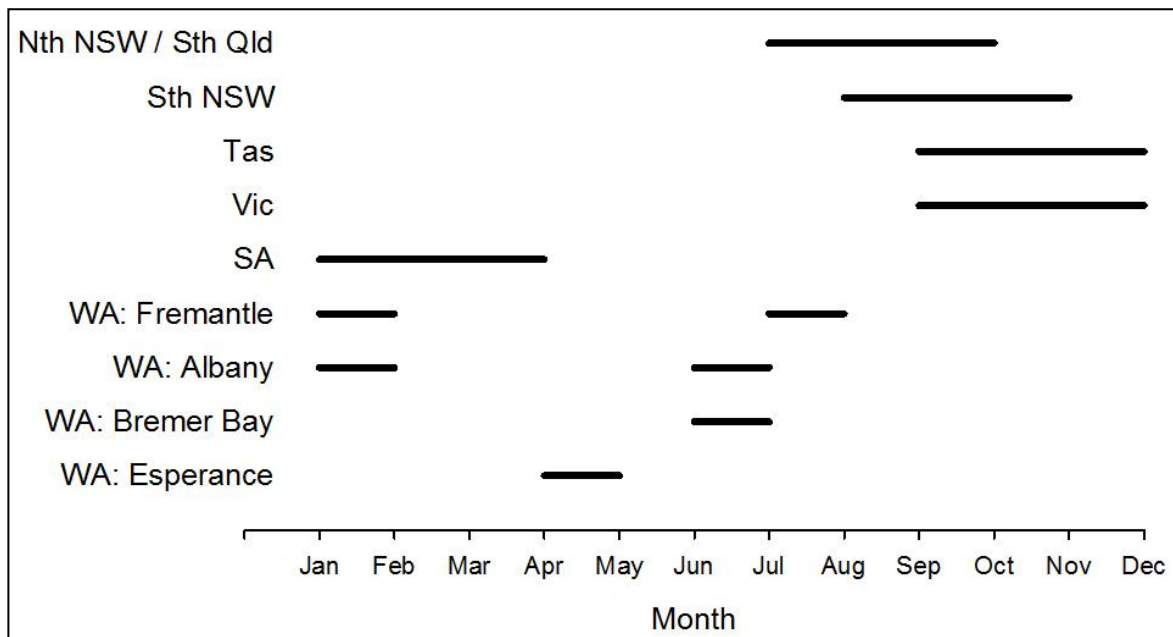


Figure A3 Periods of peak spawning of the sardine throughout its austral distribution (refer to text in Sections x & xii for specific sources of biological data).

Sardines are asynchronous multiple batch spawners (i.e. spawn more than once per season) (Fletcher et al., 1996) and spawning generally occurs in shelf waters as opposed to inshore or estuaries and bays (Fletcher, 1990; Neira et al., 1999). Those eggs spawned at sea move inshore and enter bays and inlets as larvae and juveniles after hatching (Blackburn, 1949; Neira et al., 1999; Rogers and Ward, 2007). These fish are then thought to return to adjacent shelf waters as immature fish (Blackburn, 1949).

Highly variable patterns of spawning occur in Western Australia, with bi-annual peaks in spawning evident in the west coast (Fremantle and Albany), while single peaks in spawning occur in the south coast populations (Bremer Bay and Esperance) (Gaughan et al., 2002). There is also asynchrony within the timing of spawning patterns among locations, as peaks in spawning at Fremantle occur in August and February to March (Fletcher et al., 1996), whilst Albany has two periods in July and from December to January (Fig. A3) (Fletcher and Tregonning, 1992; Fletcher et al., 1994). At Bremer Bay the peak in spawning occurs from June to July (Fletcher et al., 1994) and in south-eastern Western Australia (Esperance) and into the western Great Australian Bight the main time for spawning is between April and July (Fig. A3) (Blackburn, 1960; Fletcher et al., 1996). These differences have been used to validate the division of regional populations (Fletcher et al., 1997). No other regional Australian sardine population displays these bi-modal peaks in spawning.

Along the east coast of Australia, the spawning period of sardine appears to vary with latitude (Stewart et al., 2010). In southern New South Wales the peak in spawning occurs during summer, but is later in southern Queensland—northern New South Wales (winter/spring) (Fig. A3) (Blackburn, 1951; Staunton-Smith and Ward, 2000; Stewart et al., 2010). These peaks in the timing of spawning coincide with regional peaks in sardine landings, suggesting that sardines may migrate north from New South

Wales into Queensland to spawn in winter and return south in summer (Blackburn and Tubb, 1950; Joseph, 1981; Ward and Staunton-Smith, 2002; Stewart et al., 2010).

In South Australia the peak of the local sardine spawning season, between January and April (Fig. A3) coincides with local upwelling events throughout late summer and autumn (February to March) (Bruce and Short, 1990; Kinloch et al., 1998; Ward et al., 2006). Blackburn (1951) suggested that the eastern Great Australian Bight potentially forms a second spawning biomass in South Australia; however, peak spawning periods in the eastern Great Australian Bight occur through summer/autumn (November to May) coinciding with the general peaks throughout South Australia (Fig. A3). Similarly, in western and central Great Australian Bight, peak spawning occurred during autumn/winter (May to June) coinciding with spawning on the south coast of Western Australia (Fig. A3) (Stevens et al., 1984).

In Port Phillip Bay, Victoria the peak spawning is between September and December (spring/summer) (Fig. A3) (Blackburn, 1941, 1950; Fletcher, 1990; Hoedt and Dimmlich, 1995; Neira et al., 1997, 1998; Kinloch et al., 1998). Similarly, at Lakes Entrance, Victoria mature fish have been found throughout September and December (Blackburn, 1950); however, due to as paucity of biological research in the region, no peak spawning period has been identified.

#### ***xii. Distribution and movements of larvae and eggs***

Following spawning, the transport and distribution of the eggs and larvae are primarily dependent on the local oceanographic conditions (Logerwell et al., 2001; Logerwell and Smith, 2001; van der Lingen and Huggett, 2003). As oceanographic conditions vary temporally (i.e. current flow rates and the strength of upwelling events), the fate of the eggs and larvae as well as their relative contribution to the overall year class recruitment strength equally varies among regions (Fowler et al., 1997; Guisande et al., 2001; Galindo-Cortes et al., 2010).

There is little evidence of eggs moving from the west coast to the south coast of Western Australia (Fletcher et al., 1997). However, on the south coast where a number of discrete spawning locations exist, the Leeuwin Current facilitates mixing of the eggs and larvae (Fletcher et al., 1994, 1996; Gaughan and Fletcher, 1997). Eastward movement of eggs and larvae in south Western Australia suggests that fish recruitment comes from a common pool of recruits (Fletcher et al., 1994; Cochrane, 1999; Fletcher and Sumner, 1999). This eastward transport of eggs and larvae has been shown to vary annually, most likely due to inter-annual variability of the Leeuwin Current (Fletcher et al., 1994, 1996); nevertheless, the movement of eggs and larvae between these south coast assemblages may lead to genetic mixing; even though adult populations may be relatively separate (Gaughan et al., 2002).

The Leeuwin Current has the potential to transport larvae from the southernmost tip of Western Australia some 1000 km into southern Spencer Gulf of South Australia (Gaughan et al., 2001c). The extent of contribution of Western Australian recruits to the South Australian fishery is unclear, yet it appears unlikely that significant quantities of

larvae would be advected from Western Australian into the eastern Great Australian Bight and South Australia prior to metamorphosis and recruitment into nursery areas (Gaughan et al., 2001c).

The transport of eggs and larvae eastward along the southern Western Australian coast suggests that an analogous westward migration of fish, or the retention of a considerable quantity of eggs/larvae are required in order to maintain local sardine biomass. Mature fish appear to be present throughout the year in southern Western Australia and long-term egg retention facilitates local recruitment (Gaughan et al., 2002).

For the west coast of Western Australia (i.e. Fremantle), egg and larvae retention appears to maintain local stocks. The retention of eggs and larvae in the area is most likely to occur during the summer regional spawning peak (Muhling et al., 2008). At this time flow rates of the Leeuwin Current are dramatically decreased and local oceanic conditions are favourable for egg and larvae retention (Caputi et al., 1996; Gersbach et al., 1999), which have been observed in the adjacent Swan estuary throughout the summer months (Gaughan et al., 1990; Fowler et al., 1997).

In South Australia, sardine larvae are highly abundant at temperature and salinity fronts that form near the mouths of the two gulfs during summer and autumn (Bruce and Short, 1990) and in shelf waters off the southern Eyre Peninsula (e.g. Ward et al., 2007, 2009). Spawning coincides with the summer/autumn upwelling period in South Australia (Ward et al., 2006). As the current speeds in the region are low, larval transport is not considered significant (Rogers and Ward, 2005, 2007). High densities of sardine eggs are also present each year throughout the Great Australian Bight (Stevens et al., 1984; Ward and Staunton-Smith, 2002; Ward et al., 2009), with peak densities of eggs and larvae in the eastern Great Australian Bight in May and western Great Australian Bight between May and July (Blackburn, 1950).

Port Phillip Bay acts as a nursery area for sardines off Victoria (Jenkins, 1986; Neira et al., 1997, 1998). High densities of eggs and larvae occur in adjacent waters of the Bass Strait (Hoedt and Dimlich, 1995). Egg and larval transport is limited within this system as the adjacent Bass Strait water body undergoes long flushing times (approximately 6 months: Sandery and Kämpf, 2005).

Port Phillip Bay and Phillip Island are most likely continuous with the adjacent Bass Strait sardine population, acting as spawning grounds that facilitate a degree of juvenile migration (recruitment) into shelf waters (Hall, 2001). Regional catch compositions support this hypothesis, as juveniles dominate Port Phillip Bay catches (Jackson et al., 1998) and periodic influxes of immature fish into Bass Strait have been identified (Hobday, 1988).

There have been no reports of sardine eggs and larvae around Lakes Entrance (Blackburn, 1950). As catches of sardines from the region are dominated by large mature fish (Hall, 2001), sardines from Lakes Entrance may spawn offshore, or recruit

from adjacent regions (i.e. Port Phillip Bay and Bass Strait, or southern New South Wales).

Little information exists on the timing of spawning of Tasmanian sardines. Those few spawning fish caught seemed to coincide with that of Victorian sardines, which are believed to be late spring/summer spawners (Blackburn, 1950). No information is available on the distribution/movement of eggs and larvae in the region.

Adult sardines appear to migrate northwards into southern Queensland—northern New South Wales during early winter to spawn and peak egg and larval abundances are observed in this period (Ward and Staunton-Smith, 2002; Ward et al., 2003). It is at this time of the year that annual flows of the East Australia Current are strongest (Tilburg et al., 2001), providing poor larval retention rates, and facilitating the southward transport of sardine larvae (Ward et al., 2003). This is supported by the large densities of sardine larvae found further south in summer (Gray and Miskiewicz, 2000; Uehara et al., 2005). The potential mixing influence of the East Australian Current suggests that discrete genetic stocks are unlikely (Stewart et al., 2010). Those larvae that are entrained by the East Australian Current are thought to contribute to the southern New South Wales egg and larval biomass and in years of high flow potentially contribute to the south-eastern Australian sardine biomass (Stewart et al., 2010). This dispersal-migration pattern is similar to those observed for non-austral *Sardinops* populations in the western boundary current systems off the east coasts of North America and Africa (e.g. Hammann et al., 1988; van der Lingen and Huggett, 2003).

### **Summary**

Overall, these data suggest that a continuous spatial distribution of eggs and larvae exists throughout the range of the Australian sardine. While there appears to be eggs and larvae annually present throughout the species' distribution, seasonal abundances of eggs and larvae exist among regions due to the asynchrony of peaks in breeding that appears to inhibit inter-mixing among the populations (Table A7).

Table A7 Matrix of regional comparisons of patterns of reproductive timing, seasonality and larval duration for the Australian sardine. Where, 1 = differences, 0 = no differences among regions for each approaches, and (-) = no data available. Note: 'S' refers to the timing of spawning; 'M' refers to the size at maturity; and 'E' refers to patterns of the distribution of larvae and eggs.

Regions	wWA			sWA			SA			Vic			Tas			sNSW			nNSW		
	S	M	E	S	M	E	S	M	E	S	M	E	S	M	E	S	M	E	S	M	E
wWA																					
sWA	1	0	0																		
SA	1	1	1	1	1	0															
Vic	1	1	1	1	1	1	1	1	1												
Tas	1	-	-	1	-	-	1	-	-	0	-	-									
sNSW	1	1	1	1	1	1	1	1	1	0	0	1	0	-	-						
nNSW	1	1	1	1	1	1	1	0	1	1	1	1	1	-	-	0	1	0			

## Life history parameters

### xiii. Larval growth rates

Larval growth data have been collected from a number of Australian populations (Table A8). A standard approach to estimating larval growth was used that involved analysing daily increments in sagittal otoliths with daily length-at-age data fitted to the Laird-Gompertz growth model. Overall, larval rates of growth differed among regional populations of Australian sardines, with a distinct latitudinal gradient of growth evident, as southern regions and showed the slowest growth rates (Table A8). Even among adjacent populations of sardines, i.e. the south coast and west coast of Western Australia, larval growth was shown to be significantly different (Muhling et al., 2008).

Nevertheless, ranges of larval growth rates of Australian sardines were comparable to those of other sardine stocks in areas of high productivity: 0.40 to 0.80 mm day<sup>-1</sup> (Gaughan et al., 2001b; Muhling et al., 2008). This is most likely due to austral spawning peaks tending to coincide with localised peaks in productivity (Dimmlich et al., 2004; Strong and Ward, 2009).

Given that areas of high sardine larval density are generally associated with areas of high productivity, growth rates of clupeoid larvae are maximised (Blaxter and Hunter, 1982; Watanabe et al., 1996; Lynn, 2003; Skogen, 2005; Strong and Ward, 2009). Thus, rates of larval growth may not be an informative life history parameter for distinguishing sardine stock units. Rather, since the timing of sardine spawning coincides with oceanographic processes and localised peaks in productivity, spatial and temporal differences in these features may be more useful for identifying spatially separated sardine stocks (Daskalov et al., 2003; Curtis, 2004; Ganas et al., 2007; Ganas, 2009).



Table A8 Regional comparison of the mean and maximum of rates of instantaneous larval growth (LGR) of the Australian sardine. na – data not available.

Region	Mean LGR (mm day <sup>-1</sup> )	Max. LGR (mm day <sup>-1</sup> )	Reference
West coast WA	0.82	0.92	Muhling et al. 2008
South coast WA	0.48	0.62	Gaughan et al. 2001b
Eastern GAB	0.58	0.82	Strong & Ward 2009
South Australia	0.36	0.75	Rogers & Ward 2007
Victoria	0.30	na	Hoedt et al. 1995
Central NSW	0.53	na	Uehara et al. 2005

#### ***xiv. Adult and juvenile growth patterns***

Estimates of rates of growth of the Australian sardine are spatially (Table A9) and temporally variable (Blackburn, 1949, 1951; Hall, 2001). Overall, there appears to be a longitudinal east to west trend of increasing growth rates (Table A9). As otolith-based approaches to age determination (i.e. increment enumeration, otolith weight—age keys) provide the most biologically reasonable findings for Australian sardines (and clupeoids in general) (Fletcher, 1990, 1991b, 1995; Fletcher and Blight, 1996; Rogers and Ward, 2007); in this paper estimates of ages and growth are derived from otolith weights.

Table A9 Regional comparison of the adult/juvenile life history parameters of the Australian sardine. Von Bertalanffy growth parameters are reported:  $L_{\infty}$ : maximum length (mm) and  $k$ : growth rate. Na indicates that data are not available

Region	$L_{\infty}$ (mm)	$k$	Reference
South western WA	164 - 174	0.58 - 0.81	Fletcher & Blight 1996
South Australia	195	0.32 - 1.07	Rogers & Ward 2007
Victoria	212 - 241	0.29 - 0.38	Morison & Hall 1998; Hall 2001
Tasmania	197	na	Blackburn 1950, 1951
Southern NSW	216 - 236	0.32 - 0.37	Stewart et al. 2010
southern Qld—northern NSW	189 - 201	0.30 - 0.38	Anon 1998; Ward & Staunton-Smith 2002

While the southern New South Wales population appears to grow at a slightly faster rate than their northern counterparts (Table A9), the growth parameters among these two regions are fairly consistent (Anonymous, 1998; Ward and Staunton-Smith, 2002; Stewart et al., 2010). Moreover, these parameters conform to estimates of rates of growth for the Victorian sardine population (Table A9) (Morison and Hall, 1998). Age at length plots for samples collected from two regions in Victoria, Lakes Entrance and Port Phillip Bay did not differ markedly; however, due to the limited sample numbers a direct spatial comparison of growth parameters was not made (Morison and Hall, 1998).

Age and growth of the sardine has not been assessed for the Tasmanian population, although it is suggested that they share a similar life history to the adjacent Victorian population (Blackburn, 1951). Of those few Tasmanian sardines that were aged (using

scales increments); the majority of the fish were aged 5 or 6 years (Blackburn, 1949; Blackburn and Tubb, 1950).

The South Australian sardine population shows the greatest rates of growth of all Australian populations (Table 9) (Rogers and Ward, 2007), primarily due to the relatively high level of productivity in the region (Ward et al., 2006; van Ruth et al., 2010). Rates of growth also differ spatially throughout South Australia, with Coffin Bay having faster rates of growth than southern Spencer Gulf:  $k = 0.44$  and  $0.37$ , respectively (Morison and Hall, 1998; Hall, 2001).

For the Western Australian sardine population estimates of growth rates are only available for the Albany fishing region (the centre of the Western Australia sardine distribution), precluding the ability to assess regional variability in life history parameters. Nevertheless, the Western Australian sardine population has a relatively fast growth rate in comparison to the eastern populations and is within the range of values found for the South Australian and Great Australian Bight sardine populations (Table A9) (Stevens et al., 1984; Fletcher, 1990; Fletcher and Blight, 1996).

### Summary

There were observable patterns of variation in the life history parameters of the sardine throughout its Australian range. These differences were most likely driven by regional differences in productivity levels (Gaughan et al., 2001b). Trends in the available data indicated that differences in life histories exist among spatially separated populations (Table A10).

Table A10 Matrix of regional comparisons of life history parameters of the Australian sardine. Where, 1 = differences, 0 = no differences among regions for each approach, and (-) = no data available. Note: 'A' refers to adult and juvenile growth parameters; and 'L' refers to larval growth rates.

Regions	wWA		sWA		SA		Vic		Tas		sNSW		nNSW	
	A	L	A	L	A	L	A	L	A	L	A	L	A	L
<b>wWA</b>														
<b>sWA</b>	0	1												
<b>SA</b>	1	1	1	1										
<b>Vic</b>	1	1	1	1	1	0								
<b>Tas</b>	-	-	-	-	-	-	-	-						
<b>sNSW</b>	1	0	1	1	1	1	0	1	-	-				
<b>nNSW</b>	1	0	1	1	1	1	0	1	-	-	0	0		

### **MASS MORTALITY EVENTS**

In 1995 and 1998/1999 mass mortality events occurred in the Australian population of sardine (Gaughan et al., 2000; Ward et al., 2001b). On both occasions mortality originated near the Eyre Peninsula in South Australia, approximately the centre of the species' Australian range, and spread both east and west as 'epidemic waves' (Fig. A4) (Murray et al., 2001; Ward et al., 2001b; Gaughan, 2002).

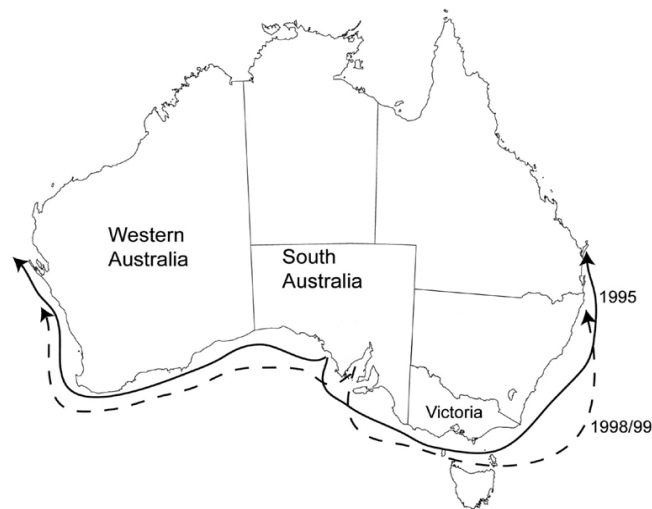


Figure A4 Extent of the 1995 (solid lines) and 1998/99 (dotted lines) mass mortalities of the sardine around southern Australia from starting points along the central South Australian coast (from Gaughan 2002).

Neither outbreak was associated with incidents of environmental stress, thus, potential vectors were investigated (and discounted) as the cause/mode of transmission of the epidemics (Fletcher et al., 1997; Griffin et al., 1997; Jones et al., 1997; Murray et al., 2001, 2003). In the end, a herpes virus was identified as the cause of both mass mortalities and named the pilchard herpes virus [PHV] (Hyatt et al., 1997; Jones et al., 1997).

Mathematical modelling indicated that in both instances the PHV epidemics originated in South Australia and in a near linear relationship between time and distance PHV was transmitted in a host-density independent manner (Fletcher et al., 1997; Whittington et al., 1997, 2008; Murray et al., 2001, 2003). The analyses of the transmission of the epidemic provide a novel analysis of patterns of sardine movement, connectivity, and stock structure (Murray et al., 2003).

The rapid, non-vector mediated transfer of the disease (i.e. fish to fish dependent transfer often against seasonally prevailing oceanographic conditions, as well as the subsequent rapid recovery of most stocks) suggests a continuous stock distribution (Murray and Gaughan, 2003a, b; Murray et al., 2003). Other factors, such as regionally variable patterns of sardine mortality and recovery (Ward and McLeay, 1999; Gaughan et al., 2000, 2008; Ward et al., 2001a, b), as well as differences in the rates of the west- and east-bound transfer of the epidemic (Murray et al., 2003), suggest that perhaps there is a more complex scenario for the stock structure of the Australian sardine.

There appears to be several sub-populations of sardines in Australian waters and the transmission of the PHV indicates that these sub-populations are not wholly isolated from one another. The mode of transmission of the PHV suggests that these different sub-populations are highly mobile and subject to a degree of mixing, i.e. the transfer of individuals (Whittington et al., 2008). This mixing of populations has not led to the development of a homogenous, single stock unit, as the species' biology appears to limit the exchange of larvae/genetic material to facilitate significant gene flow. Conversely, these sub-populations which may be comprised of smaller size dependent shoals, appear to form a single semi-continuous meta-population (Fig. A5) (Murray et al., 2003; Whittington et al., 2008).

Effectively, this 'semi-continuous meta-population' model of the stock structure of the Australian sardine suggests that the whole distribution of the species comprises a single meta-stock unit (Murray et al., 2003). Within this meta-stock there exists largely spatially and temporally independent sub-population stock units (Fig. A5) (Whittington et al., 2008). This scenario is largely consistent with the findings of the direct and indirect approaches to delineating the stock structure of the sardine.

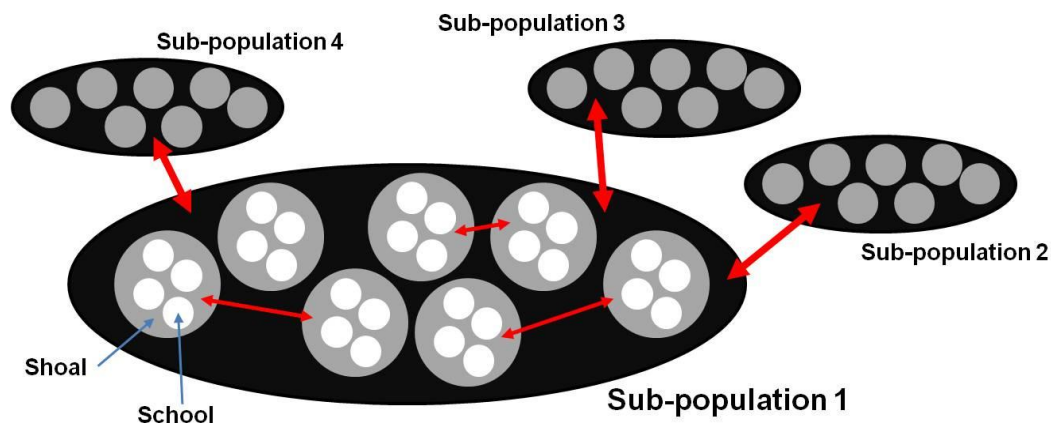


Figure A5. Stylised model of the population structure of the Australian sardine, where sub-populations of sardines are comprised of shoals which consist of schools. Population mixing is facilitated by individual fish migrating from one school to another (thin arrows) during close contact of schools and shoals exchange individuals both within and among subpopulations (thick arrows) (from Whittington et al. 2008).

### **INTEGRATION OF THE AVAILABLE DATA**

Refer to the Results/Discussion chapter of this report (section 7.1.1: *Integration of the available data: definition of Australian sardine management units*) for the final semi-quantitative analysis of the multiple Stock Difference Index matrices.

## 18. APPENDIX 5: MEDIA RELEASE

### South Australian Research and Development Institute (SARDI) Aquatic Sciences

**Media Release:** 9 December 2009

[http://www.sardi.sa.gov.au/information\\_and\\_news/2010\\_media\\_releases2/hi-tech\\_laser\\_reveals\\_the\\_travels\\_of\\_the\\_australian\\_sardine](http://www.sardi.sa.gov.au/information_and_news/2010_media_releases2/hi-tech_laser_reveals_the_travels_of_the_australian_sardine)

### Hi-tech laser reveals the travels of the Australian sardine



Thousands of tiny earbones of fish collected over the past 15 years will play a critical role in future management of the growing Australian sardine fisheries.

Scientists are studying the earbones, or otoliths, to increase understanding of the movement patterns of the Australian sardine throughout southern Australia.

The otoliths, which are just a few millimetres long, will also reveal how many sub-populations of sardines there may be in Australian waters.

The two-year project, led by SARDI Aquatic Sciences and funded by the Fisheries Research and Development Corporation (FRDC), will provide information to enhance the management of Australia's sardine fisheries. The project, was highlighted at the SARDI Aquatic Sciences Open Day held November 22, and involves scientists from the University of Adelaide, New South Wales Department of Industry and Investment, University of NSW and Queensland Primary Industries and Fisheries Victoria.

Project leader and SARDI Wild Fisheries program leader, Dr Tim Ward says the South Australian sardine fishery is the largest fishery in Australia, with annual catches from Spencer Gulf of around 30,000 tonnes."Up until now, the catch has mainly been used to feed farmed Southern Bluefin Tuna and as bait by recreational fishers," he said. "However, the Australian sardine is quickly gaining in popularity as a table fish and there are significant opportunities to export sardines to countries such as China. We

need to know about the movement patterns to ensure that we can make the most of these export opportunities and optimise the management of this fishery.”

The project will use modern scientific equipment - a laser and a mass spectrometer - at the University of Adelaide, to compare the chemistry of otoliths currently archived in South Australia, New South Wales, Queensland, Victoria and Western Australia.

“The Australian sardine plays a vital role in the marine webs being a key prey item for a wide range of predatory fishes, marine mammals and sea birds. We need to know about the movement patterns of this species to ensure that the fisheries are managed sustainably and do not compromise the needs of their predators”, said Dr Ward. “Otoliths have traditionally been used to ascertain the age of fish, with growth rings or annuli being used to estimate their age – like rings on a tree.”

Co-investigator Bronwyn Gillanders from the University of Adelaide added that modern technology now allowed scientists to get information about the population structure and movement patterns of fish. “We will use a laser to remove calcium carbonate from points along a transect across the otolith. A spectrometer will tell us what is in the material, giving us a measure of changes in the otolith’s chemistry over time,” she says.

“Changes in otolith chemistry over time may reflect either the movement of fish between different environments or seasonal changes in environmental conditions, or both. Otoliths from fish of the same species living in the same environment at the same time usually have similar chemistry. By comparing the otolith chemistry of fish taken from different places we can work out their patterns of movement”.

Dr Ward said that this study would focus on movement patterns in some key areas, such as between the Great Australian Bight and Spencer Gulf and along the east coast of Australia.

“Previous studies have suggested that some sardines move up the east coast during winter into southern Queensland and northern NSW to spawn, and that their larvae are returned to southern waters by the East Australian Current. What we want to find out is what proportion of the east coast population undertakes this spawning migration and what proportion stays at home in southern NSW and western Victoria. This information will tell us how well a biomass estimate for the spawning aggregation in southern Queensland and northern NSW reflects the size of the entire east coast stock.

“For South Australia, we particularly want to know about the rates of movement of sardine between Spencer Gulf and the Great Australian Bight.”

The project is a national collaborative effort involving a steering committee with representatives of the Commonwealth Government, State fisheries and research agencies, universities and commercial fishing sector.

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## 19. APPENDIX 6: ASFB 2010 CONFERENCE PRESENTATION ABSTRACT

### **Movement patterns and stock structure of the Australian sardine throughout Australia**

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**Keywords:** Australian sardine, otolith chemistry, laser ablation ICP-MS, management

#### **Abstract**

The Australian sardine (*Sardinops sagax*) is distributed throughout southern Australia, where multiple fisheries have grown rapidly over the last decade. A better understanding of the biomass/abundance, distribution and patterns of movement of Australian sardine stocks within and among jurisdictions are required to support future increases in the regional TAC's and the definition of spatial management (zones) throughout the species' range. We used profile-based otolith chemistry techniques (analysis of elements by laser ablation ICP-MS) to determine the stock structure and movement patterns of sardine between gulf and shelf waters of South Australia and along the East Coast of Australia. From the resulting data, we focused on the early life history and collection period (edge of otolith) to determine whether there were spatial differences in otolith chemistry, as well as two collection periods to determine whether there was temporal variation. We also analysed profiles across the otolith of all fish to determine variation through time and estimate whether some fish are resident versus migratory. These findings will be addressed in relation to the implications for future stock assessment and management of the species.