Implementation of an Age Structured Stock Assessment Model for Sardine (*Sardinops sagax*) in South Australia

T. M. Ward, P. J. Rogers, P. Stephenson, D. W. Schmarr, N. Strong and L. J. McLeay

Final Report to the Fisheries Research and Development Corporation

FRDC Project 2000/125

Implementation of an Age Structured Stock Assessment Model for Sardine (*Sardinops sagax*) in South Australia

T.M. Ward, P.J. Rogers, P. Stephenson,

D.W. Schmarr, N. Strong and L.J. McLeay.

Implementation of an Age Structured Stock Assessment Model for Sardine (*Sardinops sagax*) in South Australia

Authors: T.M. Ward, P.J. Rogers, **P. Stephenson, D.W. Schmarr, N. Strong, and L.J. McLeay.

Addresses: South Australian Research and Development Institute, 2 Hamra Avenue, West Beach, Adelaide South Australia, 5024.

**Western Australian Marine Research Laboratories, PO Box 20, North Beach, Western Australia, 6020.

Telephone: (08) 8207 5400
Facsimile: (08) 8207 5406
http://www.sardi.sa.gov.au

Cover photograph by Paul Rogers.

Printed in Adelaide 2004.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NON TECHNICAL SUMMARY

TITLE: IMPLEMENTATION OF AN AGE STRUCTURED STOCK ASSESSMENT MODEL FOR SARDINE (*SARDINOPS SAGAX*) IN SOUTH AUSTRALIA.

FRDC PROJECT NO: 2000/125

PRINCIPAL INVESTIGATOR: Dr Tim Ward

EMAIL: ward.tim@saugov.sa.gov.au

PRESENT ADDRESS: South Australian Research and Development Institute (SARDI) Aquatic Sciences, 2 Hamra Ave, West Beach, 5024, South Australia.

Objectives:
1. To develop a reliable age determination method for South Australian sardine and investigate the patterns of age and growth for the population.
2. To investigate the effects of primary productivity on growth rates of larval sardine in the eastern Great Australian Bight, South Australia.
3. To determine the age structure of the South Australian sardine population using commercial and fishery independent data.
4. To assess the potential for using data on the abundance of pre-recruits in catches as an index of recruitment.
5. To implement an age structured stock assessment model for the South Australian Sardine Fishery.

The South Australian Sardine Fishery is the largest fishery in Australian waters with a total catch of approximately 24,248 tonnes in 2003. The catch is mostly used as fodder for the southern bluefin tuna (SBT) mariculture industry off Port Lincoln, which has an annual value of >AUD $280 million. Several processing facilities have been established in Port Lincoln and increasing quantities of the catch are being used for recreational fishing bait and human consumption.

This project (FRDC Project 2000/125) was initiated in response to the pressing need to establish a reliable age determination method for South Australian sardine and to determine the extent to which catch-at-age data from commercial fishery and fishery-independent sampling represents the age structure of the population and is useful for

stock assessment. Prior to this study, the absence of reliable age determination methods hindered both investigation of the effects of two mass mortality events on the age structure of the population and the application of age-based stock assessment models to the rapidly expanding fishery.

The approach taken to developing suitable age determination methods, was to estimate the age of larval, juvenile and adult sardine from samples obtained throughout South Australian waters using a variety of otolith based techniques.

Sardine larvae were sampled using plankton nets during Daily Egg Production Method (DEPM) spawning biomass research cruises on *RV Ngerin* in 2002. High larval densities were recorded at the entrance of Spencer Gulf, where a hydrographic frontal system concentrates zooplankton during summer and autumn, and in the eastern Great Australian Bight (GAB), downstream from upwelling centres along the coast of the southern Eyre Peninsula. Larvae were aged by counting daily increments in otoliths, for which daily deposition rates have been validated elsewhere. Larval growth rates in the eastern GAB were higher than those recorded in less productive waters of southern Western Australia and comparable to those recorded in the productive eastern boundary current systems off Africa and the Americas. Results support the findings of other studies, which suggest that waters off South Australia form part of a northern boundary current system and are more productive than other Australian seas.

This is the first Australian study that has succeeded in capturing and determining the age and growth patterns of juvenile sardine. Juveniles were mainly collected using gillnets and underwater lights. This method resolved previous sampling difficulties and resulted in the capture of 1023 juveniles. Juveniles were aged by counting daily growth increments in sagittal otoliths. Juveniles older than 200 days were difficult to age due to narrow increment widths in the first translucent zone and near the outer edges of otoliths. Average growth rates for juveniles were significantly lower in Gulf St Vincent than in Spencer Gulf. Average growth rates in South Australian waters were slightly lower than those in the productive systems off California and South Africa.

Adult sardine were collected from the commercial catch between 1995 and 2001 and fishery independent samples were collected from shelf waters between 1998 and 2001 using multi-panelled gillnets and underwater lights. Samples of dead sardine were collected from the beach during the second mass mortality event in 1998/9.

Age-specific marginal increment analysis was not suitable for validating the periodicity of annuli in otoliths from adult sardine due to difficulties in accurately identifying the margins between translucent and opaque zones. Edge analysis indicated translucent edges mostly formed between November and August and opaque edges were dominant in September and October (spring). The presence of opaque edges coincided with seasonal increases in sea-surface temperature in southern gulf waters. The trajectories of age-otolith weight relationships for juveniles and adults reflected changes in growth rates as fish approached sexual maturity and showed otoliths continued to develop with age. Therefore, based on available evidence we decided that otoliths from sardine satisfied the three main requirements used for justifying the use of daily increment and translucent zone counts for age determination, i.e. that otoliths must contain interpretable increments in the form of alternating translucent and opaque zones that reflect a measurable time scale and continue to develop throughout the lifespan. Based on our findings and other published studies on sardine ageing, we assumed translucent zones in otoliths from adults represented annuli. Further direct validation is required for otoliths from adult sardine to reduce the uncertainty associated with the timing of deposition of the first translucent zone.

Whole otoliths of all adults collected were scored using a readability index, where 1 = excellent, 2 = very good, 3 = average, 4 = poor and 5 = unreadable. Ages were assigned to adult sardine by counting translucent zones in whole otoliths with readability index scores between 1 and 4. Most otoliths from adult sardine were considered to be of average or poor readability. Bias corrected average percent error (APE) estimates for sardine in South Australia were of similar magnitude (9-19%) to those for sardine aged in a similar study in California (Butler *et al.* 1996).

Age and growth analysis showed sardine in the South Australian fishery catch ranged in age between 1 and 6 years old, whereas sardine collected in offshore shelf regions using fishery independent methods were aged between 2 and 7 years old. Most sardine sampled in the offshore shelf regions were ≥3 years old, whereas in Spencer Gulf most were 1 to 4 years old. Von Bertalanffy growth constants for adults and all life history stages indicated sardine growth rates in South Australia were higher than in Western Australian waters, but lower than in the productive California and Benguela Current systems.

Ages were adjusted using an algorithm that transformed translucent zone counts into estimates of decimal age. Following this step, age–otolith weight relationships for otoliths with high readabilities (1 and 2) were used to calculate ages for fish whose otoliths had low readabilities between 3 and 5. Cost-benefit analyses showed the otolith weight method

was the most cost and time efficient method for determining the age of large samples of sardine from the South Australian fishery.

When adults collected by all methods were combined, the mean quantity of otolith weight deposited during the first year of development was similar to findings for juveniles aged by counting daily increments. Annual otolith weight deposition rates for adult (>1 years old) sardine in South Australia were identical (0.23 mg.year^{-1}) to those for Western Australian sardine (0.22 mg.year^{-1}). The age distributions estimated from the otolith-weight age relationships were significantly different to those determined from translucent zone counts. However, the otolith weight-age method only underestimated age by ~3-4 months compared to the translucent counting method. As described by Francis and Campana (2004) this underestimation or "smoothing" of the age distribution was mostly evident in the older age classes, which were difficult to age regardless of the approach used.

The two sardine mass mortality events in 1995 (ME1) and 1998 (ME2) led to significant declines in sardine populations and allowed us to investigate changes in size and age structure after the events. There was no decline in the size and age structure of fishery independent sardine samples collected in offshore waters. In Spencer Gulf, a decline in the size and age structure in commercial catch samples was observed after ME1, but this was not repeated after ME2, and the inverse occurred off Coffin Bay. Fishing effort declined off Coffin Bay after ME2 and as a result, the commercial size and age structure dataset for this region was less robust. Analysis of the size and age structure of dead sardine collected from the beach during ME2 provided further evidence of regional differences in the size and age classes affected by the mortalities. During ME2, dead juveniles were found in several regions of South Australia, yet none were found in Spencer Gulf. Surviving juvenile cohorts may have remained in southern Spencer Gulf and supported the recovery of the spawning biomass, which increased rapidly following ME2. This recovery time is consistent with findings by Murray and Gaughan (2003) who predicted that if juveniles survived, the recovery would take approximately 5 years.

The South Australian Sardine Fishery rarely catches 0+ year old juveniles (<110 mm CFL). Therefore monitoring the abundance of juveniles in commercial catches is not appropriate as an index of pre-recruit abundance. The first recruits to the fishery were one and two years old and the relationship between the abundance of one year olds and two year olds in the following year was poor. The abundance of recruits was not correlated with spawning biomass in future years.

The age structured stock assessment model developed for Western Australia sardine was implemented for the South Australian Sardine Fishery. Uncertainties surrounding key parameters limited the usefulness of this model as a tool for managing the South Australian Sardine Fishery. In particular, the lack of data on natural mortality rates, biases in the age samples from the commercial catch and difficulties encountered in the development of a recruitment index limited the capacity of the model to predict future trends in biomass. Hence, annual DEPM estimates will remain the basis for managing the South Australian Sardine Fishery in the foreseeable future. The age structured model will be used to conduct a retrospective management strategy evaluation of the performance indicators, reference points and decision rules that have been established for the fishery.

1.0 GENERAL INTRODUCTION

1.1 Background

The South Australian sardine *Sardinops sagax* fishery was established in the early 1990's to supply fodder for the southern bluefin tuna (SBT) *Thunnus maccoyii* mariculture industry that developed from the tuna purse seine fishery (Mackie 1995). The sardine fishery is managed by PIRSA Fisheries under a regime of input and output controls that involve entry limitations, gear restrictions, an annual total allowable commercial catch (TACC) and individual transferable quotas (ITQs). The daily egg production method (DEPM) has been used by SARDI Aquatic Sciences to provide PIRSA fisheries management with conservative estimates of the spawning biomass of sardine since 1995. The TACC for the fishery is set at 10 to 17.5% of the spawning biomass estimate, based on decision rules outlined in the interim management plan, which is summarised in Shanks (2004).

Separate mass mortality events in 1995 and 1998 each killed more sardine over a larger area than any other documented mono-specific fish-kill (Griffin *et al.* 1997; Hyatt *et al.* 1997; Ward *et al.* 2001a). Herpesvirus was the only pathogen that was consistently isolated from dead sardine and was identified as the likely disease agent during both mortality events. The spawning biomass estimate in 1995 was 165,000 tonnes, but this decreased to 37,000 tonnes in 1996 after the first mass mortality event killed ~75% of the spawning stock (Ward *et al.* 2001a). Following the second mass mortality event in 1998 the spawning biomass decreased again to 36,000 tonnes in 1999. The second recovery of the stock was monitored annually and the biomass increased rapidly to 91,000 tonnes in 2000, to 240,600 tonnes in 2002 and 269,065 tonnes in 2003. During the recovery from the second mass mortality event, the fishery expanded considerably and in 2003 the TACC was set at 40,350 tonnes and the total catch was approximately 24,248 tonnes (Rogers *et al.* 2004).

Cochrane (1999) indicated management of sardine fisheries is improved by integrating annual spawning biomass estimates with models using age structure and fishery data. This provided the impetus for the development of reliable and cost effective age determination methods for South Australian sardine. Age structured stock assessment models were used as a tool to predict biomass recovery after mass mortality events in Western Australia (Murray and Gaughan 2003).

Prior to this project, the lack of suitable methods for age determination hindered investigation of the effects of the mortality events on the age structure of sardine in South Australia. Previous age determination studies on sardine have used zone counts in

otoliths, analysed marginal increment widths and tracked modes in otolith weight (Fletcher 1991, 1995; Fletcher and Blight 1996; Kerstan 2000). At the Third International Symposium on Fish Otolith Research and Application in Townsville, several fisheries scientists suggested that cost-benefit analyses of otolith weight age determination methods should be conducted on a species by species basis, prior to the adoption of these techniques (Stephenson 2004; Francis and Campana 2004). These previous studies and recent presentations provided further confirmation and support for otolith weight methods used for sardine during this study.

The daily deposition of increments in otoliths has been validated for laboratory reared sardine larvae (Hayashi *et al.* 1989). With the exception of Waldron (1998), few studies have investigated the periodicity of zone formation in otoliths from adult sardine. Waldron (1998) showed the first translucent zones in South African sardine formed at approximately one year of age and successive zones formed at a mean age of every 218 days. However, these results were inconclusive as some daily increments within the translucent zones of fish older than one year were difficult to count, even using scanning electron microscopy (SEM).

Sardine growth patterns have mostly been studied in productive upwelling regions in the Benguela and California Current systems (Butler *et al.* 1996; Beckley and van der Lingen 1999; Kerstan 2000). Few published sardine age determination studies based on translucent zone counting techniques have focused on Australian stocks with the exception of Fletcher and Blight (1996). Sardine larvae are fast growing (up to 0.82 mm.day^{-1}) and growth rates are influenced by variation in primary production (Watanabe and Kuroki 1997; Gaughan *et al.* 2001a). There have been no previous studies of the growth patterns of juvenile sardine from Australian waters. Juveniles in the Gulf of California have fast growth rates that range from 0.48 to 0.63 mm.day^{-1} (Quinonez-Velazquez *et al.* 2000). Von Bertalanffy growth parameters for adult sardine in Western Australia suggest sardine grow slower and attain smaller maximum sizes (L_∞ = 164 to 174 mm, k = 0.58 to 0.81 yr^{-1}) than sardine from South African (L_∞ = 221 mm, k = 1.09 yr^{-1}) and southern Californian waters (L_∞ = 205 mm, k = 1.19 yr^{-1}) (Fletcher and Blight 1996; Butler *et al.* 1996; Beckley and van der Lingen 1999).

1.2 Need

The South Australian Sardine Fishery has undergone rapid growth with significant investment in infrastructure during the recovery from the 1998 mass mortality event. Large-scale inter-annual fluctuations in abundance caused by two mass mortality events

coupled with the rapidly expanding fishery emphasized the need to monitor the size, age structure and growth patterns of the sardine population. These issues and the ecological importance of sardine led to the need for development of reliable age determination methods for South Australian stocks. Reliable age structure information is critical to the sustainable management of sardine populations and for the implementation of age based stock assessment models. In addition, there was a need to determine if catch at age data from commercial fishery represented the age structure of the wider South Australian sardine population.

The objectives stated below are based on those from the original project proposal. Objective 2 is an additional objective that was incorporated into the project as part of a graduate study undertaken in conjunction with University of Adelaide.

1.3 Objectives

1. To develop a reliable age determination method for South Australian sardine and investigate the patterns of age and growth for the population.

2. To investigate the effects of primary productivity on growth rates of larval sardine in the eastern Great Australian Bight, South Australia.

3. To determine the age structure of the South Australian sardine population using commercial and fishery independent data.

4. To assess the potential for using data on the abundance of pre-recruits in catches as an index of recruitment.

5. To implement an age structured stock assessment model for the South Australian Sardine Fishery.

2.0 DEVELOPMENT OF A RELIABLE AGE DETERMINATION METHOD: PATTERNS OF AGE AND GROWTH OF SOUTH AUSTRALIAN SARDINE (*SARDINOPS SAGAX*)

Objectives:

1. To develop a reliable age determination method for South Australian sardine.

2. To investigate the patterns of age and growth for South Australian sardine.

The first objective was achieved using adults collected from the commercial catch and larvae, juveniles and adults were also sampled using fishery independent methods. Larvae and juveniles were aged by counting daily increments in sagittae. Two independent readers estimated the age of a sub-sample (n = 526) of otoliths from adult sardine. The average ageing bias between readers ranged between -0.51 and -0.64. Bias-corrected average percentage error (APE) ranged between 9.75 and 19.52%. Cost-benefit analysis showed otolith weight-age based methods were appropriate for assigning ages to adult sardine. Ages were assigned to samples of adults with otoliths of average to poor readabilities (3 to 5) using otolith weight-age relationships for otoliths with high readabilities of 1 and 2. The mean quantities of otolith weight deposited during the first year in juveniles collected using fishery independent methods were significantly different in Spencer Gulf (1.06 mg) and Gulf St Vincent (0.73 mg). The mean quantities of otolith weight deposited during the first year were 0.69 mg in fish aged by translucent zone counts and 0.86 mg in fish aged by daily increment counts. Age-specific marginal increment analysis was not a suitable age validation technique for adult sardine. Edge analysis showed deposition of narrow translucent edges peaked in mid winter and wider opaque edges mostly formed in spring. Significant variation in length at age was observed during all sardine life history stages. Maximum growth rates for larvae were higher (0.75 mm.day^{-1}) than in other regions of southern Australia. Average growth rates for juveniles were significantly different (*ANCOVA, P < 0.05*) in southern Spencer Gulf and upper Gulf St. Vincent at 0.44 and 0.25 mm.day^{-1}, respectively. The von Bertalanffy growth constants for all life history stages combined indicated sardine growth rates in South Australia were higher than in Western Australia, but lower than in more productive regions overseas that support significant small pelagic fisheries.

2.1 Methods

2.1.1 Approach to data organisation

Commercial, fishery independent and mortality event samples were subdivided by separating South Australian waters into four regions: Gulf St Vincent (GSV), Spencer Gulf (SG), Coffin Bay (CB) and shelf waters (S). Coffin Bay refers to the broad area off Point Sir Isaac, on Coffin Bay Peninsula (Fig. 2.1). Shelf waters include those around Kangaroo Island (KI) and the offshore islands of the Great Australian Bight (GAB), including Flinders Island, Greenly Island, Waldegrave Island, St Francis Island, Corvisart Bay and Franklin Island (Fig. 2.1).

2.1.2 Sample collection

Samples of larvae, juveniles and adults were collected in Gulf St Vincent, Spencer Gulf and in the GAB between January 1995 and February 2002 from the commercial catch and by fishery independent methods. The locations and regions sampled are shown in Fig. 2.1 and a summary of sample sources, methods, regions, numbers of samples and individuals of each life history stage is shown in Table 2.1.

Larvae

Ichthyoplankton samples were collected from 89 stations in shelf waters during February and March 2002 from *RV Ngerin* (See Ch. 3, Fig. 3.14b). Samples were collected at the surface using bongo plankton nets (500 μm mesh) with mouth diameters of 0.6 m and total lengths of 4 metres. The nets were towed at speeds of 2-3 knots and the duration of each tow was ten minutes. Ichthyoplankton samples were stored in 70% ethanol. Larvae analysed in this section were also collected opportunistically between 2000-2002, using dab-nets and underwater lights while sampling juveniles.

Fishery independent juveniles

Juveniles were collected after dusk at several locations (Fig. 2.1) in Spencer Gulf and Gulf St Vincent using multi-panelled monofilament gillnets (10 x 12 x 14 mm, stretch mesh). Nets were suspended 3-10 m under the research vessel using weights and an underwater light was used to attract schools. Juveniles were stored on ice before being frozen at the SARDI Aquatic Sciences research facility.

Fishery independent adults

Samples of adult sardine were collected off Kangaroo Island, Greenly Island, Flinders Island and Waldegrave Island in the eastern Great Australian Bight (GAB) between 1998 and 2001 (Fig 2.1 and 2.2; Table 2.1). Adults were collected after dusk using multi-

panelled, multifilament gillnets (25 x 28 x 32 mm, stretch, double diamond mesh) (Ward *et al.* 2001a) (Fig. 2.2). Schools of adult sardine were located using a Furuno CH-240 (60 and 180 kHz) sonar on *RV Ngerin*. Nets were suspended 5-20 m under the research vessel using head ropes and footropes. Weights were attached to the ends of the footropes. Underwater lights were suspended under the research vessel to attract sardines. Soak times varied widely and were dependent on the number of sardines aggregating near the lights.

Samples were used for two purposes: Sub-samples were frozen onboard for age determination and additional samples were stored in 5% formalin solution for estimation of adult reproductive parameters for the DEPM. DEPM samples were weighed onboard yet no measures of caudal-fork length (CFL) were recorded. Caudal fork lengths (CFL) for these samples were estimated separately for males and females using length-weight relationships (T. Ward unpublished data).

Commercial samples

Monthly samples were collected from the South Australian commercial catch between November 1995 and December 2001. Fig. 2.1 shows the location of the sardine fishery. The fishery used purse seine nets that were 600 m long, with a drop of 50 m and a minimum mesh size of 12 mm. Sardine are transferred from the nets to the vessel using fish pumps. Sub-samples of sardine from the commercial catch were frozen fresh by processors (SA Premium Pilchards) at Proper Bay, Port Lincoln and transported to SARDI Aquatic Sciences for biological analysis.

Mortality event samples

During the 1998 mass mortality event samples of dead juvenile and adult sardine were collected from the beach at several locations in Gulf St Vincent, Spencer Gulf and shelf waters, which included the area from the south east of South Australia, near Kingston to Point Brown on the west coast of Eyre Peninsula (Fig. 2.1). All samples were stored on ice before being frozen at -40º C in the laboratory.
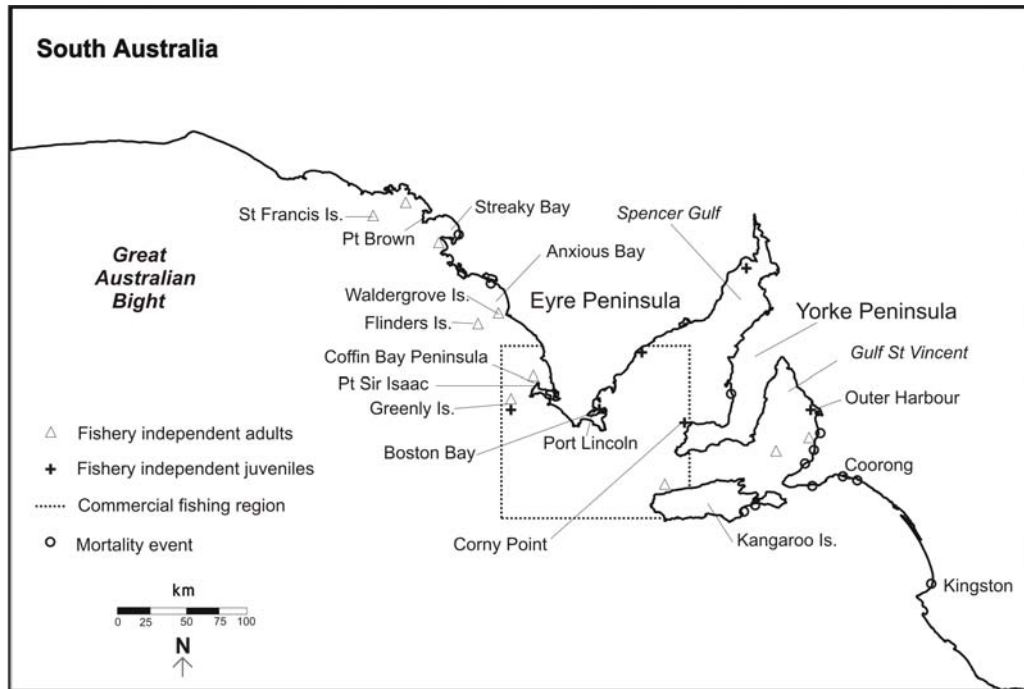
Figure 2.1. Locations where commercial and fishery independent samples of juvenile and adult sardine were collected between 1995 and 2001. Locations where dead sardine were collected from the beach during the second mass mortality event (ME2) in 1998.
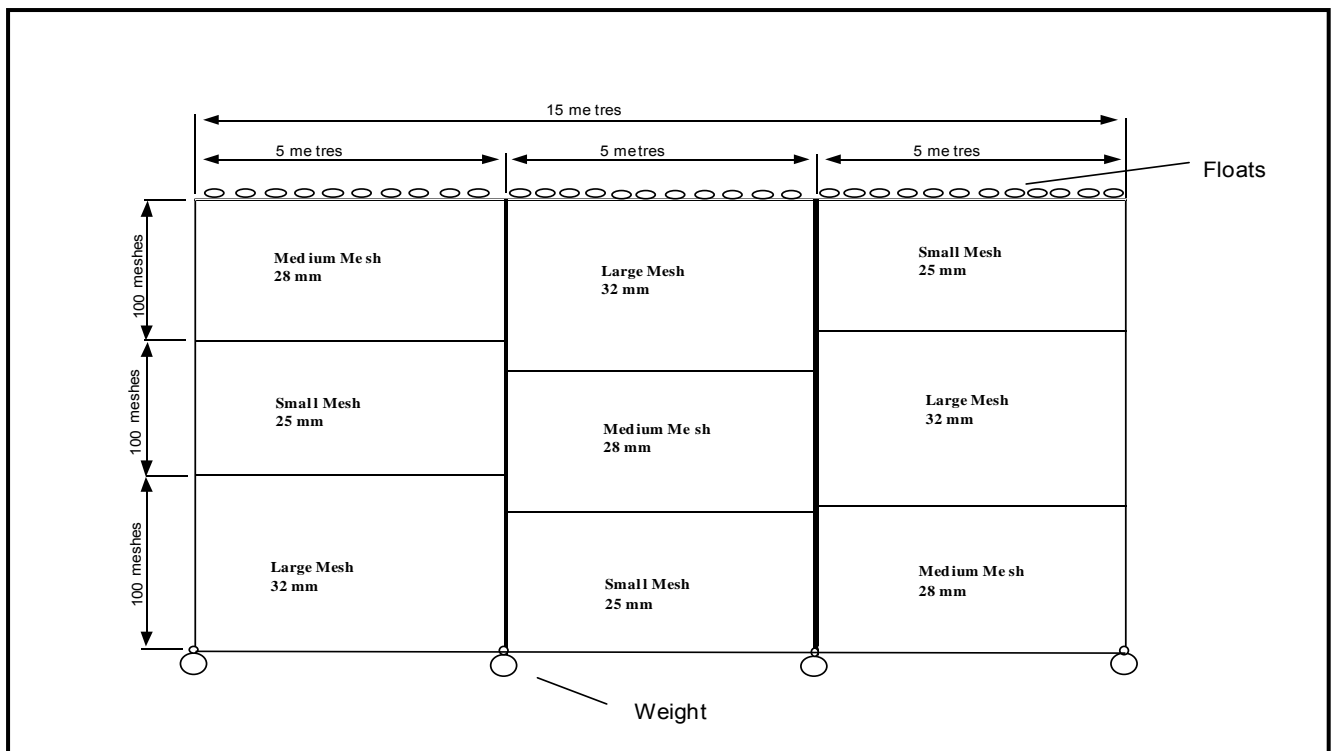
Figure 2.2. Schematic representation of the multi-panelled gillnet used to collect samples of adult sardine (above). Research vessel crew removing adult sardine from the multi-panelled gillnet at Greenly Island in the eastern Great Australian Bight (below)

Table 2.1. Sample sources, methods, regions and numbers of sardine collected between 1995 and 2001 (based on all fish measured). MPMOGN = Multi-panelled monofilament gill-net. MPMGN = Multi-panelled multifilament gill-net and beach = moribund fish collected from the beach. ME II = samples collected from beach during the second mass mortality event in 1998.

Source	Life history stage	Regions	Sampling method	N samples	n
Commercial	Adults	Coffin Bay	Purse seine	274	8,996
		Spencer Gulf	Purse seine	415	16,743
Fishery Independent	Larvae	Shelf/ SG	500µm net	89	549
	Juveniles	Gulf St Vincent	MPMOGN	10	729
		Spencer Gulf	MPMOGN	10	292
		Shelf waters	MPMOGN	1	2
	Adults	Shelf waters	MPMGN	24	6,525
ME II	Adults	Spencer Gulf	beach	2	51
	Juveniles/ adults	Gulf St Vincent	beach	3	339
	Juveniles/ adults	Shelf waters	beach	9	734
Grand Total				837	34,960

2.1.3. Laboratory analysis

Age determination-Larvae

Ichthyoplankton samples were sorted for sardine larvae using light microscopes at 4-40x magnifications. Sardine larvae were identified using keys and descriptions in Neira *et al.* (1998). Representative sub-samples of 30-40 larvae were selected for age determination from each sample or if less were present in the sample, all were aged. Larvae were measured to ±0.01 mm (total length, T.L) using digital vernier callipers. The head of each specimen was removed and re-hydrated in distilled water. Otoliths (sagittae) were located using a cross-polarising filter under a light microscope at 40x magnification. Otoliths were removed with fine needles, cleaned in distilled water, dried and fixed whole to microscope slides using crystal bond™ thermal adhesive. Daily increments were viewed at magnifications of 250-625x using a Leitz (Diaplan) compound microscope and Image Capture™ software. Daily increment counts were made using a mechanical counter.

Age determination-Juveniles

Juveniles were measured to ±1.0 mm (caudal-fork length, CFL) and weighed to ±0.01g. Representative sub-samples were used for age determination. Both otoliths (sagittae) were removed from the semi-circular canals using fine forceps. Otoliths were cleaned in 10% sodium hypochlorite, rinsed in distilled water, and dried in IWAKI™ plastic microplates. Otoliths were dried in the oven for 24 hours at $50^{o}C$ and weighed to ±0.00001g. Sagittal sections were prepared by fixing each otolith to a microscope slide and grinding both surfaces using 3 and 9 µm lapping film and polished with silk cloth. Viewing and counting techniques were as described for larvae.

Age determination-Adults

Adults were measured to the nearest mm (caudal-fork length, CFL) and weighed to ±0.01g. Sub-samples of 30-50 individuals from fishery independent, commercial and mortality samples were used for ageing. Both otoliths (sagittae) were removed as per the juveniles. Otoliths were soaked overnight in 10% Sodium Hypochlorite solution to remove excess tissue, rinsed in distilled water and dried in IWAKI™ plastic microplates. One otolith from each fish was weighed using methods previously described for juveniles (NB: no significant difference between weights of left and right otoliths, *ANOVA, P > 0.05*). Translucent zone counts were made for one otolith from each fish.

An image of each otolith was taken using Image Capture™ software and reflected light against a flat black background. Sigmascan™ software was used to measure the width of each translucent zone and the distance between the outermost translucent zone and the

peripheral edge in each otolith for marginal increment analysis. In some otoliths from adults a thin translucent zone, which forms during the first winter of juvenile (0+) growth was visible within the large central opaque mass. In otoliths from adults this first translucent zone was not counted as the first zone.

2.1.4. Data analysis

Age-specific marginal increment analysis (MI)

The width of outer translucent zones was measured as the distance between the outer edge of the forming zone and the outer edge in otoliths with RI scores of 1 and 2, along the longest posterior axis (Hyndes *et al.* 1992). The age-specific marginal increment (MI) was expressed as the width of the outer (forming) translucent zone as a proportion of the width of the previous zone (if = 1) or the mean width of the previous translucent zones (if >1), based on the equations:

$$M.I. = \frac{TZW_{outer}}{TZW_{\substack{previous \\ zone}}} \ or \ \frac{TZW_{outer}}{\overline{XTZW}_{\substack{previous \\ zones}}}$$

; where TZW is the translucent zone width. Mean age-specific marginal increments (±s.e.) were plotted by month to determine if formation of the translucent zones in the 2 and 3 year old age classes occurred annually.

Edge analysis

Seasonal patterns of translucent and opaque zone formation at the posterior edge of otoliths from adults were analysed for a sub-sample (n = 467) of otoliths. Transmitted light (16-40x magnification) was used and outer zone edges were classified as opaque or translucent. If light refraction made identification of edge types difficult the otolith edge type was not classified as either opaque or translucent.

Readability (RI) and estimation of between reader error

A subjective readability index (RI) was established and each otolith was given a readability score where; 1 = excellent, 2 = very good, 3 = average, 4 = poor and 5 = unreadable. Independent translucent zone counts were undertaken by readers for a subset of otoliths from MAFRI and SARDI Aquatic Sciences. Mean between reader bias was calculated from the SARDI age minus the MAFRI age. Average percent error (APE) was estimated for all RI scores pooled and when agers from each agency independently

agreed on RI scores. Average percent error (APE) was calculated using methods in Beamish and Fournier (1981), where:

$$APE = \frac{100}{N} \sum_{i=1}^{R} \left[\frac{1}{R} \sum_{i=1}^{R} \frac{\left| X_{ij} - X_j \right|}{X_j} \right]$$

and N is the number of sardine aged, R is the number of times each otolith is aged by different readers, X_{ij} is the ith age estimation for the jth individual, and X_j is the mean age for the jth individual.

The original estimates of APE were randomly re-sampled with replacement 5000 times via bootstrap techniques used in Green *et al.* (2002). The means and standard errors were calculated from bootstrapped APEs. To correct bias in the original APE estimate, the mean bootstrapped APE was subtracted from the original APE estimate and added to the original APE estimate, where

$$BiascorrectedAPE = originalAPE + \left(originalAPE - meanbootstrappedAPE \right)$$

95% confidence intervals were calculated using the equation:

$$95\% C.I. = BiascorrectedAPE \pm (1.96.SEbootstrapAPE)$$

Decimal age-otolith weight calibration

To estimate decimal age for adults (≥1 year(s) old), an arbitrary birth-date of March 1 was assigned, which represented the peak of the spawning season (T. Ward unpublished data). The midpoint of translucent zone formation was assumed to be mid-winter. Decimal ages (*D.A.*) were calculated using the following IF statement algorithms:

$$IF\, \alpha < \beta_s, D.A. = \left(\alpha - \beta_p\right)/365 + TZC + 0.334$$
$$\&$$
$$IF\, \alpha > \beta_s, D.A. = (\alpha - \beta_s)/365 + TZC + 0.334$$

where α is the capture date, β_s is the assumed translucent zone formation date from the same year as α, β_P is the assumed translucent zone formation date from the previous year, *TZC* is the translucent zone count and 0.334 (4 decimal months) adjusts for the difference between the assigned birth-date and the approximate timing of the first translucent zone.

Ages for fish with otoliths with low RI scores of 3-5 were calculated using otolith weight-age relationships for otoliths with high RI scores of 1 and 2. Kolmogorov–Smirnov (KS) tests were used to compare age distributions from the otolith weight-age derived and algorithm derived ages (all RI scores). Age structure biases resulting from the otolith weight-age method were estimated from the algorithm derived decimal age minus the otolith weight derived age as in Worthington *et al.* (1995). Age structures for each sub-set of the data (region/year/sample source) were determined by calculating the percentage of sardine in each age class from the otolith weight-age derived ages (See results in Chapter 4). KS and Chi-squared tests were used to test for differences in size and age structure distributions after the mass mortality events in 1995 and 1998.

2.1.5. Costs and benefits of annuli counting and otolith weight-age calibration

A summary of the costs and benefits of translucent zone counting using whole otoliths and otolith weight-age techniques were compiled. This assessment assumed sample collection and fish dissection were completed, otolith weight-age relationships had been developed and weighing otoliths was the only laboratory work required. The processing costs and time taken per otolith were based on standard laboratory techniques and hourly rates for an OPS 2 technical officer (+40% on-costs). The laboratory times in weeks to process 5000 otoliths were based on 37.5 hour working weeks. These times were likely to be underestimated for both methods, as breaks were not included. Benefits were assessed in terms of the tradeoffs between (1) efficient use of laboratory time (2) the magnitude of ageing biases when methods were compared and, (3) the timeliness of outputs for monitoring the age compositions of fishery catch samples.

2.1.6. Age and growth patterns

Larvae

The Laird-Gompertz (*LG*) growth model was used to describe the relationship between age and length for larvae and juveniles and is represented by the equation:

$$L(t) = a \exp\left(\frac{b}{c} \left[1 - \exp\left(-c\left(t - t_0 \right) \right) \right] \right)$$

where $L(t)$ is the length at age *t*, *a* is length at age t_0, *b* is the specific growth rate at age t_0, and *c* is the rate of exponential decay of the growth rate. Levenberg-Marquardt non-linear curve fitting routines in Table Curve™ software were used to fit the growth curves. Instantaneous growth rates were calculated from the first derivative of the *LG* growth equation at daily age intervals:

$$\frac{dL(t)}{dt} = b \exp\left(-c\left(t - t_0 \right) \right) L(t)$$

$$= \left\{ b - c \ln\left(\frac{L(t)}{a} \right) \right\} L(t)$$

Juveniles

The relationships between age in days and caudal fork length (CFL) in Spencer Gulf and Gulf St Vincent were analysed using linear regression analyses. Average somatic growth rates were determined from slopes of the regression equations. Slopes (somatic growth rates) were compared by analysis of covariance (*ANCOVA*).

Adults

Von Bertalanffy (*VB*) growth model fits to adult length at age data were estimated via non-linear least-squared procedures and Levenberg-Marquardt iterations. The modified version of the original von Bertalanffy model (Xiao 1996) is represented by the equation:

$$L(t) = L_\infty - \left(L_\infty - L(t_0) \right)^{\left(-k(t-t_0) \right)}$$

where $L(t_0)$ = 2.2 mm (Size at hatch for *S. sagax* larvae from Neira *et al.* 1998), and t_0 = 0 decimal years, L_∞ is the asymptotic length predicted by the equation, k is a constant that describes the rate at which the asymptotic length is reached. *VB* models were fitted to a range of different subsets of the length at age data based on sample sources and region.

VB parameters were compared between regions, sexes and years using Kimura's (1980) maximum likelihood methods represented by the equation;

$$X_r^2 = -N.\ln \left(\sigma_\Omega{}^2 / \sigma_\omega{}^2 \right)$$

where – N was the number of observations used in the *VB* growth model, σ_Ω^2 = full model mean residual sum of squares and σ_ω^2 = partial model mean residual sum of squares.

2.2 Results

2.2.1 Larvae

Readability and interpretation

A total of 549 of 625 (88%) otoliths from larvae were aged by counting daily increments. The concentric increments in larval otoliths were readily interpreted at 250-625x magnifications. The area near the primordium was the most difficult to resolve. Figures 2.3a and 2.3b show the microstructure of daily increments in otoliths from larvae.

Age and growth patterns

Larval length at age data were collected between 2000 and 2002 to determine the broad scale patterns of larval growth in South Australian waters (Chapter 3 shows comparison of larval growth rates in upwelling and non-upwelling regions). Larvae ranged in size between 6.51 and 40.11 mm, TL. The mean growth rate for sardine larvae was 0.39 ±0.03 mm.day^{-1}. Instantaneous growth rates ranged from 0.52 mm.day^{-1} at hatch to a maximum of 0.76 mm.day^{-1} at ages of approximately 18 days old. Following this peak, growth rates gradually decreased to ~0.06 mm.day^{-1} as larvae approached transformation at ~40 mm, TL. Fig. 2.4 and Table 2.2 show the Laird Gompertz parameters and instantaneous growth rates for larvae sampled throughout South Australian waters.

Table 2.2. Laird-Gompertz parameters for sardine larvae collected in South Australian waters between 2000 and 2002. Upper and lower 95% C.I.s in italics below parameter estimates.

Laird-Gompertz parameter	Length at age t (a)	Growth rate at t_0 (b)	Exponential decay of growth rate (c)	r^2
South Australian waters Larvae	3.93 *3.35 & 4.51*	0.12 *0.11 & 0.14*	0.06 *0.05 & 0.06*	0.86
Larvae and juveniles	9.17 *8.22 & 10.12*	0.04 *0.03 & 0.04*	0.01 *0.01 & 0.02*	0.90
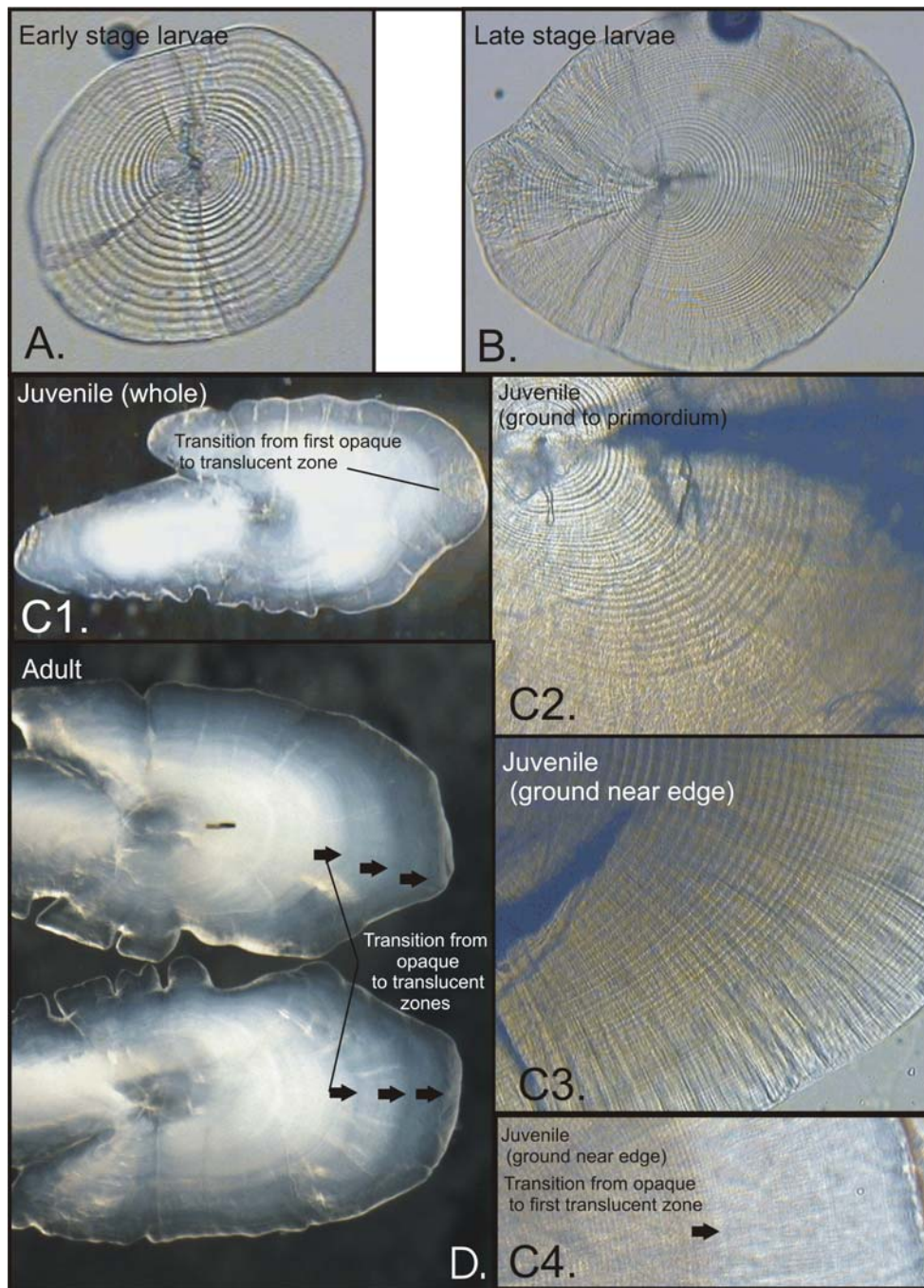
Figure 2.3. **A**. Otolith from early stage larvae at 625x magnification. **B**. Otolith from late stage larvae at 250x magnification. **C1**. Otolith from juvenile at 60x magnification showing the formation of the first translucent zone and the inner opaque zone that represents larval and early juvenile growth. **C2 and 3.** Sections of otolith from a juvenile at 250x magnification. **C4.** Edge of first translucent zone in otolith from juvenile at 250x magnification. **D.** Translucent and opaque zones in otoliths from an adult.
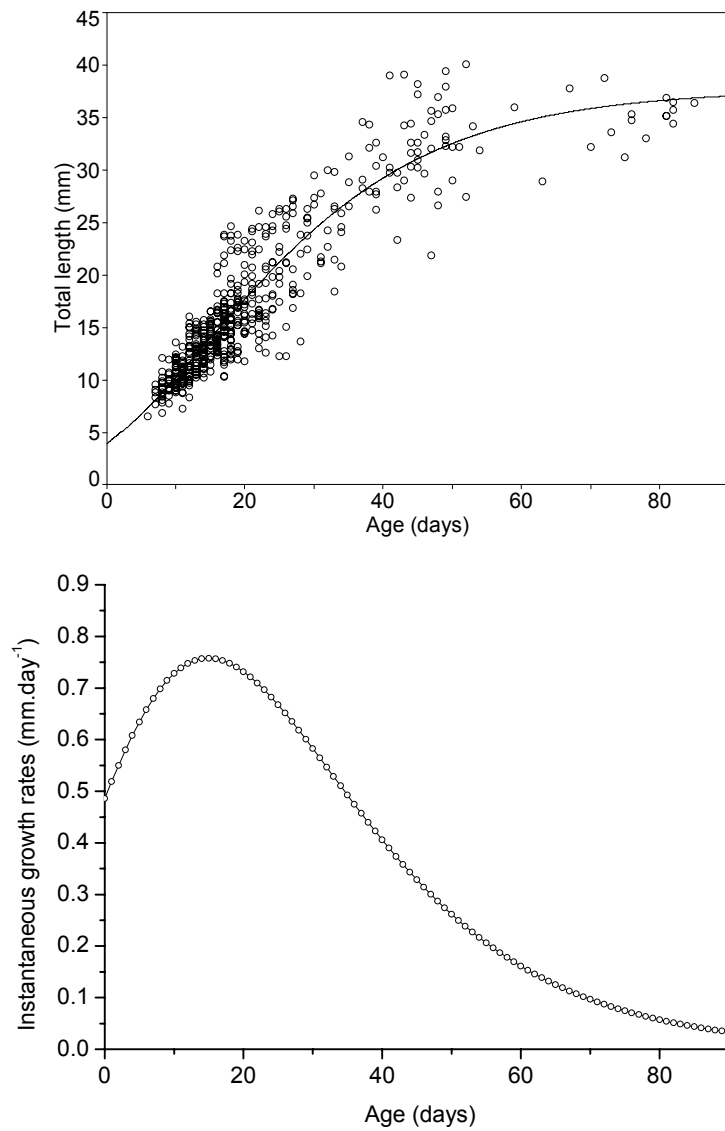
Figure 2.4. Laird Gompertz fit to age and total length for sardine larvae (n = 549) in South Australian waters (top). Instantaneous growth rates over daily intervals (bottom).

2.2.2 Juveniles

Readability and interpretation

Daily increment counts were made for 562 of 667 (84%) otoliths removed from juveniles. Daily increments became increasingly difficult to interpret at ages >200 days, yet counts of up to 306 daily increments were made. Figure 2.3c.1-4 show the structure of increments in ground and unground otoliths from juveniles. The first opaque zone that extends out from the primordium represents the larval and early juvenile growth. In some otoliths this central opaque zone contained a thin translucent zone that coincided with the first winter after hatch or ~3 to 7 months of age (Fig. 2.3).

Age and otolith weight relationships

The relationships between age and otolith weight for juveniles sampled in Spencer Gulf and Gulf St Vincent were significantly different (*ANCOVA, P < 0.05*). Linear regressions predicted that means of 1.06 and 0.73 mg of otolith weight, respectively, were deposited during the first year in otoliths from juveniles in Spencer Gulf and Gulf St Vincent (Fig. 2.5). Although these linear regressions were significantly different, the Spencer Gulf and Gulf St Vincent datasets were combined to determine the mean quantity of otolith weight deposited during the first year (0.86 mg) in otoliths from juveniles. This allowed comparisons with other ageing methods.

Growth patterns

Juveniles ranged in size between 35 and 145 mm, CFL and ages of 62 to 306 days old. The linear relationships between age and length for samples collected in Spencer Gulf and Gulf St Vincent between 2000 and 2001 are shown in Fig. 2.6 (Table 2.4). Juveniles in Spencer Gulf were between 64 and 257 days old. The average somatic growth rate for juveniles (35 to 145 mm, CFL) from Spencer Gulf was 0.44 mm.day^{-1}. Juveniles from Gulf St. Vincent were between 71 and 267 days old. The average somatic growth rate for juveniles (35 to 109 mm, CFL) from Gulf St. Vincent was 0.25 mm.day^{-1}. Average somatic growth rates for juveniles collected in Spencer Gulf and Gulf St Vincent were significantly different (*ANCOVA, P < 0.05*) (Table 2.4).

The maximum instantaneous growth rate determined using the Laird Gompertz model for larvae and juveniles (combined) was 0.59 mm.day^{-1} at 65 days old (Fig. 2.7). Table 2.2 shows the parameter estimates for the Laird Gompertz fit to larval and juvenile life history stages.
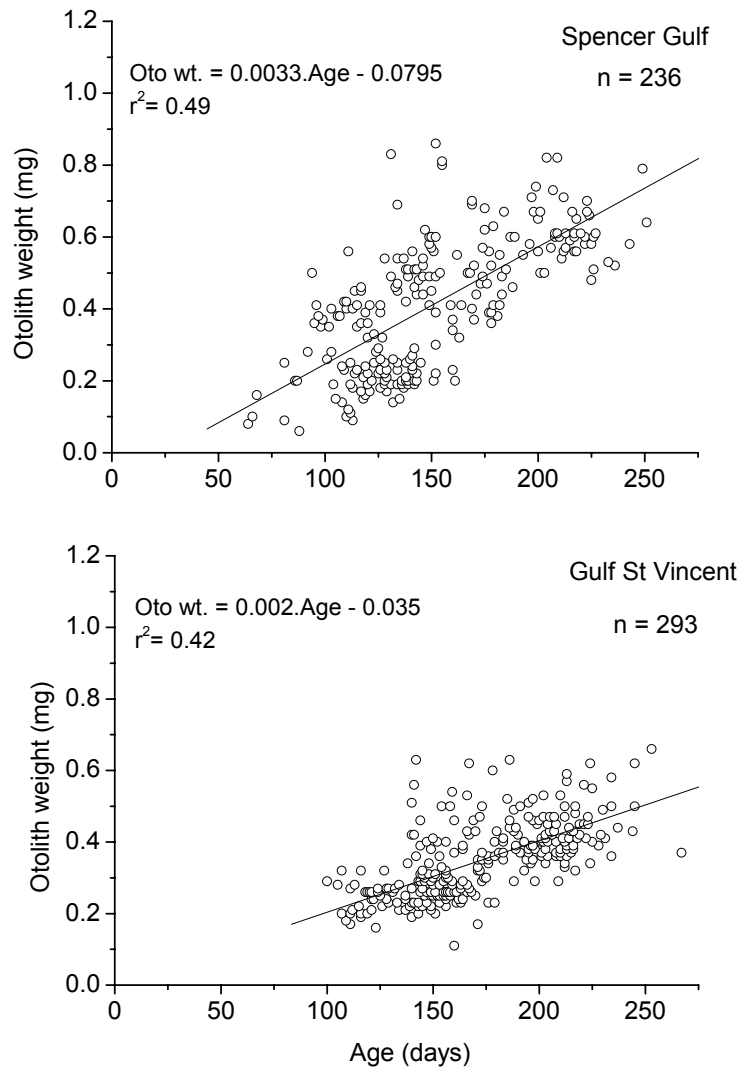
Figure 2.5. Relationship between age and otolith weight for juvenile sardine sampled in Spencer Gulf and Gulf St Vincent in 2000 and 2001.

Table 2.3. Juveniles. A. Regression lines for age and otolith weight (Oto wt) relationships for juveniles sampled in Spencer Gulf and Gulf St. Vincent. B. Comparison between regions - Analysis of covariance (*ANCOVA*) for regression lines in A. *P* values-* significant.

A. *REGRESSIONS*

Region	Relationship	Linear regression equation	r^2	n
Spencer Gulf	AGE – Oto wt.	Oto wt = 0.003.Age – 0.0795	0.49	236
Gulf St Vincent	AGE – Oto wt.	Oto wt = 0.002.Age + 0.035	0.42	293

B. *ANCOVA*

AGE-Oto.wt *F*-ratio	d.f	*P-value*
25.02	1,525	0.00*
143.34	1,528	0.00*
420.31	1,528	0.00*

Table 2.4A-B. Juveniles. A. Regression lines for Age and Caudal–fork length (CFL) relationships for juveniles sampled in Spencer Gulf and Gulf St. Vincent. B. Comparison between regions - Analysis of covariance (*ANCOVA*) for regression lines in A. *P* values -* represents significant (*P < 0.05*).

A. *REGRESSIONS*

Region	Relationship	Equation	r^2	n
Spencer Gulf	AGE - CFL	CFL = 0.44.Age + 20.84	0.71	257
Gulf St Vincent	AGE - CFL	CFL = 0.25.Age + 29.87	0.66	296

B. *ANCOVA*

Source	AGE-CFL *F* ratio	*d.f.*	*P-value*
Slope	84.27	1,549	0.00*
Intercept	613.00	1,552	0.00*
r^2	1,042.81	1,552	0.00*
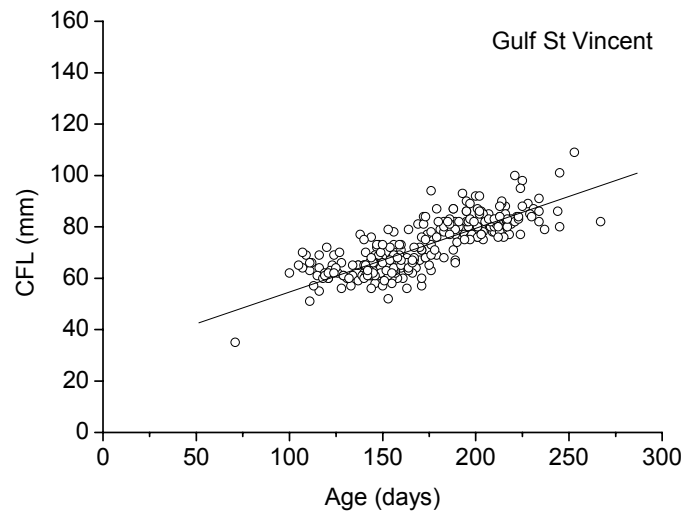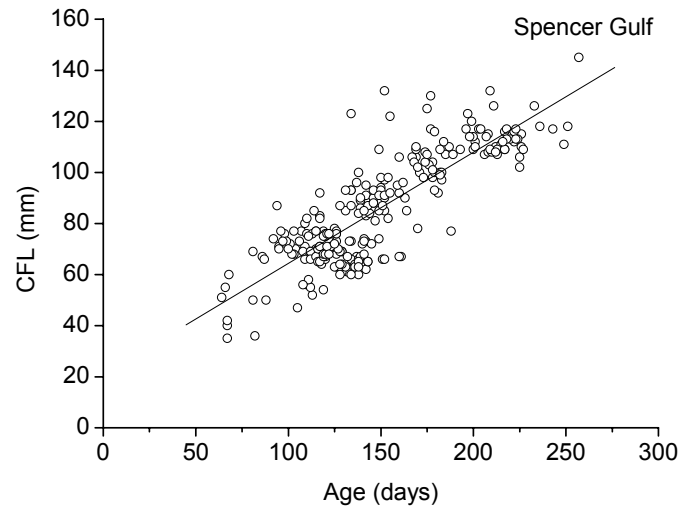
Figure 2.6. Regressions for estimates of size and age of juvenile sardine collected in Spencer Gulf and Gulf St Vincent during 2000 and 2001 (See Table 2.4 for regression equations).
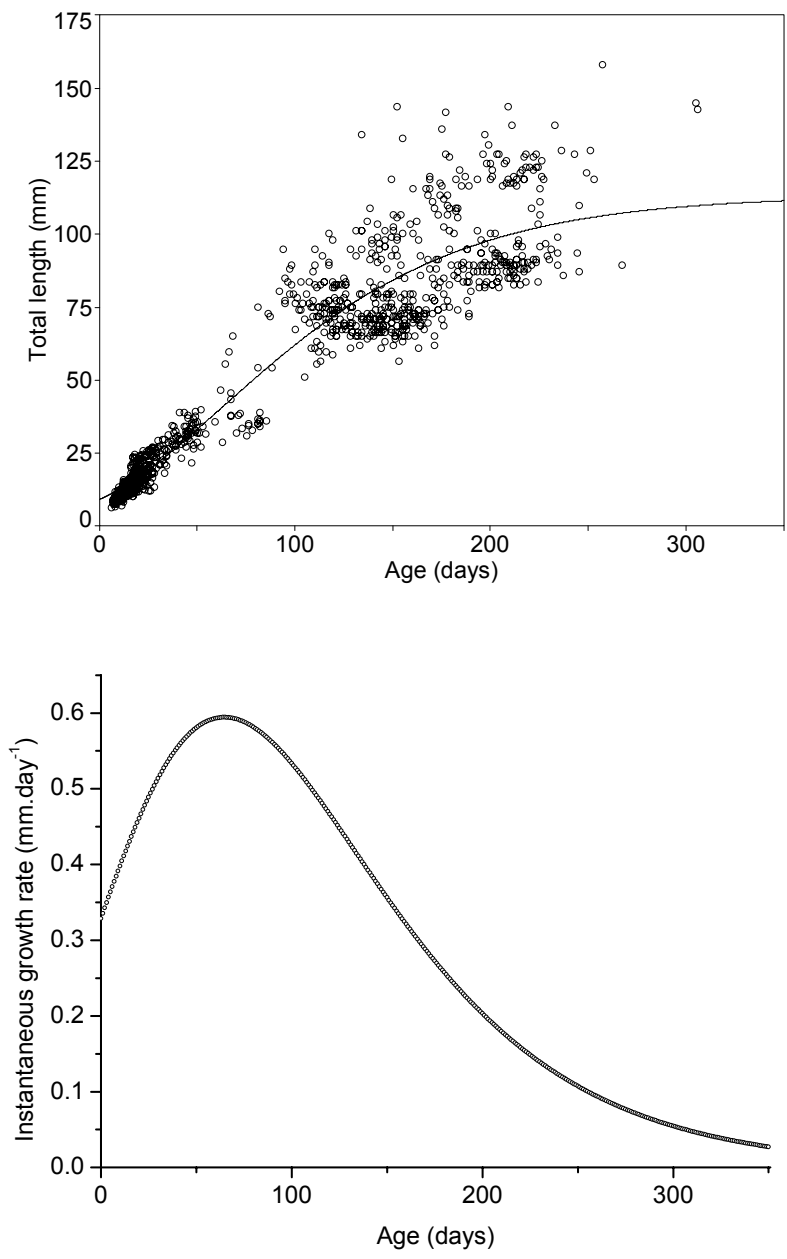
Figure 2.7. Laird Gompertz fit to size at age data for larval and juvenile sardine collected during 2000 and 2002 (top). Instantaneous growth rates for larvae and juveniles (below).

2.2.3 Adults

Age-specific-marginal increment analysis (MI)

Analysis of mean age-specific marginal increments in 2 year olds showed only minor seasonal increases in mean MI occurred between March (0.09) and July (0.12). The lowest mean MIs (0.08) occurred in September (Fig. 2.8). In otoliths from 3 year olds, mean MI increased slightly in March. However, unlike in the 2 year olds, the increase in mean MI continued through mid-winter and peaked in October at 0.08, before decreasing to 0.05 in December.

Both age classes showed minimal change in mean MI throughout the year. The transition between translucent and opaque zones in otoliths was often blurred or contained opaque checks. The presence of these irregularities meant that measurements and calculated MI's were imprecise and unreliable.

Edge analysis

Deposition of translucent edges peaked in June (85%) (winter) and opaque edges were most common in September and October (58-65%) (spring) (Fig. 2.9). Translucent zones were the most common edge type. It was difficult to accurately classify the edge types for some otoliths and these were excluded from the analysis.

Costs and benefits of age determination methods

The estimated costs of ageing large samples of sardine using translucent zone counts and otolith weight-age calibration methods are shown in Table 2.5. Otolith weight-age methods were more cost and time efficient than translucent zone counting techniques. Mean negative bias resulting from using the otolith weight-age method was low (~0.28 years). It is important to note that laboratory times estimated for each method (based on n = 5000 sardine otoliths) are likely to be underestimated as breaks were not included.

Readability indices (RI)

Fig. 2.3d shows the alternating translucent and opaque zones in otoliths from an adult sardine. Of the otoliths used to estimate the age of sardine from the commercial catch (n = 4,047), 15.8%, 70.6% and 11% had readabilities of 2, 3 and 4, respectively (Fig. 2.10). Similarly, of otoliths used to age fishery independent samples (n = 1435), 4.5%, 68.7% and 23.6% had readabilities of 2, 3 and 4, respectively. Only 0.6% of otoliths collected from both sampling sources had the highest readability score of 1 and ~5% were completely unreadable (5s).

There was no significant difference (*ANOVA*, *P > 0.05*) between RI scores assigned to a sub-sample (n = 526) of otoliths aged by independent readers from MAFRI and SARDI Aquatic Sciences (D.S). The second reader (D.S.) was slightly more conservative in assigning the higher readability scores (2 and 3) and his counts were used for subsequent analyses.

Estimation of between reader ageing error

A summary of between reader ageing variation including average biases, average percentage errors (APE), bias corrected APE and the confidence intervals is shown in Table 2.6. Bias corrected APEs ranged between 9.75 and 19.51%, depending on the partitioning of the data-set in terms of agreed RI scores assigned by MAFRI and SARDI agers. The bias corrected APE for all otoliths aged (RI scores of 1-4), was 14.40%. Average between-reader bias for translucent zone counts for all RI scores was -0.56 (D.S.). This was mostly caused by inconsistencies between readers when identifying the first translucent zone.

Age and otolith weight relationships

The linear relationships between decimal age and otolith weight were determined from otoliths assigned RI scores of 1 and 2 in commercial (n = 662) and fishery independent samples (n = 135) (Fig. 2.11). Analysis of covariance showed the slopes and intercepts of the commercial and fishery independent otolith weight-age regressions were significantly different (*ANCOVA*, *P < 0.05*). Mean quantities of otolith weight deposited during the first year of growth were 0.59 and 0.35 mg, respectively, in commercial and fishery independent samples.

To determine the otolith weight-age relationship for the wider South Australian sardine population, data from commercial and fishery independent samples were combined. The mean quantity of otolith weight deposited during the first year of growth described by the broken stick plot was 0.69 mg (Fig. 2.12). Table 2.7 shows the mean amount of otolith weight deposited by each age class between 1995 and 2001 based on the combined regression for adults in Fig. 2.12, otolith wt = 0.23.Age + 0.46. The slope of the regression indicated approximately 0.23 mg of otolith weight is deposited in the otoliths of adults each year after year 1.

39

Decimal age-otolith weight calibration

Otolith weight-decimal age relationships were used to assign ages to 4,047 fish from commercial samples and 1,573 fish from fishery independent samples (n_{total} = 5,620) collected between 1995 and 2001. Linear equations used to calculate ages were, Commercial Age = 1.95.Oto.wt + 0.43, r^2 = 0.63, Fishery Independent Age = 2.46.Oto.wt +1.33, r^2 = 0.67). The mean biases calculated from the algorithm-derived age minus the otolith weight derived age were 0.28 years for both the fishery independent and commercial datasets. This showed the otolith weight-age method underestimated the age of some fish in the older age classes. Age distributions determined using the otolith weight-age method were significantly different to algorithm-derived age distributions in both the commercial (*KS statistic* = 0.28, *P < 0.05*) and fishery independent datasets (*KS statistic* = 0.26, *P < 0.05*).

Table 2.5. Laboratory time estimates, costs and cost ratio comparison of otolith weight-age calibration and whole otolith techniques for assigning ages to sardine

Translucent zone counting method	Lab time per otolith (minutes)	Lab time (hours per 5000 otoliths)	Cost (per otolith)	Cost (per 5000)	Cost ratio
Annulus counting (Read whole)	4.14	345 (9.2 wks)	$1.84	$9,200	
Otolith weight	1.9	158 (4.2 wks)	$0.84	$4,200	**2.2:1**
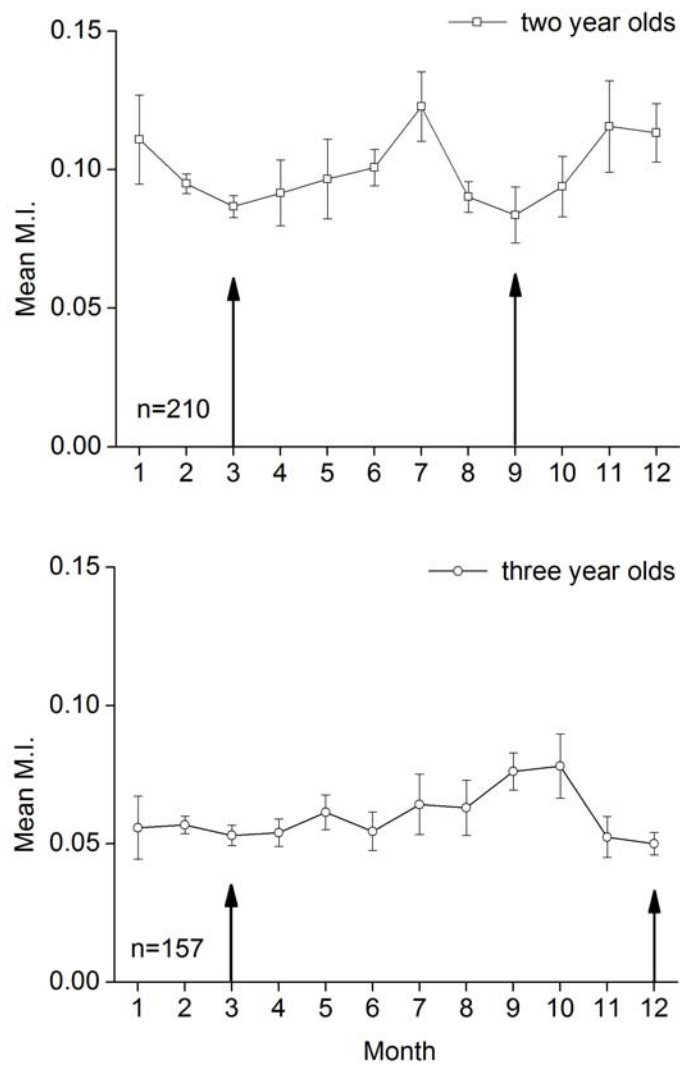
Figure 2.8. Mean age-specific marginal increment (±SE) for whole otoliths with two and three translucent zones. Arrows indicate minimum values.
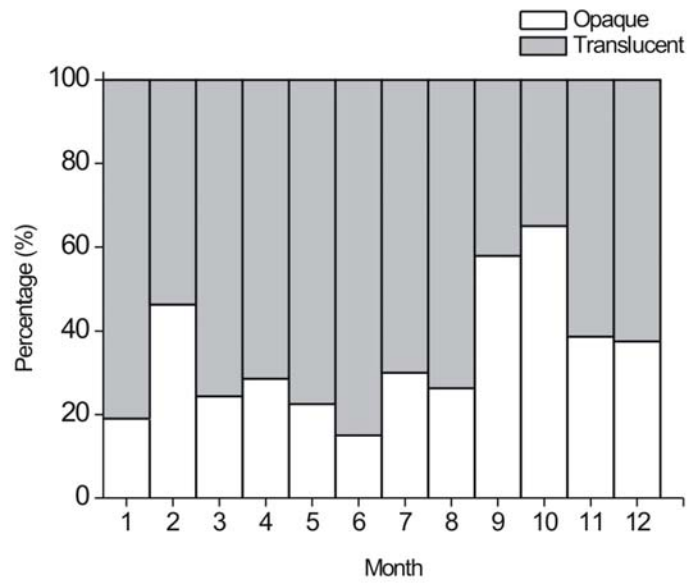
Figure 2.9. Seasonal patterns of edge deposition in a sub-sample of otoliths from adult sardine (n = 467)

Table 2.6. Mean between reader bias, APE, bias corrected APEs and confidence intervals between independent readers for all otoliths and those separated based on agreed readability indices (RI).

Readability Index (RI)	APE (Average percentage error)	Bias-corrected APE (upper and lower 95% C.I.)	Bootstrapped mean (S.E.)	Mean between reader bias (years)	N aged (both readers)
All (1-4)	14.40	14.40 (*14.38 & 14.42*)	14.40 (0.01)	-0.56	526
1&2	19.52	19.51 (*19.47 & 19.53*)	19.75 (0.02)	-0.64	178
3	9.77	9.75 (*9.73 & 9.78*)	9.79 (0.01)	-0.51	152

Table 2.7. Overall otolith weight-age relation for sardine in South Australia based on overall relationship between age and otolith weight for samples collected between 1995 and 2001.

Age (years)	Otolith weight (mg)
1	0.69
2	0.92
3	1.16
4	1.40
5	1.62
6	1.85
7	2.08
8	2.32
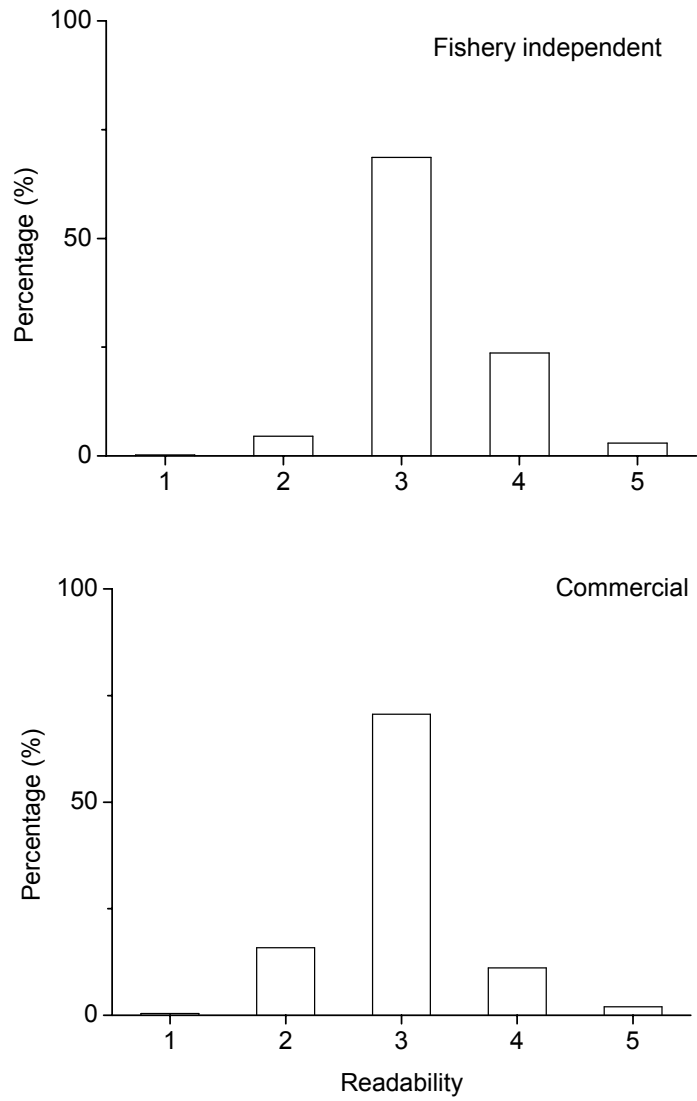
Figure 2.10. Readability index (RI) for otoliths sampled from by fishery independent (n = 1435) methods and from the commercial catch (n = 4047) between 1995 and 2001.
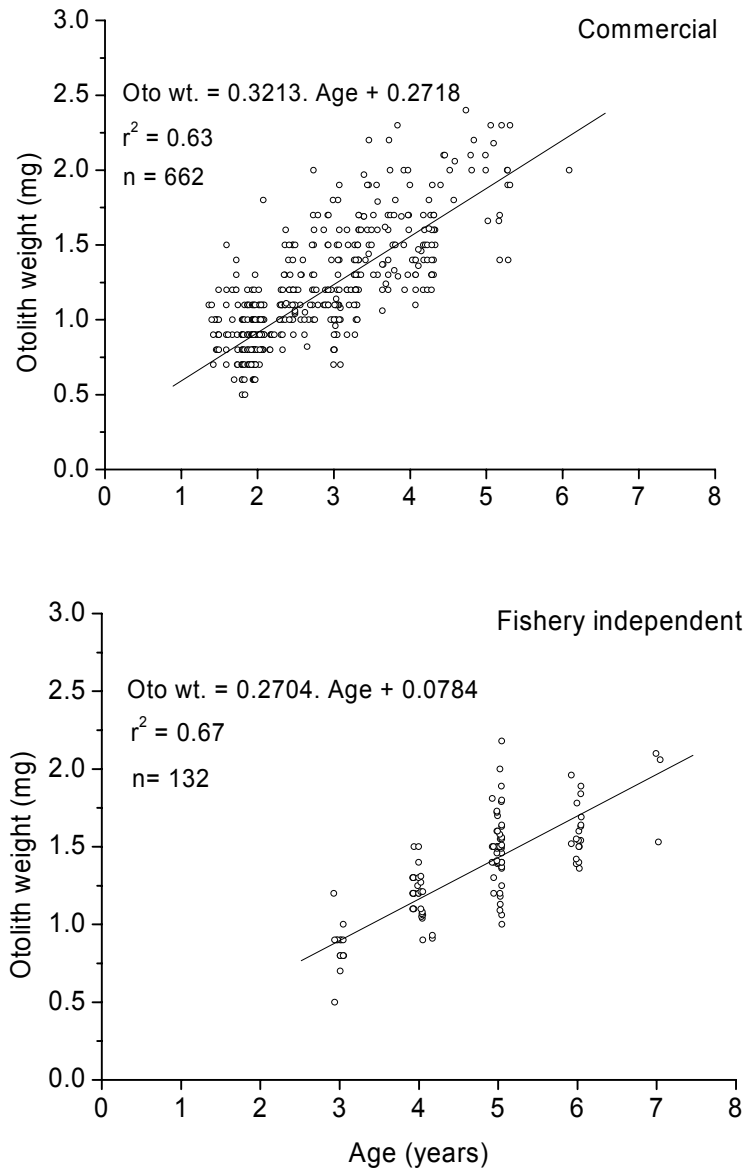
Figure 2.11. Age otolith weight relationships for adults from commercial and fishery independent samples collected between 1995 and 2001 (readabilities of 1 and 2).
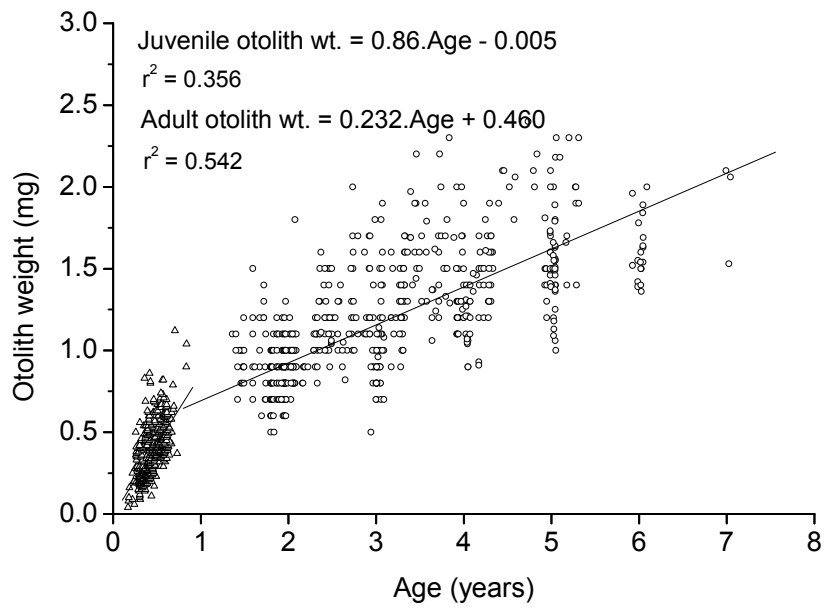
Figure 2.12. Broken stick plot of otolith-weight age relationship for South Australian juvenile and adult sardine between 1995 and 2001 (n = 1335).

Growth patterns

Adult sardine sampled in shelf waters using fishery independent methods were between 2 and 7 years old and most fish were 3 to 6 years old. The magnitude of the von Bertalanffy growth constants reflected the larger size and proportion of older fish in these samples. Von Bertalanffy growth curves are shown in Fig. 2.13. The values for L_∞ and k for fishery independent samples for all years combined (L_∞= 203.05 mm and k = 0.33 yr^{-1}) showed sardine reached a larger asymptotic size in offshore shelf waters than in Coffin Bay and Spencer Gulf (Table 2.8).

Adults collected from commercial catch samples were between 1 and 6 years old and most fish were 1 to 4 years old. Von Bertalanffy growth constants for adult sardine collected in Coffin Bay were L_∞ = 181.54 mm and k = 0.67 yr^{-1}, whereas sardine from Spencer Gulf reached a larger asymptotic size (L_∞ = 192.67 mm) and had lower k values (k = 0.55 yr^{-1}) (Fig. 2.13, Table 2.8). Kimura's (1980) maximum likelihood tests showed there were significant differences between growth curves for samples collected in Spencer Gulf, Coffin Bay and shelf waters between 1995 and 2001 (x^2, P < 0.0001).

The values of the von Bertalanffy growth constants for all sardine life history stages combined were k = 1.05 yr^{-1} and L_∞ = 161 mm (Fig. 2.13, Table 2.8). This suggests the magnitude of the L_∞ estimate was constrained by the larval and juvenile length at age data. A gap in the length at age data occurred between the larger, older juveniles (~0.8 yrs) and the youngest adults (~1.3 yrs). This may have been due to under-estimation of age in late stage juveniles due to difficulties in counting narrow daily increments near the peripheral edge and overestimation of age in some young adults (R. McGarvey pers comm).

Kimura's (1980) maximum likelihood tests showed there were significant differences in growth patterns between sexes in samples collected between 1995 and 2001. Females reached larger asymptotic sizes (L_∞ = 182.22 to 219.19 mm) than males (L_∞ = 178.91 to 194.59 mm) (x^2; P< 0.0001) and had slower growth rates (k = 0.29 yr^{-1} to 0.68 yr^{-1}) than males (k = 0.37 yr^{-1} to 0.69 yr^{-1}) in Spencer Gulf, Coffin Bay and shelf waters (Table 2.8).
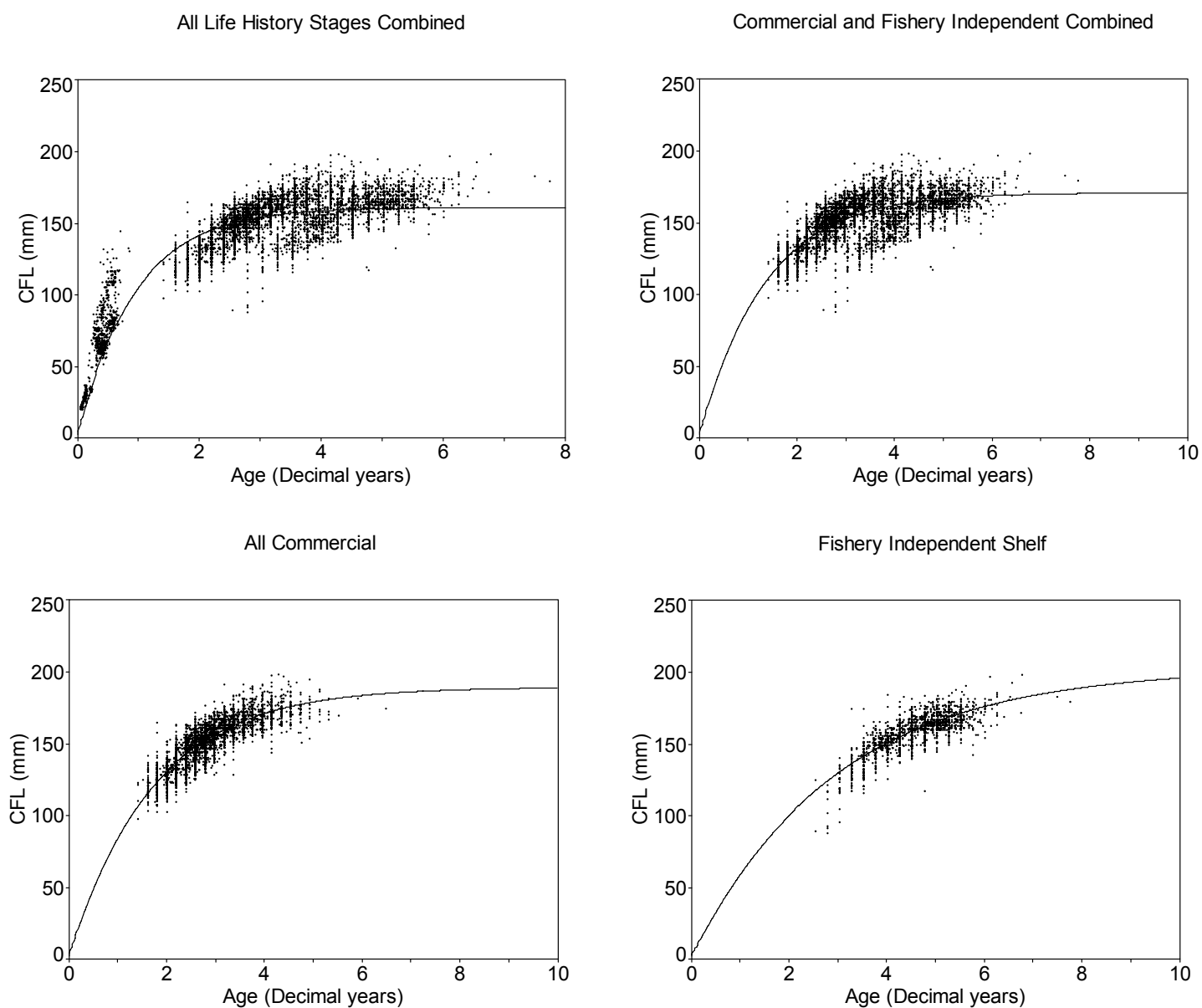
Figure 2.13. Von Bertalanffy growth curves for adult sardine sampled from Coffin Bay and Spencer Gulf (Commercial) and shelf waters of the eastern GAB (Fishery independent).

Table 2.8. Von Bertalanffy growth parameters and *95% C.I.s* for sardine sampled by commercial and fishery independent methods. The correlation coefficients (r^2) for each non-linear fit are shown.

Source	L_∞ (mm)	k (yr^{-1})	r^2	n
All life history stages combined	161.14 (*160.56, 161.72*)	1.05(*1.03, 1.07*)	0.81	6,290
Commercial & Fishery Independent	170.94 (*170.18, 171.69*)	0.74 (*0.73, 0.75*)	0.54	5,620
All Commercial	189.44 (*188.12, 190.75*)	0.58 (*0.57, 0.59*)	0.75	4,047
Fishery Independent Shelf	203.05 (*199.31, 206.8*)	0.33 (*0.32, 0.35*)	0.66	1,435
Fishery Independent Shelf Males	194.59 (*190.74, 198.43*)	0.37 (*0.35, 0.39*)	0.56	1,083
Females	219.19 (*210.67, 227.71*)	0.29 (*0.26, 0.31*)	0.78	340
Commercial Spencer Gulf	192. 67 (*190.94, 194.41*)	0.55 (*0.54, 0.57*)	0.76	3,094
Commercial Coffin Bay	181.54 (*179.57, 183.51*)	0.67 (*0.65, 0.70*)	0.65	953
Commercial Coffin Bay Males	178.91 (*176.04, 181.77*)	0.69 (*0.65, 0.73*)	0.66	402
Females	182.22 (*179.64, 184.79*)	0.68 (*0.64, 0.71*)	0.63	545
Commercial Spencer Gulf Males	183.98 (*181.26, 186.71*)	0.61 (*0.59, 0.63*)	0.68	1,252
Females	194.49 (*192.28, 196.70*)	0.55 (*0.54, 0.57*)	0.77	1,699

2.3 Discussion

This is the first study to describe the age and growth patterns of sardine (*S. sagax*) from all life history stages in Australian waters. Daily increment deposition has been validated in otoliths from larval sardine and these structures were used to estimate the age of larvae and juveniles (Hayashi *et al.* 1989). Daily increment counts for juveniles became less reliable with increasing age (>200 days) due to ambiguities in the otolith microstructure near the outer edge. The outer edge present in sagittae from older juveniles comprises the first translucent zone in sagittae from adults and represents winter growth. The first translucent zone was often difficult to interpret due to inconsistencies in microstructure between the central opaque zone and this first zone. Accurate interpretation of translucent zones in otoliths from adults was only possible for a low percentage of individuals (<20%), which made it necessary to investigate use of otolith weight methods for assigning ages.

Previous age validation studies involving capture and husbandry of clupeoids have resulted in high mortalities and low success rates (Fletcher and Blight 1996; Gaughan *et al.* 2001b; Hoedt 2002). Age-specific marginal increment analysis did not describe the typical seasonal zone deposition pattern observed in other temperate species and was determined to be an unsuitable age validation technique for sardine (Hyndes *et al.* 1992). The weakness of this method for sardine was attributed to difficulties in accurately measuring the marginal increment due to poorly defined margins between consecutive opaque and translucent zones. In a similar study, Gaughan and Mitchell (2000) also found marginal increment analysis was unsuitable for validating the deposition of growth zones in otoliths of the tropical sardine *Sardinella lemuru*.

Preliminary edge analysis showed deposition of translucent edges in sardine otoliths occurred throughout the year with a peak in mid-winter and the opaque edges were most common in spring. Similarly, opaque zones were most common in otoliths from Californian sardine during late spring and translucent zones were dominant during the remainder of the year (Barnes and Foreman 1994). These results should be viewed cautiously as problems associated with subjectivity when classifying these zones and variability in light refraction between otoliths have been recognised in previous studies (Campana 2001). However, the deposition pattern observed for sardine during this study are consistent with findings for other species that occupy southern Australian waters, including King George whiting *Sillaginodes punctata* and the wrasse *Notolabrus fucicola* (Fowler and Short 1998; Ewing *et al.* 2003). This seasonal pattern coincides with increases in SST during spring and higher growth rates in southern Australian temperate waters (Fowler and Short 1998; Rogers *et al.* 2003). South Australian shelf waters are characterised by minimal seasonal

variation in SST due to upwelling events that transport cool water (15-17°C) to the surface layer near the coast between December and April. Offshore shelf waters also remain cool during winter (13-15°C) (McClatchie and Ward accepted). These extended periods characterised by cool surface water may help to explain why translucent edges in sardine otoliths were dominant throughout most of the year and why opaque edges mostly occurred in spring.

When adult sardine collected from the commercial catch and by fishery independent methods were combined, the mean amount of otolith weight deposited during the first year (0.69 mg) was similar to the amount deposited in otoliths from juveniles (0.86 mg). This similarity in otolith weight deposition during the first year is evidence that the two independent methods (daily increment counts and otolith weight-age relationships) led to similar estimates of age. Fletcher and Blight (1996) found otolith weight in adult sardine increased at an average rate of 0.22 mg.year^{-1} in Western Australia, which was identical to findings during this study (0.23 mg.year^{-1}).

The change in rates of otolith weight deposition during the transition from juvenile to adult stages reflected the changes in growth rates that occur during the onset of sexual maturity. The continuum of increasing otolith weight over the lifespan provided further evidence of the likelihood that daily increments and translucent zones in otoliths represent daily and annual time series, respectively. Evidence that otolith structures continue to develop throughout the lifespan can also be observed in the physical changes in otolith microstructure that occur with increasing age (Fig. 2.3), e.g. larval and early juvenile growth is visible as the first opaque zone that radiates out from the primordium in sectioned and whole otoliths from 0+ year old juveniles and adults. Sardine otoliths satisfy the three basic principles described in Fowler and Doherty (1992) necessary for justifying use of these structures for age determination. These are that otoliths must contain interpretable increments that reflect a time scale and continue to develop throughout the lifespan.

The first thin translucent zone present in sagittae from some adults represents the juveniles growth period or the first winter after spawning when sardine are approximately 3 to 7 months old. This first translucent zone was often obscured by ancillary opaque material and our reader consistently did not count this first zone. The first translucent zone was visible at the edge of the large opaque mass (larval and early juvenile period) that radiated out from the primordium and was more likely to represent approximately 13-17 months of age rather than one calendar year. In support of this conclusion, Waldron (1998)

51

found the first translucent zone approximated 1 year of age, but may also represent 13-17 months of age. Similarly, Barnes and Foreman (1994) concluded that there was likely to be significant inter-annual variation in the timing of deposition of opaque and translucent zones in sardine otoliths in response to plasticity in feeding, spawning and movement patterns. It was these issues that led to the development of the algorithm for estimating decimal ages from zone count data. In summary, our findings suggested opaque and translucent zones were suitable for grouping sardine into age classes, once the seasonally and environmentally driven variability in zone deposition have been considered.

Most adult sardine otoliths were assigned RI scores of three (FI = 69%, Com = 71%) and changes in mean between reader biases (-0.56 to -0.64 years) did not directly reflect RI scores assigned by independent readers. Estimates of APE for sardine were high (9.75-19.51%) compared to ageing studies of other fishes and this reflects the difficulties involved in interpreting these otoliths (Campana 2001). In a similar ageing study on sardine in California, APE estimates were of similar magnitude (16%) and agreement between five independent readers was low (31%) (Butler *et al.* 1996). High between-reader error was driven by difficulties in identifying the first translucent zone. This may be due to a lack of discussion of interpretation issues by readers from different agencies and highlights the importance of experience when interpreting sardine otoliths.

Problems experienced when ageing adult sardine and the costs associated with processing large samples led to further investigation of the use of otolith weight for calculating individual ages. Otolith weight has been used extensively for assigning ages to sardine in Western Australia (Fletcher 1991,1995: Fletcher and Blight 1996). Worthington *et al.* (1995) also discussed use of otolith weight-age relationships to calculate age structures for small reef fishes. In addition, Cardinale and Arrhenius (2004) suggested otolith weight methods were suitable for constructing age structures for short-lived species. We adopted the otolith weight-age regression method of Worthington *et al.* (1995), as it was the most suitable and cost effective option. The ages used in these otolith weight-age regressions represented our reader's best estimates and were rated the highest readability scores. Negative bias generated by using the otolith weight-age method as opposed to translucent zone counting methods was low (-0.28 years) and was mostly apparent in the older age classes, which are often difficult to age regardless of the approach used.

Cost-benefit analysis showed the otolith weight-age method was twice (2.2:1) as efficient as traditional translucent zone counting methods. However, it is important that costs of recalibrating the otolith weight-age relationship are considered when comparing the

relative benefits of these methods. Advantages of the otolith weight-age method include a shorter training time for technical staff, no subjectivity and higher precision when ageing difficult/older fish. The main disadvantages of translucent zone counting methods were that only ~20% of otoliths are assigned high RI scores of <3 and these rejected otoliths represented a significant research cost. Identification of the first translucent zone led to confusion between independent readers and represented significant potential for ageing error in sardine. Further investigation of this issue is warranted, as the implications of under-estimating the age of short-lived species such as sardine may be significant.

Significant variation in length at age was apparent during all life history stages of sardine in South Australian waters. Sardine aggregations are highly mobile and are likely to experience a wide range of oceanographic conditions and feeding regimes, which may contribute to this high variability in growth (Misund *et al.* 2003). Maximum growth rates (0.75 mm.day^{-1}) for sardine larvae (6.51 to 40.11 mm, TL) in South Australian waters were higher than those found for sardine larvae in other less productive regions of southern Australia (Gaughan *et al.* 2001a). As found by Gaughan *et al.* (2001a), sardine growth rates during this study were highest during mid-late flexion and declined as they approached transformation to the juvenile stage. A detailed investigation of the effects of environmental factors on sardine larval growth is shown in Chapter 3.

This study represents the first comprehensive investigation of the age and growth patterns and relationships between age and otolith weight for juvenile sardine in Australian waters. A recent study in Western Australia found aggregations of juvenile sardine were difficult to locate due to bad weather and only 23 individuals were collected (Gaughan *et al.* 2001b). During this study, smaller juveniles (40-70 mm, CFL) were elusive, however robust samples were obtained at Outer Harbour in northern Gulf St Vincent and off Corny Point and Port Neill in southern Spencer Gulf. Average growth rates for juveniles were significantly different between southern Spencer Gulf (0.44 mm.day^{-1}) and upper Gulf St Vincent (0.25 mm.day^{-1}), which may be related to the disparate SST regimes in the northern and southern extremes of these gulfs (Rogers *et al.* 2003). Instantaneous growth rates determined from the Laird-Gompertz growth model for *Sardinops caeruleus* (*S.sagax*) in California were higher (0.71 mm.day^{-1}) than those determined for larvae and juveniles (0.59 mm.day^{-1}) during this study (Quinonez-Velazquez 2000). In South African waters, average growth rates of juveniles range between 0.55 and 0.60 mm.day^{-1} (Beckley and van der Lingen 1999). This suggests growth rates of juveniles may also vary in response to localised variation in primary and secondary productivity.

Sardine in South Australian waters reach slightly larger maximum sizes (250 mm, TL) than in other regions of southern Australia, but do not grow as large as those found in more productive regions, including the Benguela, Agulhas and California Current systems where they reach lengths up to 320 mm, TL (Fishbase Website 2004). Spatial, sampling (commercial and fishery independent) and sex-based partitioning of the datasets had a considerable effect on von Bertalanffy parameter estimates. The range of von Bertalanffy parameters suggest growth rates (k = 0.33-1.05 yr^{-1}) and asymptotic sizes (161-219 mm) for sardine in South Australia were higher than in Western Australia (k = 0.58 to 0.81 yr^{-1}, L_∞= 164 to 174 mm) and lower than for sardine in South African (L_∞ = 306 mm, k = 1.50 yr^{-1}) and southern Californian waters (L_∞ = 205.4 mm, k = 1.19 yr^{-1}) (Fletcher and Blight 1996; Butler *et al.* 1996; Beckley and van der Lingen 1999). Kimura's (1980) maximum likelihood tests showed there were significant differences between *VB* curves for sardine in offshore shelf and gulf waters, which may be attributed to the high statistical sensitivity of these tests, sampling biases, actual differences or a combination of these factors.

3.0 EFFECTS OF PRIMARY PRODUCTIVITY ON GROWTH RATES OF LARVAL SARDINE IN THE EASTERN GREAT AUSTRALIAN BIGHT, SOUTH AUSTRALIA.

Note: The data used in this section was collected during Nathan Strong's honours study. This chapter has been submitted for publication in Marine Biology.

<u>Additional Objective:</u> To investigate the effects of primary productivity on growth rates of larval sardine in the eastern Great Australian Bight, South Australia.

Specifically, this section examines the previously published conclusion that the growth rates of larval sardine throughout southern Australian waters are at the lower end of those recorded for this species worldwide, due to low levels of primary production. Observations of patches of cool surface water (~15°C) in coastal waters during February and March 2002 support previous suggestions that coastal upwelling occurs in the eastern GAB during each austral summer and autumn. Elevated concentrations of chlorophyll-*a* (~1.0 μg L^{-1}) recorded in these patches suggest that upwelling enhances primary production. High zooplankton densities (up to 29 ml m^{-3}) to the west of the cool productive patches suggest that the products of upwelling are transported into shelf waters of the eastern GAB by the prevailing south-easterly winds. Elevated densities of zooplankton were also recorded in the mouth of Spencer Gulf, where a frontal system develops during summer and autumn. High larval densities were recorded in shelf waters of the eastern GAB (500-1,000 per 100 m^3) and in the mouth of Spencer Gulf (2,000-8,000 per 100 m^3). Maximum and mean growth rates in upwelling (0.82 and 0.56 ±0.04 mm.day^{-1} respectively) and non-upwelling (0.72 and 0.61 ±0.02 mm.day^{-1}) areas were similar, and higher than those previously reported for southern Australia. Findings suggest that waters of southern Australia are not universally unproductive, and that the growth rates of larval sardine in the world's only northern boundary current system are similar to those in the eastern boundary current systems.

3.1 Introduction

Large populations of sardine (*S. sagax*) occur in the eastern boundary current systems off the west coasts of northern and southern Africa and North and South America. Upwelling enhances primary production in these systems (Mann and Lazier 1996; Schwartzlose *et al.* 1999). A smaller sardine population occurs off southern Australia, where primary production is generally lower than in these eastern boundary current systems (Schwartzlose *et al.* 1999; Gaughan *et al.* 2001). However, recent studies suggest that the spawning biomass of sardine in the eastern Great Australian Bight (GAB, Fig. 3.1) is considerably larger than previously suggested, and has recovered from the effects of mass mortality events in 1995 and 1998 to reach ~290,000 tonnes in 2004 (Ward *et al.* 2004).

Gaughan *et al.* (2001a) suggested that growth rates of sardine larvae in waters off southern Australia are lower than those off the coasts of southern Africa, the Americas and Japan, due to the lower levels of production. However, this conclusion was drawn from specimens collected mainly from the relatively unproductive waters of southern Western Australia (Schwartzlose *et al.* 1999), and from the eastern GAB outside the peak spawning period. Importantly, Gaughan *et al.* (2001a) did not consider the potential for intra-regional variations in levels of primary production or the growth rates of sardine larvae within southern Australia.

Shelf waters between Cape Otway and the Head of the GAB (Fig. 3.1) form part of the world's only northern boundary current system (Middleton and Cirano 2002). Each summer and autumn, cool (~13°C) nutrient-rich water intrudes into shelf waters and intermittently reaches the surface at sites around the western tip of Kangaroo Island and along the western Eyre Peninsula coastline (Lewis 1981; Kämpf *et al.* 2004). Elevated levels of primary production are often associated with these patches of cool surface water, and levels of zooplankton abundance are often enhanced in waters to the west of the main upwelling areas (Ward *et al.* 2002). Frontal systems at the mouth of Spencer Gulf and in Investigator Strait also concentrate planktonic organisms during this period (Bruce and Short 1990). The peak spawning season of *S. sagax* in the eastern GAB coincides with upwelling and formation of frontal systems Ward *et al*. 2001a; 2002, 2004).

Recent studies suggest the northern boundary ecosystem between Cape Otway and the Head of the GAB has oceanographic, biological and ecological similarities to the eastern boundary current ecosystems off southern Africa and the Americas. However, detailed comparison of these regions is impeded by the lack of biological and ecological data for this part of southern Australia. *S. sagax*, which is an important component of the pelagic

communities of both the eastern GAB and eastern boundary current ecosystems, provides a good example of this problem. Considerable information is available on the biology and ecology of *S. sagax* in waters off southern Africa and the Americas (Schwartzlose *et al.* 1999). However, current knowledge of *S. sagax* in southern Australia is based mainly on information from Western Australia (Schwartzlose *et al.* 1999), which is the western edge of the Australian distribution for this species and supports a relatively small part of the Australian population (spawning biomass ~75,000 t, http://www.fish.wa.gov.au/sof/1999). In contrast, few data are available for *S. sagax* in the eastern GAB, which is the centre of the Australian distribution of the species and supports a much larger proportion of the Australian population.

This paper provides *in situ* biological oceanographic data for shelf waters of the eastern GAB and compares the growth rates of larval *S. sagax* in this region with those previously recorded in southern Australia by Gaughan *et al.* 2001a (Western and South Australia combined) and the eastern boundary systems. Specifically, the study examines the hypothesis that the growth rates of larval sardine throughout southern Australian waters are at the lower end of those recorded for this species worldwide, due to the low levels of production.
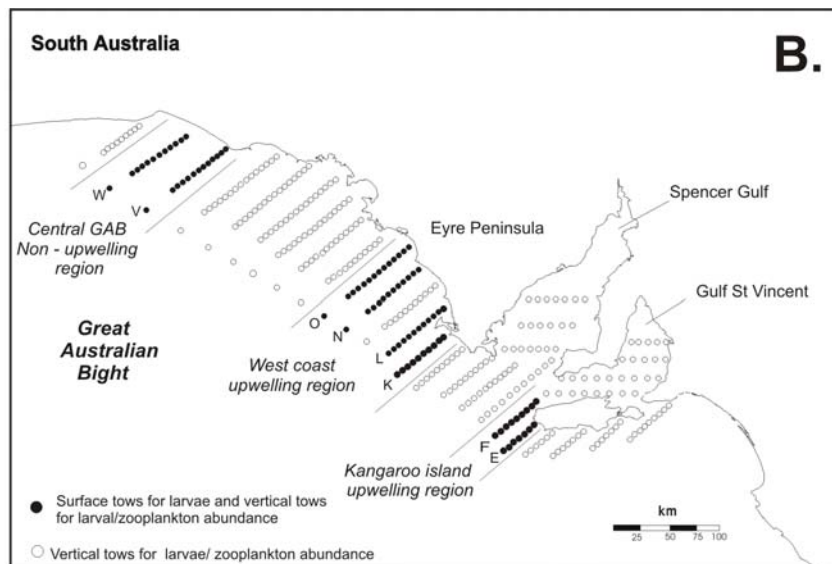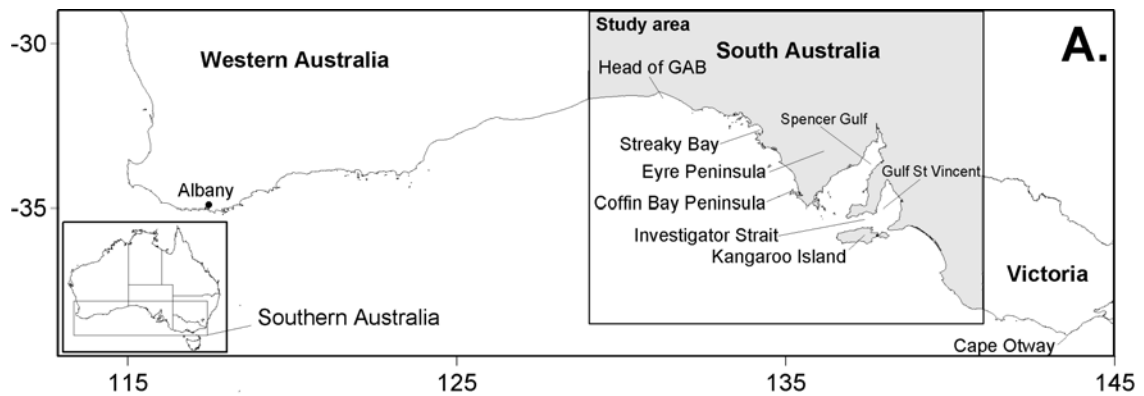
Figure 3.1 A. Locations in southern Australia **B**. Location of stations in South Australia where larvae and oceanographic data were collected during the sardine DEPM cruise in February and March 2002

3.2 Methods

3.2.1. Sample collection and processing

Research cruises were conducted aboard the *RV Ngerin* in shelf waters of South Australia during 1-10 February and 7-21 March 2002 (Fig. 3.1). Sea surface temperature (SST °C) and fluorescence were recorded at each site using a *Sea-Bird* Conductivity-Temperature-Depth (CTD) recorder with a mounted fluorometer. For the purpose of this report fluorescence was used as an indicator of chlorophyll-*a* concentration (μg L^{-1}). Sites were grouped into upwelling and non-upwelling regions based on chlorophyll-*a* concentrations observed during previous research cruises.

The relative abundance of zooplankton and *S. sagax* larvae at each site were determined from samples collected using paired CalVET nets (0.225 m diameter, 355 μm mesh). Nets were deployed vertically to within 10 m from the seabed, or to 70 m in depths >80 m and retrieved at 1m s^{-1}. Samples from the two nets taken at each site were pooled and preserved in 5% formaldehyde. Flow meters in each net were used to estimate the distance towed. The volume of water filtered was calculated by multiplying the distance towed (m) by the surface area of the net (m^2). An index of the abundance of zooplankton at each site was estimated by dividing the settled volume of zooplankton (ml) by the total volume of water sampled (m^3). *S. sagax* larvae were identified under a dissecting microscope (magnification 6.4-40x) using descriptions from Neira *et al.* (1998). Densities of larvae at each site were calculated by dividing the number collected by the total volume of water sampled (m^3).

Larvae used for the growth studies were collected from sites located on eight transects in upwelling areas near the western tip of Kangaroo Island and off the western coast of Eyre Peninsula and a non-upwelling area near Head of Great Australian Bight (GAB) (Fig.3.1). Paired bongo nets (0.6 m diameter, 500 μm mesh) were towed along the surface for 10 minutes at 4 knots. Samples from each net were pooled and preserved in 70% ethanol. The total length (TL) of each larva was measured to the nearest 0.01 mm using electronic callipers.

3.2.2. Otolith preparation and age determination

Sub-samples of 25-30 larvae from sites with >25 specimens were used for age determination. Larvae were re-hydrated in distilled water. Otoliths were removed using fine needles under a dissecting microscope, air-dried and fixed whole to a slide with a clear resin. A compound microscope (magnification 250-625x) and image analysis software

were used to examine otoliths. It was assumed that concentric increments in otoliths (Fig. 3.2) were deposited with daily periodicity, based on a previous validation study on *S. sagax* (Hayashi *et al.* 1989). Only otoliths with clear increments were included in the analysis. Three counts were made along the longest axis of each otolith from the primordium to the posterior edge. The mean of the increment counts were used in the analysis if replicate counts varied by <5%. The first increment in *S. sagax* is deposited 2.5-3 days after hatching so two days was added to each count to estimate the post-hatch age of larvae (Hayashi *et al.* 1989).

3.2.3. Growth rate

The relationship between TL and age was initially examined using three methods: the generalized von Bertalanffy growth function, the Laird-Gompertz form of the von Bertalanffy growth function, and a power function. Examination of the residual sums of squares showed that the Laird-Gompertz growth function provided the best fit to the data. This model was also used by Gaughan *et al.* (2001) and has the form shown in equation 1:

$$L(t) = a \exp\left(\frac{b}{c}\left[1 - \exp\left(-c\left(t - t_0\right)\right)\right]\right) \quad (1)$$

where $L(t)$ is the length of an average larva at age t, *a* is its length at age t_0, *b* is the specific rate of growth at age t_0, and *c* is the instantaneous rate of decay of the growth rate. The rate of somatic growth was calculated as the first derivative of *L(t)* with respect to age *t* and has the form shown in equation 2:

$$\frac{dL(t)}{dt} = b \exp\left(-c\left(t - t_0\right)\right)L(t)$$
$$= \left\{b - c \ln\left(\frac{L(t)}{a}\right)\right\}L(t) \quad (2)$$

3.2.4. Effect of environmental factors on growth rate

The effects of surface chlorophyll-a concentration, SST, zooplankton density and age on larval length were explored using a general linear model. Following this exploratory analysis, the data were fitted to a general linear model to calculate the rate of larval growth. Use of a general linear model to calculate the rate of larval growth is justified because for the narrow range of larval ages, a linear model approximates a nonlinear growth model, which describes growth better over a wider range of ages.

3.3 Results

3.3.1. Environmental factors

SST ranged from 15.3 to 22.2°C (Fig. 3.3). Low SSTs were mostly recorded off the western tip of Kangaroo Island and along the southern coast of the western Eyre Peninsula as far west as Streaky Bay (Figs 3.1 and 3.3). High SSTs (>19°C) were recorded in central Spencer Gulf, Gulf St Vincent and offshore waters of the central GAB (Fig. 3.3).

Surface chlorophyll-*a* concentrations ranged from 0.07 μg L^{-1} to 1.06 μg L^{-1} (Fig. 3.4) and generally declined with increasing distance from shore. Highest levels of chlorophyll-*a* were recorded off the south-western coast of Kangaroo Island and along the coast of the western Eyre Peninsula, within patches of water with low SSTs.

Densities of zooplankton ranged from approximately 0.5 ml m^{-3} to 29 ml m^{-3} (Fig. 3.5). High estimates of relative zooplankton density were mostly recorded sites with intermediate water temperatures, lying to the west of sites with low SST and elevated chlorophyll-*a* levels. High zooplankton densities were also recorded in southern Spencer Gulf.

3.3.2. Larval density

High densities of larvae (per 100m^{3}) occurred in areas between the Head of the GAB and Kangaroo Island (Fig. 3.6). The highest densities were recorded in the mouth of Spencer Gulf and in Investigator Strait within patches of water with high chlorophyll-*a* concentrations and zooplankton densities.

3.3.3. Age and growth patterns

Larvae collected from the upwelling areas were between 6 and 27 days old and between 6.51 and 24.1 mm, TL; most were 14 to 24 days old and 10 to 25 mm, TL (Fig. 3.7 and 3.8). Larvae collected from the non-upwelling area near Head of the Bight were 7 to 24 days old, and from 7 to 18 mm TL; most larvae were 10 to 18 days old and 10 to 16 mm, TL (Fig. 3.7 and 3.9).

The growth parameters in upwelling and non-upwelling areas are shown in Table 3.1. The maximum instantaneous growth rates for larvae from the upwelling regions was 0.82 mm.day^{-1} at 8 days old, compared with 0.72 mm.day^{-1} at 8 days of age for larvae from the non-upwelling region. The mean growth rates (±se) in the upwelling and non-upwelling regions were 0.61 ±0.02 mm.day^{-1} and 0.56 ±0.04 mm.day^{-1}, respectively

3.3.4. Effect of environmental factors on growth rate

SST and surface chlorophyll-*a* concentration and larval age significantly affected the rate of larval growth. For a particular age, the length of larvae increased linearly with SST and surface chlorophyll-*a* concentration. For a particular value of SST or surface chlorophyll-*a* concentration, fish length always increased linearly with age. Thus, a generalized linear model that incorporates the interactions of age with SST and surface chlorophyll-*a* concentration was appropriate for analysing these data. This model indicated a significant interaction of age with SST, region and surface chlorophyll-a concentration (Table 3.2). The GLM and its parameters estimates in Table 3.3 are represented by equation 3:

$$L = \{a(\text{region}) + b\text{SST} + c(\text{surface chlorophyll-}a)\} \times \text{age} + 5.92 \qquad (3)$$

where *L* is the total length of the larvae, *a* is the effect of region, *b* is the effect of SST, and *c* is the effect of surface chlorophyll-*a* concentration on length.

The first derivative of the equation for total length with respect to age (equation 4), as produced using the GLM was used to show growth rate dependent of SST and surface chlorophyll concentrations in three-dimensions (Figs 3.10 and 3.11).

$$\frac{dL}{d\text{age}} = a(\text{region}) + b\text{SST} + c(\text{surface chlorophyll-}a) \qquad (4)$$

Temperature and surface chlorophyll-*a* values were taken from Table 3.4. The growth rates derived from the model using SST and surface chlorophyll-*a* for the upwelling region (Fig. 3.10) were higher than in the non-upwelling region in the central GAB (Fig. 3.11). In all cases, the growth rate of larvae was highest at the maximum SST and chlorophyll-*a* concentration.
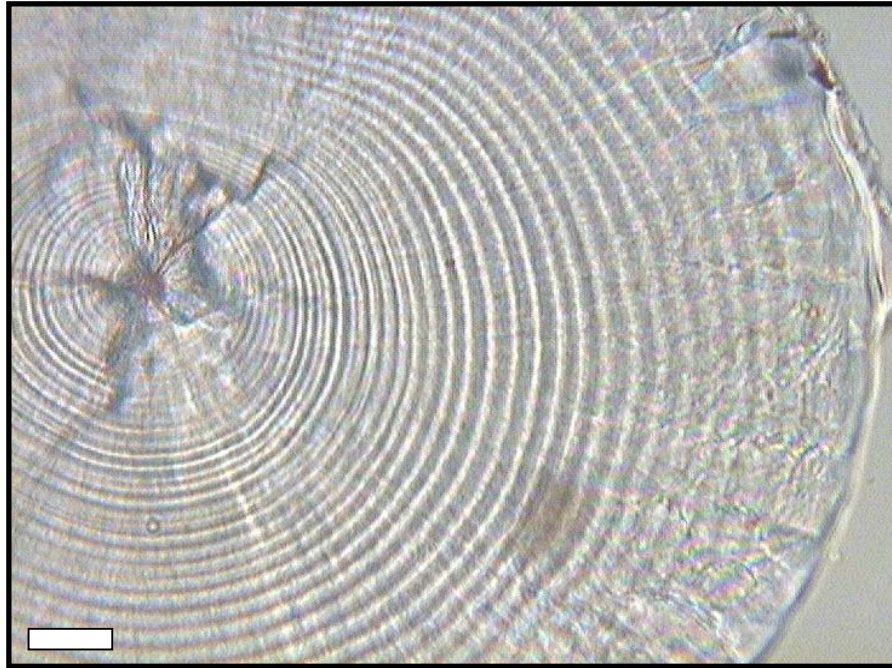
Figure 3.2. Sagitta from sardine larvae showing the microstructure of the daily increments at 625x magnification (*scale bar* = ~10 μm)
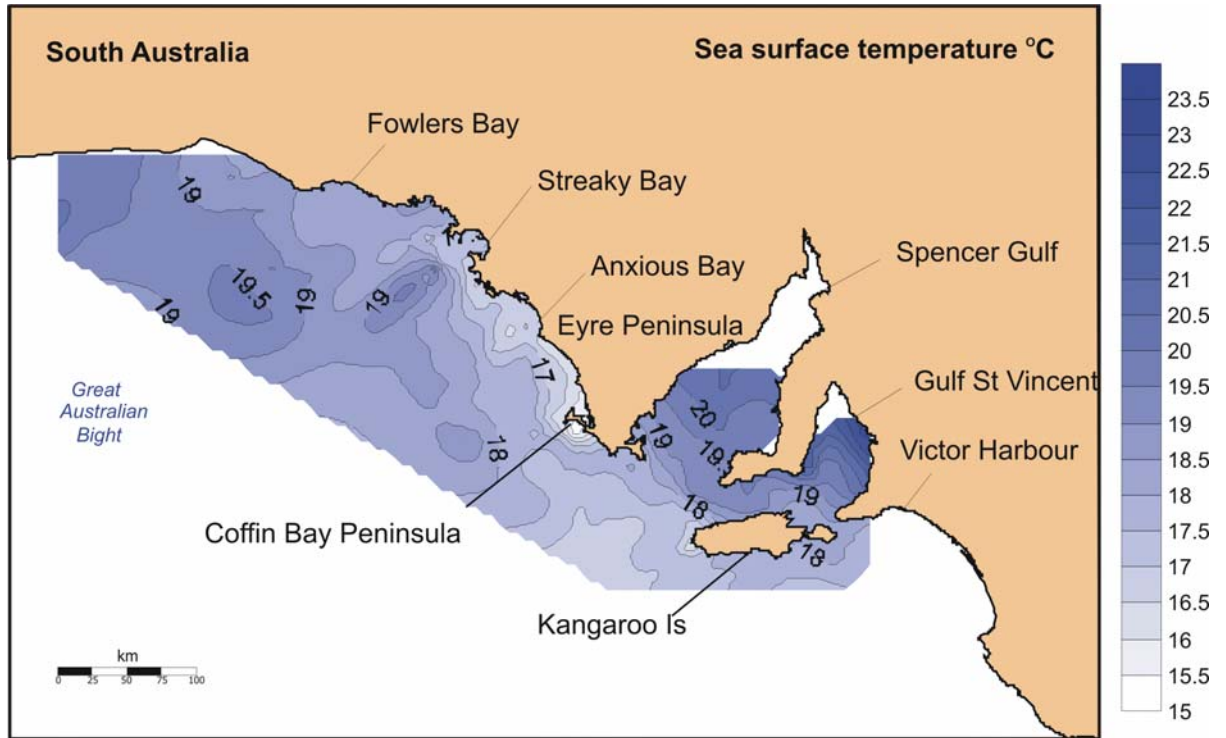
Figure 3.3. Sea surface temperatures (^{o}C) based on *in situ* CTD measurements from the *RV Ngerin* in 2002
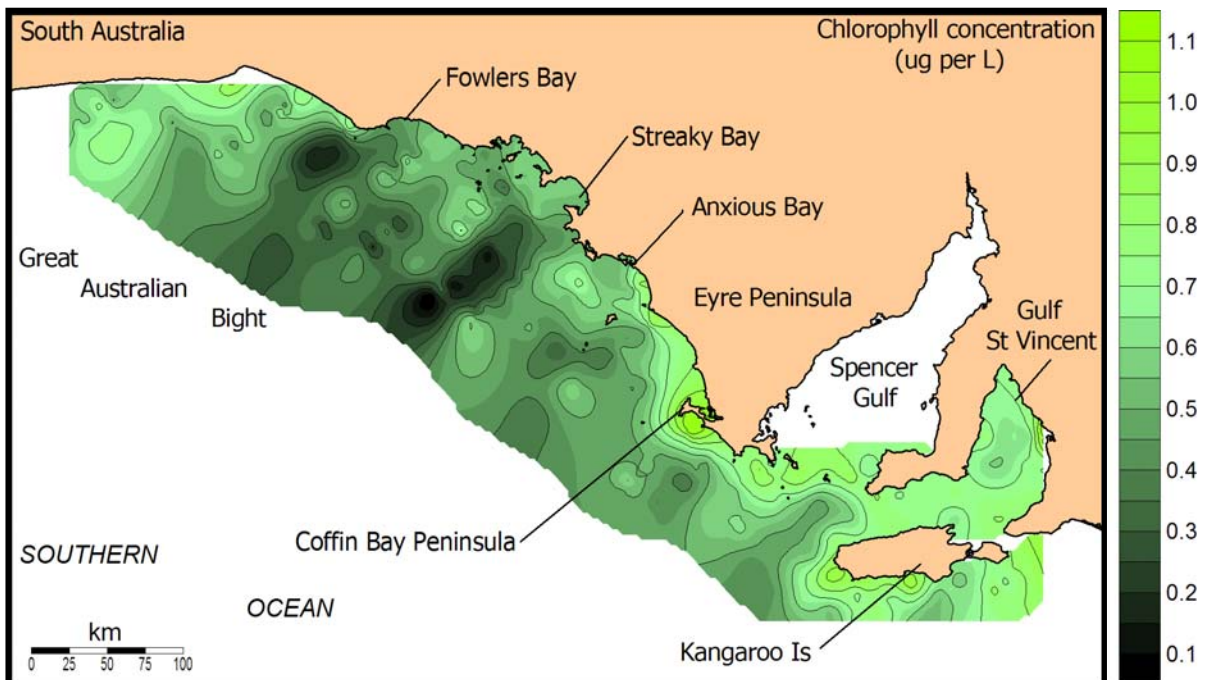
Figure 3.4. Surface chlorophyll-*a* concentrations (µg.litre^{-1}) based on *in situ* measurements using the CTD mounted fluorometer in 2002
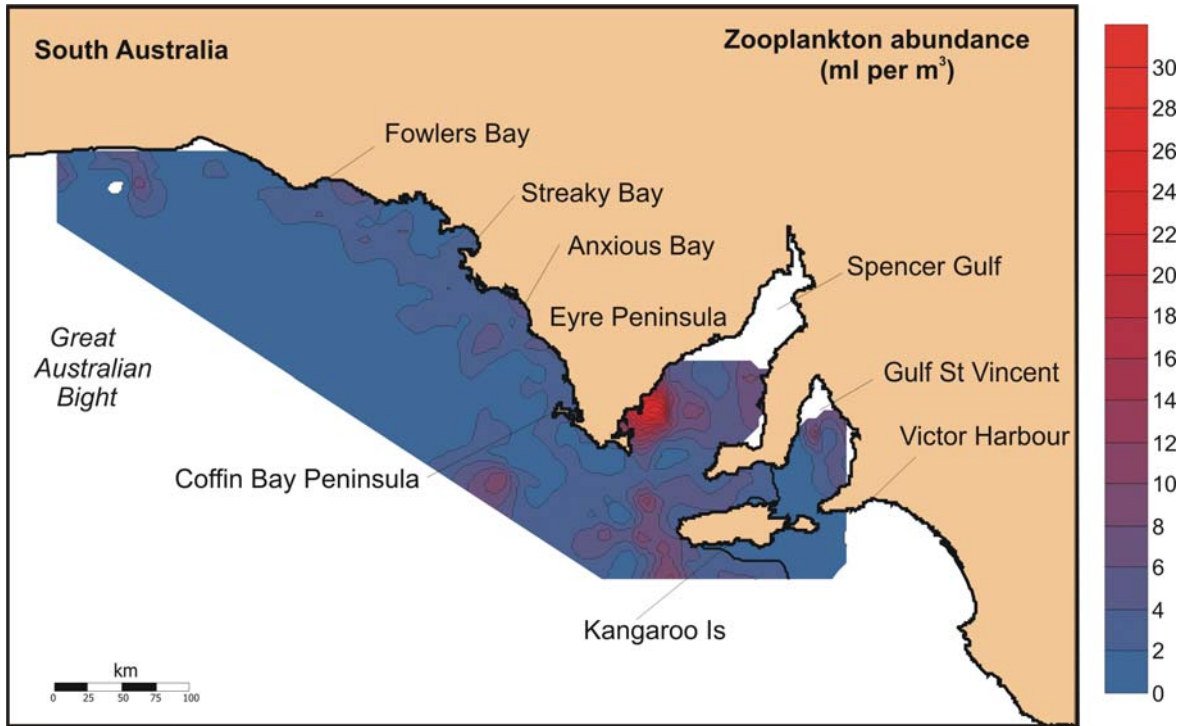
Figure 3.5. Zooplankton abundance (ml per m^3) based on samples collected from the *RV Ngerin* in 2002
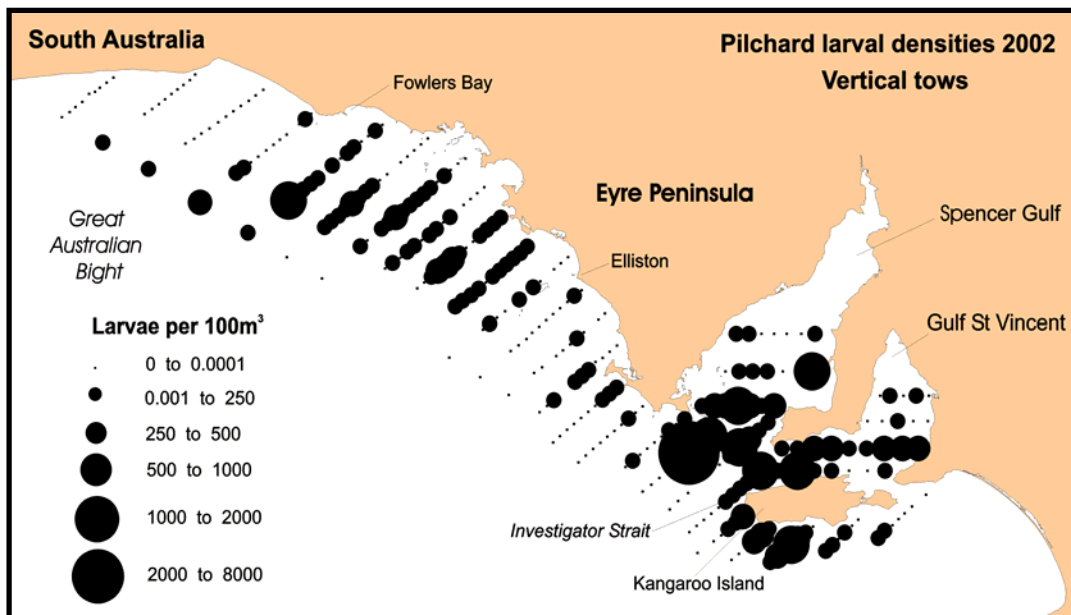
Figure 3.6. Density of larvae (per 100m^3) in the eastern GAB in 2002
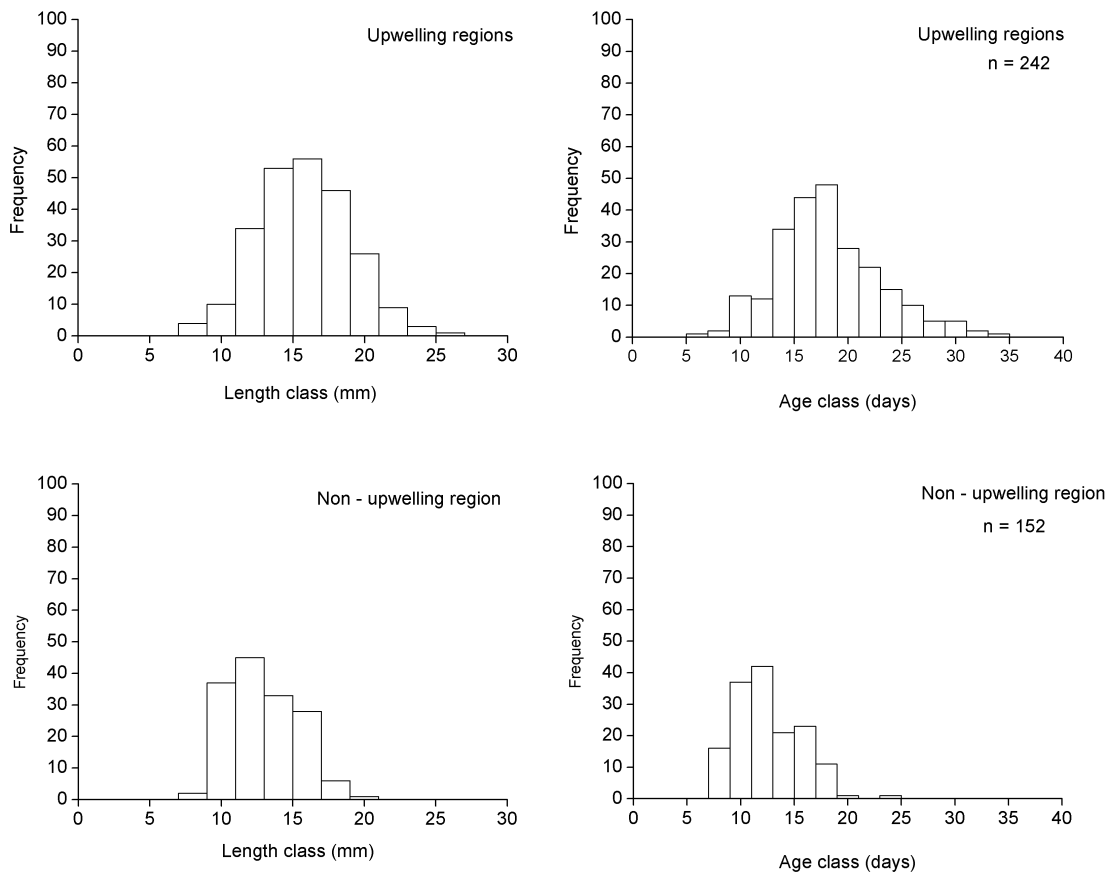
Figure 3.7. Size and age distributions for sardine larvae in the upwelling and non-upwelling regions
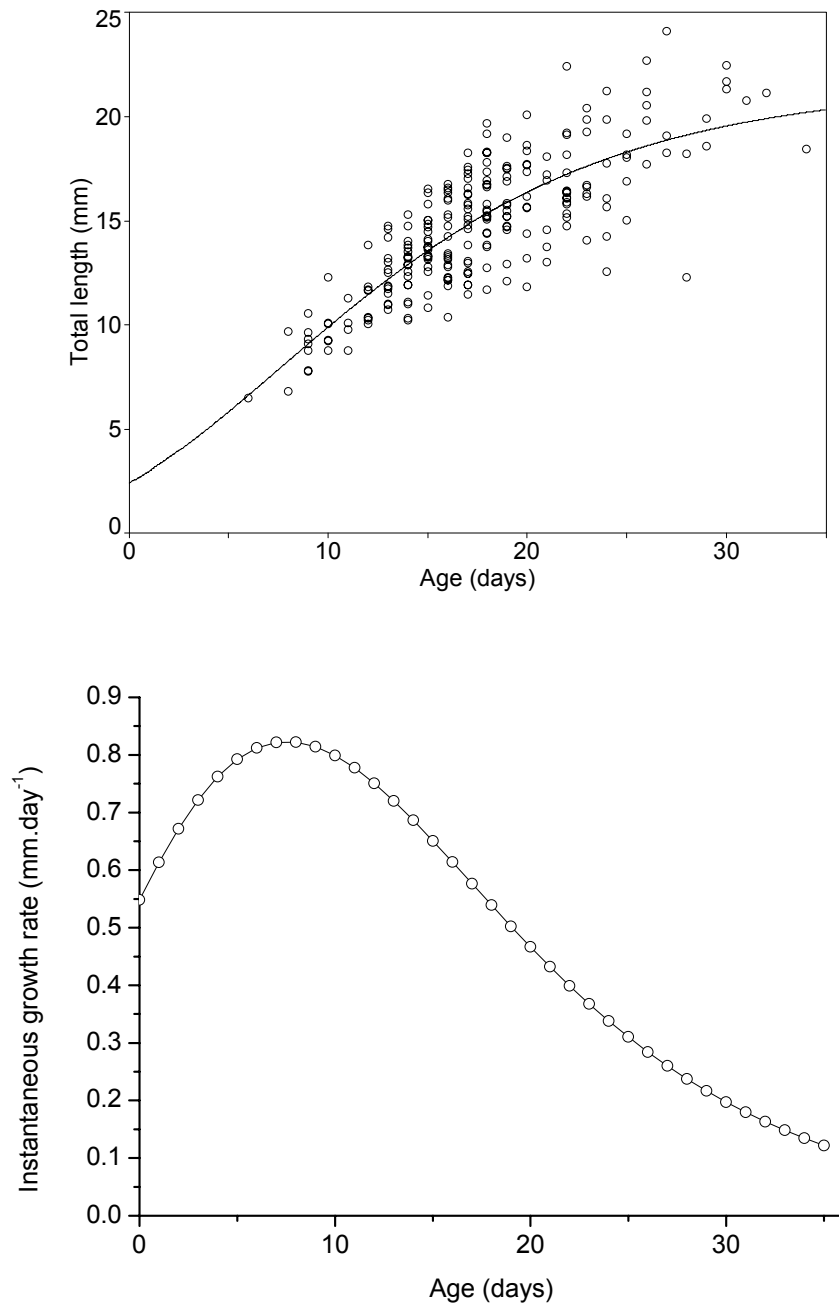
Figure 3.8. Laird Gompertz fit to age and total length for sardine larvae (n = 242) sampled in the upwelling regions off Kangaroo Island and in the eastern Great Australian Bight. Instantaneous growth rates over daily intervals
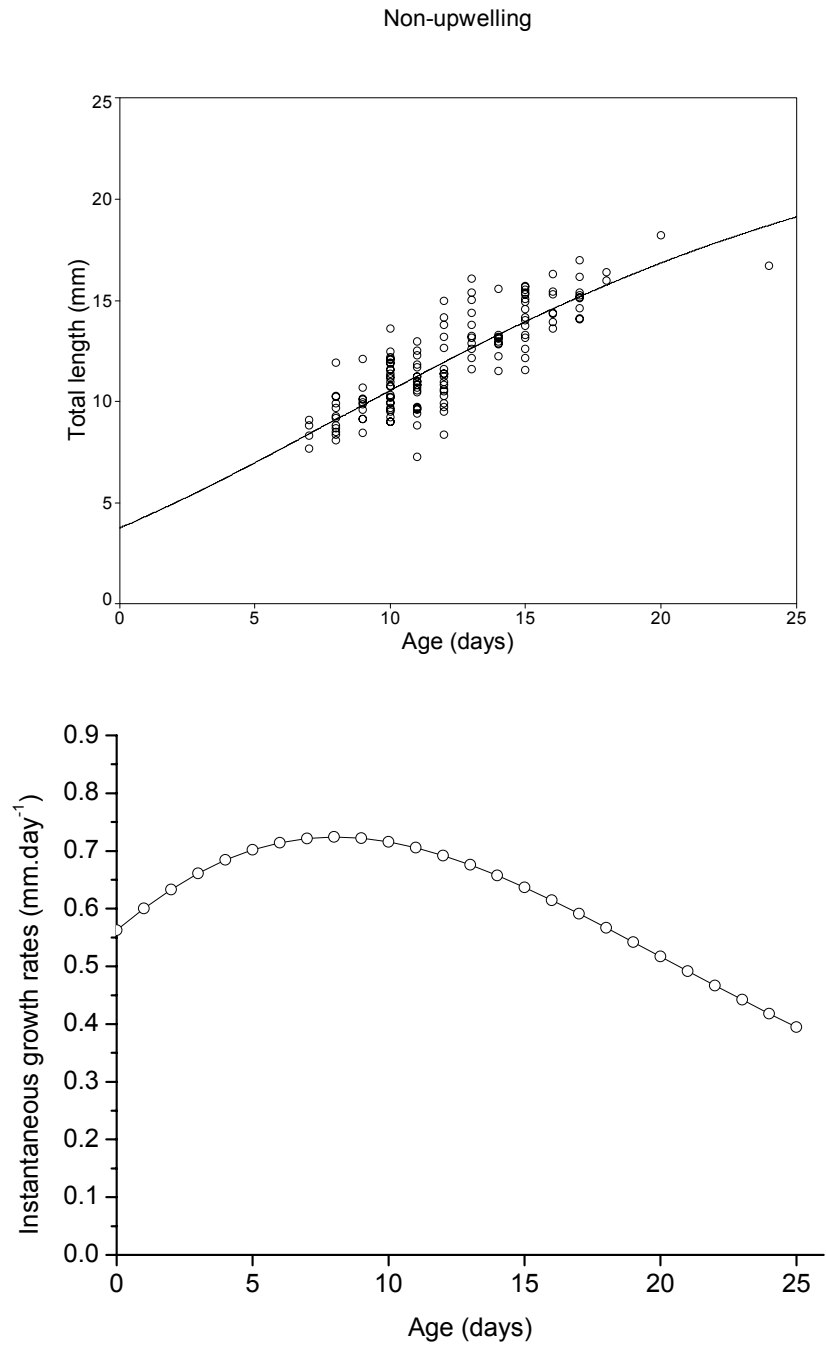
Non-upwelling

Figure 3.9. Laird Gompertz fit to age and total length for sardine larvae (n =152) sampled in the non-upwelling region in the central Great Australian Bight. Instantaneous growth rates over daily intervals
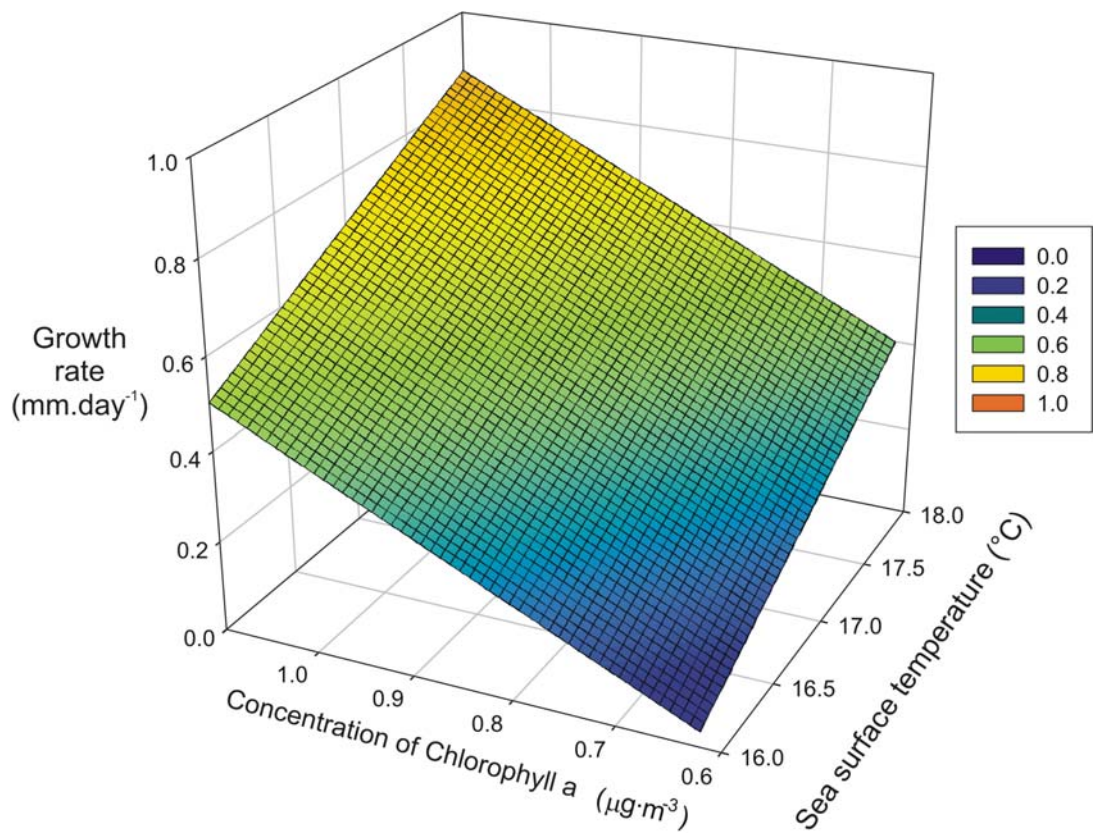
Figure 3.10. Three-dimensional plot of the growth rate of larvae dependent on SST (°C) and concentrations of surface chlorophyll-*a* (µg m^3) from upwelling regions. Colour scale = growth rate (mm.day^{-1}).
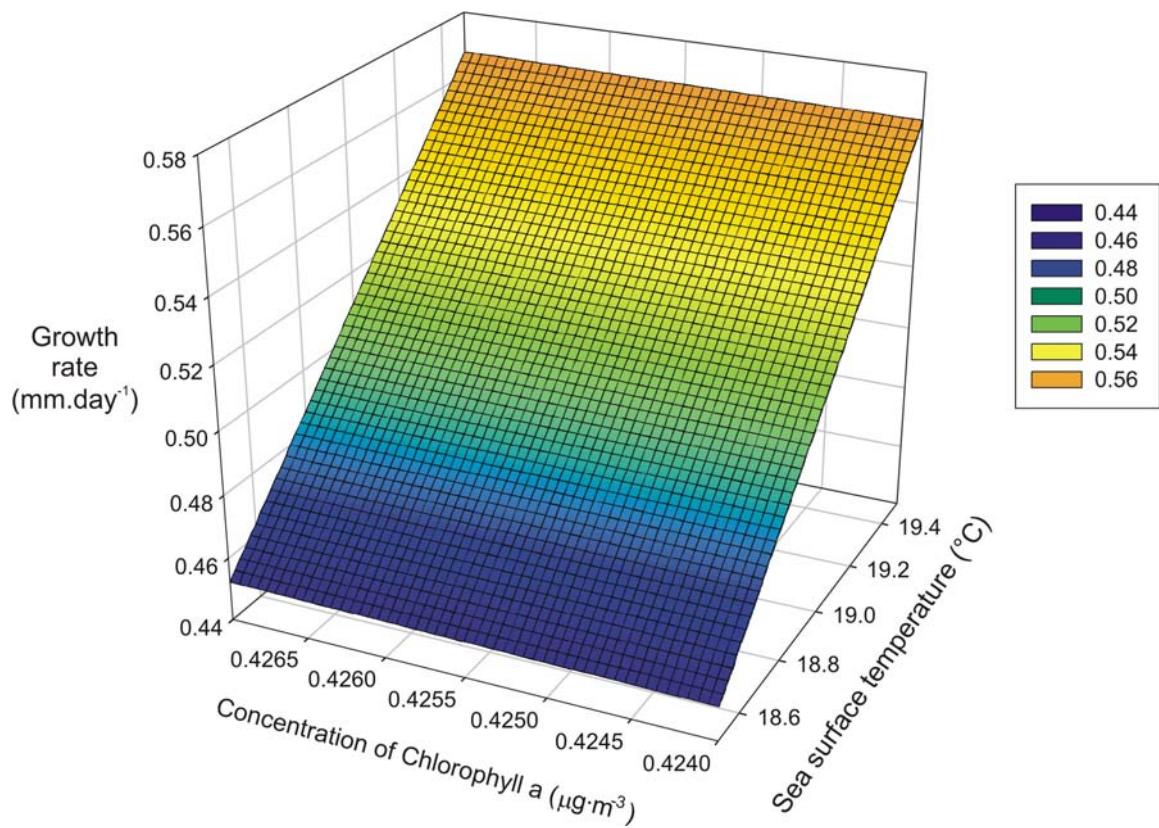
Figure 3.11. Three-dimensional plot of the growth rate of larvae dependent on SST (°C) and concentrations of surface chlorophyll-*a* (µg m³) from non-upwelling region. Colour scale = growth rate (mm.day^{-1}).

Table 3.1. Laird-Gompertz parameters for sardine larvae collected in upwelling and non-upwelling regions in 2002. Upper and lower 95% C.I.s in italics below parameter estimates. ** Parameter estimates (±s.e.) from Gaughan *et al.* 2001

Laird-Gompertz parameters	Length at age t (a)	Growth rate at t_0 (b)	Exponential decay of growth rate (c)	r^2	n
Upwelling region	2.41 *0.822 & 3.995*	0.23 *0.10 & 0.36*	0.10 *0.072 & 0.14*	0.65	242
Non upwelling region	3.80 *1.918 & 5.595*	0.15 *0.05 & 0.25*	0.079 *0.026 & 0.13*	0.70	152
Regions combined (current study)	3.36 *2.315 & 4.404*	0.17 *0.11 & 0.23*	0.091 *0.07 & 0.11*	0.72	394
Southern Australia**	1.8 (0.31)	0.198 (0.03)	0.080 (0.01)	0.98	240

Table 3.2. Results from the General Linear Model (Type III sum of squares) for factors that had significant effects on growth rate.

Source	d.f.	Type III SS	MS	*F* - value	*P > F*
AGE*Region	2	221.39	110.69	49.57	**<0.0001**
AGE*SST	1	262.76	262.76	117.66	**<0.0001**
AGE*Surf Chlorophyll -*a*	1	303.06	303.06	135.70	**<0.0001**

Table 3.3. Parameter estimates and error for each region, SST and Surface Chlorophyll-*a* for equation 3.

Parameter	Estimate	Error
Intercept	5.93	0.31
Age*Region (Non-upwelling)	-2.68	0.29
Age*Region (Upwelling)	-2.67	0.28
Age*SST	0.15	0.01
Age*Surf Chlorophyll-*a*	0.81	0.07

Table 3.4. Environmental data summarised for each region as used in the GLM of age and length for sardine larvae (See Table 10) (mean ±s.e.)

Environmental variable	Non upwelling	Upwelling
Surf SST	19.38 (0.02)	17.01 (0.04)
Min water T	18.80 (0.02)	14.96(0.04)
Surf Chlorophyll-*a* µg/L	0.43 (0.01)	0.82 (0.01)
Min Chlorophyll - a µg/L	0.42 (0.01)	0.74 (0.09)
Zooplankton den ml.m^3	1.22 (0.04)	3.35 (0.17)
Depth (m)	61.66 (0.72)	56.68 (1.02)

3.4 Discussion

The maximum growth rates of sardine larvae recorded during this study in both upwelling (maximum = 0.82 mm.day^{-1} at 8 days old) and non-upwelling areas (maximum = 0.72 mm.day^{-1}) of the eastern Great Australian Bight were higher than those reported for southern Australia combined (maximum = 0.62 mm.day^{-1} at 9 days of age by Gaughan *et al.* 2001a). This difference in peak larval growth rates within southern Australia presumably results from within region differences in levels of primary and secondary production, and hence the quantities of food available for larval sardine. In Western Australia, where >50% of the larvae analysed by Gaughan *et al.* (2001a) were collected, no large-scale, predictable nutrient enrichment processes are known to occur (Schwartzlose *et al.* 1999) and levels of primary production are low by world standards (Pearce *et al.* 2000). Larvae obtained from South Australia by Gaughan *et al.* (2001a) were collected in July, which is outside the peak spawning period and upwelling season in the eastern GAB, and these data were unsuitable for characterizing growth rates of sardine larvae in this broad region.

Upwelling processes that coincide with the spawning season of sardine in the eastern GAB increase the levels of primary production to levels that are within the lower range of those reported in productive eastern boundary current systems and above those reported for most other parts of southern Australia. The general linear model suggested region (non-upwelling), SST and surface chlorophyll-*a* concentrations had significant effects on growth rates of sardine larvae in South Australian shelf waters. The hypothesis of Gaughan *et al.* (2001a) that the growth rates of larval sardine in southern Australian waters are at the lower end of those for this species worldwide was not supported by data obtained during the present study. Growth rates for sardine larvae in upwelling areas of the eastern GAB were comparable to growth rates reported worldwide by Gaughan *et al.* (2001a). More specifically, the mean growth rate (0.56 mm.day^{-1} for larval sardine in upwelling areas of the eastern GAB is comparable to those observed in the productive boundary current systems off Africa (0.59 mm day^{-1}, Thomas 1986) and America (0.65 mm day^{-1}, Castillio *et al.* 1985) and lower than off Japan (0.85 mm day^{-1}, Watanabe & Kuroki 1997).

The similarity between the growth rates of sardine larvae in the eastern GAB and other ecosystems may be due to similarities in the oceanographic conditions during the spawning season. The low SSTs (<17°C) recorded around the western tip of Kangaroo Island and along the southwestern coast of Eyre Peninsula confirm previous findings that upwelling occurs in this region during this period (Schahinger 1987; Ward *et al.* 2004; Kämpf *et al.* in press). During the upwelling period, SSTs in the eastern GAB are

comparable to those observed in the Humboldt Current off Peru/Chile (e.g. 16-18 $^{\circ}$C, Mann and Lazier 1996, Escribano and Hidalgo 2000), but are warmer than those recorded in the Benguela Current off western Africa (e.g. 10-12 $^{\circ}$C, Verheye *et al.* 1992). It is clear that the oceanographic conditions in the eastern GAB are different to those off southern Western Australia, where most of the larvae analysed by Gaughan *et al.* (2001a) were collected, and where SSTs during the spawning season for sardine typically range between 17 and 20°C (Fletcher *et al.* 1994).

Lewis (1981) showed nitrate concentrations in upwelled water off the southeastern coast of South Australia were at least 70 times greater than the background levels of 0.01-0.9 mmol m^{-3}. Enhanced nutrients levels during the upwelling season in the eastern GAB may contribute to increases in primary production to levels above those recorded in other parts of southern Australia. For example, the concentrations of chlorophyll-*a* recorded in coastal waters off the western Eyre Peninsula during the present study (0.07 µg L^{-1} to 1.1 µg L^{-1}) and during previous years were substantially higher than ranges (0.2-0.3 µg L^{-1}) reported by Pearce *et al.* (2000) in southern Western Australia. Whilst chlorophyll-*a* concentrations recorded in South Australia were higher than those reported in other regions of southern Australia, they lie at the lower end of ranges of chlorophyll-*a* concentrations observed in the Californian/Humbolt and southern Benguela systems, which range from 1 to 30 and 0.8 to 24 µg L^{-1} during upwelling events, respectively (Small and Menzies 1981; Peterson *et al.* 1988; Brown 1984).

As is the case in other upwelling systems, enhanced concentrations of chlorophyll-*a* produced by upwelling appear to enhance zooplankton production (e.g. Escribano and Hidalgo 2000). The products of this enhanced biological production appear to be dispersed westward into the GAB by prevailing southeasterly winds, resulting in increased zooplankton densities (4-5 ml m^{-3}) in areas to the southwest of the upwelling cells. However, high densities of zooplankton were also recorded in southern Spencer Gulf, where frontal systems form during summer and autumn (Bruce and Short 1990). High densities of sardine larvae were recorded in both areas where high levels of zooplankton abundance were recorded. Other studies have shown that the abundance and density of plankton patches affect the growth rates of clupeoid larvae (Blaxter and Hunter 1982; Arrenius and Hansson 1993). It is likely that the high abundance and density of plankton patches in the eastern GAB, compared to southern Western Australia, increases the probability of larvae encountering and remaining within a suitable plankton patch and thus enhances their growth rates. This is certainly the case in the Kuroshio Current (Nakata *et al.* 1995) and off southwest Africa (Beckley and van der Lingen 1999), where the spawning

areas and season of sardine also coincide with spatial and temporal peaks in secondary production.

This study shows that growth rates of sardine larvae in upwelling areas of the eastern GAB are higher than those in parts of southern Australia where large-scale, predictable nutrient enrichment processes do not occur, such as southern Western Australia. The results do not support the hypothesis of Gaughan *et al*. (2001a) which suggests that the growth rates of sardine larvae throughout southern Australia are lower than those in the productive boundary current systems off the coasts of southern Africa, the Americas and Japan. Findings of this study emphasize the importance of considering intra-regional variability when attempting to characterize the life history characteristics of pelagic fishes across regions as large and diverse as southern Australia.

4.0 EFFECTS OF THE 1995 AND 1998 MORTALITY EVENTS ON THE SIZE AND AGE STRUCTURE OF SARDINE IN SOUTH AUSTRALIA

Objective: To determine the size and age structure of the South Australian sardine population using commercial and fishery independent data and assess the impacts of two mass mortality events.

In this chapter we investigated the size and age structure of South Australian sardine, using samples collected from commercial catches and by fishery independent methods. Changes in size and age structures after the mass mortalities in 1995 (ME1) and 1998 (ME2) were investigated in shelf, Spencer Gulf and Coffin Bay waters between 1995 and 2001. In Spencer Gulf there were significant declines in the modal size and age after ME1, however this was not repeated after ME2. Conversely, off Coffin Bay a decline in the modal size and age did not occur after ME1, yet did occur after ME2. Unfortunately, the Coffin Bay size and age structure datasets were less robust after ME2 due to a reduction in commercial fishing effort in this region. Fishery independent samples collected in offshore shelf waters after ME2 mostly comprised large, adult fish and no declines in modal size or age were observed. However, there were declines in the oldest (6 and 7 year old) age classes following ME2. There were significant differences in the size and age structure of dead sardine collected from the beach in Gulf St Vincent, Spencer Gulf and shelf waters. No juveniles (<130 mm, CFL, 0 years old) were present in a small number of samples collected in Spencer Gulf during ME2.

4.1. Methods

See Chapter 2 for methods used in this section.

4.2. Results

4.2.1. Size structure

Fishery independent - juveniles

During 2000, juveniles collected in Spencer Gulf ranged in size between 40 and 140 mm, CFL (Fig. 4.1). The distribution was bimodal with modes at 70 and 110 mm. In 2001, lengths ranged between 70 and 110 mm with a single mode present at 80 mm. Size distributions were significantly different (X^2 = 119.77, df = 88, *P < 0.05*) between years in Spencer Gulf. Juveniles collected in Gulf St Vincent during 2000 ranged in size between 40 and 100 mm with a single mode at 70 mm. During 2001 juveniles ranged in size between 60 and 110 mm with modes at 70 and 90 mm. In Gulf St Vincent, size distributions were significantly different between years (X^2 = 80, df = 36, *P = 0*). In addition, size distributions of juvenile sardine collected in Spencer Gulf and Gulf St Vincent were significantly different in both 2000 (X^2 = 96, df = 66, *P < 0.05*) and 2001 (X^2 = 71.8, df = 42, *P < 0.05*).

Fishery independent - ageing samples

Size frequency distributions for samples collected specifically for age determination in shelf waters between 1998 and 2001 showed sardine ranged in size between 110 and 200 mm, CFL (Fig. 4.2). Most sardine sampled were 150 to 170 mm. During 1998, size classes ranged from 130 to 190 mm and dual modes were present at 130 and 170 mm. In 1999, after ME1, the modal size remained at 170 and 180 mm, which represented ~51% of sardine collected. In 1998 and 1999 (after ME2) size distributions in shelf waters were significantly different (X^2 = 182, df = 64, *P < 0.05*). During 2000, the 150 and 180 mm size classes dominated samples, with a mode present at 160 mm. The robust 150 to 180 mm size classes present during 2000 were also present during 2001 and represented ~97% of adults sampled.

Fishery independent - DEPM samples

Length frequencies for sardine collected during the DEPM cruises, show sizes in shelf waters ranged from 130 to 210 mm, CFL between 1997 and 2001 (Fig. 4.3). Larger size classes (170 to 190 mm) were more common in these regions than in commercial samples from Spencer Gulf over the same period. During 1998, there was a clear mode in the size structure represented by the 170 mm size class (33%), which progressed to the 180 mm

size class (25%) in 1999. Similarly, the 180 mm size class in 1998 progressed to the 190 mm size class in 1999. Size distributions in 1998 and 1999 (post ME2) were significantly different (X^2 = 144, df = 56, *P < 0.05*).

1998 Mortality event (ME2) samples

During ME2, dead sardine were collected from the beach throughout South Australian shelf and gulf waters. Locations ranged from Kingston to Pt Brown on the west coast of Eyre Peninsula (Fig. 2.1). In shelf waters, dead fish between 60 and 210 mm, CFL were collected (Fig. 4.4) and two distinct size modes were present at 120 and 170 mm, respectively. In Spencer Gulf, sardine between 130 and 170 mm were collected and the 150 to 160 mm size classes were most common. Length frequency distributions in shelf and Spencer Gulf waters were significantly different (X^2 = 78, df = 42, *P < 0.05*). In Gulf St Vincent, dead fish ranged in size from 70 to 200 mm and size frequency distributions were significantly different to those from shelf waters (X^2 =298, df = 168, *P < 0.05*). Length frequency distributions were significantly different in Gulf St Vincent and Spencer Gulf (X^2 = 71, df = 36, *P < 0.05*). No dead juveniles were collected in Spencer Gulf during ME2.

Commercial - Coffin Bay

Sardine sampled in Coffin Bay between 1995 and 2000 ranged in size between 110 and 210 mm, CFL. The 160 and 170 mm size classes dominated during 1995, comprising 77.6% of samples (Fig. 4.5). In 1995 the 170 mm size class represented 46.9% of fish collected. Length frequency distributions in 1995 were significantly different to in 1996 (post ME1) (X^2 = 208, df = 80, *P < 0.05*). In 1996, after ME1 the 150 to 180 mm size classes (82.3%) were prominent. During 1997, the 150 and 160 mm size classes comprised 66.1% of samples and the modal size class declined from 170 to 160 mm. During 1998 the mode at 160 mm persisted and 160 and 170 mm size classes dominated. In 1999, following ME2, the modal size declined significantly (X^2 = 208, df = 88, *P < 0.05*) to 130 mm and the smaller size classes (130-150 mm) were common (55.7%). Unfortunately, sample sizes declined significantly after ME2 due to movement of the fleet into Spencer Gulf. During 2000, the 150 mm size class was most abundant (52.2%), which suggests 130 and 140 mm cohorts from the previous year may have progressed to the 150 mm size class.

Commercial - Spencer Gulf

Between 1995 and 2001, sardine from the commercial catch in Spencer Gulf ranged in size between 110 and 210 mm, CFL. During 1995, 140 to 180 mm size classes comprised 94.8% of catch samples with a mode at 140 mm (Fig. 4.5). During 1996, (after ME1) the modal size declined from 140 to 130 mm and length frequency distributions were significantly different (X^2 = 208, df = 90, *P < 0.05*) between 1995 and 1996 (after ME1). In 1997 the mode from the previous year had progressed to 140 mm and combined with the 150 mm size class comprised 79.9% of samples. In 1998 samples consisted of a broad range of size classes from 120 to 180 mm and the modal size had decreased to 130 mm. There was a significant difference between the length distributions in 1998 and 1999, after ME2 (X^2 = 260, df = 100, *P < 0.05*). The modal size increased to 140 mm in 1999 from 130 mm in the previous year. As observed in 1998, the 120 to 180 mm size classes continued to dominate, comprising 96.7% of samples. In 2000, a narrower range of size classes from 140 to 170 mm dominated samples (91.5%) and the modal size continued to progress to the 160 mm size class. This modal size was sustained during 2001 and catch samples continued to consist of 3 strong size classes ranging from 150 to 180 mm.

4.2.2. Age structure

Fishery independent juveniles

In Gulf St Vincent during 2000 juvenile age structures were bimodally distributed with modes present at 150 and 200 days old. Juveniles ranging in size between 60 and 110 mm, CFL, were 100 to 260 days old and had a mean age (±SE) of 177 ±0.13 days (Fig. 4.6). In 2001, juveniles of the same size range belonged to a broad range of age classes, from 100 to 250 days old and had a mean age (±SE) of 147 ±0.65 days. As in 2000, dual modes were present at 110 and 140 mm, CFL. The age distributions were significantly different between 2000 and 2001 (*KS statistic* = 0.47, *P < 0.05*)

In Spencer Gulf during 2000 there was a bimodal distribution of age classes with modes at 110 and 210 days old. Juveniles ranging in size between 40 and 140 mm, CFL belonged to a broad range of age classes ranging from 110 to 230 days old and had a mean age (±SE) of 158 ±0.25 days (Fig. 4.6). During 2001 juveniles ranging in size between 70 and 150 mm, belonged to a broad range of age classes from 60 to 250 days old and had a mean age (±SE) of 131 ±0.34 days: These age distributions were significantly different between 2000 and 2001 (*KS statistic* = 0.35, *P < 0.05*). In addition, age distributions in juveniles were significantly different between regions during 2000 (*KS statistic* = 0.35, *P < 0.05*) and 2001 (*KS statistic* = 0.24, *P < 0.05*).

Fishery independent ageing samples

During 1998 adult samples collected in offshore shelf waters ranged in age between 2 and 7 years old. The 3, 4 and 5 year old age classes were dominant and comprised 36.7%, 20.3% and 30.4% of samples, respectively (Fig. 4.7). In 1999, after ME2 there was a significant shift in the age distribution from the previous year (*KS statistic* = 0.3, *P < 0.05*). The 6 year old age classes that comprised >10% of samples in 1998 had declined to ~4% after ME2. There were no 7 year olds present in samples after ME2. In 2000, 3 and 4 year olds continued to dominate, comprising 22.5% and 61.3% of samples, respectively. In 2001, the 3 and 4 year old age classes progressed to become robust 4 (43.3%) and 5 (35.8%) year old age classes and the 6 year old age class recovered slightly to comprise ~1.7% of samples.

1998 Mortality event (ME2) samples

Ages were determined for 247 (21%) of the dead sardine collected from several locations in shelf, Gulf St Vincent and Spencer Gulf waters (Fig. 2.1) during ME2. Of these individuals, n = 163 were collected from shelf waters, n = 56 from Gulf St Vincent and n = 28 from Spencer Gulf. In shelf waters, the distribution of ages was bimodal with 1 to 3 year old age classes most common (Fig. 4.8). In Spencer Gulf, no juveniles (0s) were collected and samples mainly consisted of fish in the 2 (57.1%) and 3 (39.3%) year old age classes. The age distributions of moribund sardine collected in Gulf St Vincent and Spencer Gulf were significantly different (*KS statistic* = 0.38, *P < 0.05*). In Gulf St Vincent the 1(26.8%), 2 (28.6%) and 3 (25%) year old age classes were dominant and age distributions in Gulf St Vincent and shelf waters were significantly different (*KS statistic* = 0.22, *P < 0.05*). Similarly, the age distributions in samples of dead fish collected from beaches in shelf waters were significantly different to those from Spencer Gulf (*KS statistic* = 0.39, *P < 0.05*). It is notable, however that sample sizes in Spencer Gulf were small therefore interpretation of these analyses should be treated with caution.

Commercial - Coffin Bay

In Coffin Bay during 1995, 2 and 3 year old age classes dominated, comprising 24.5% and 59.4% of samples (Fig. 4.9). In 1996, after ME1 the age structure continued to be dominated by 2 (25.6%), 3 (47.2%) and 4 (23.3%) year olds. During 1997 and 1998, catch samples were mostly comprised of 2, 3 and 4 year olds. In 1999, directly after ME2 there was a significant (*KS statistic* = 0.68, *P < 0.05*.) decline in the age structure and samples mostly comprised 1 and 2 year olds (82.8%), however fewer samples were collected and aged. During 2000, the strong 1 year old age class from the previous year progressed to become a strong 2 year old age class which comprised 79.6% of the catch samples. The 3 and 4 year old age classes that were robust prior to ME2 did not recover in 2000.

Commercial - Spencer Gulf

In Spencer Gulf during 1995, samples were mostly comprised of 2 (46.7%) and 3 (32.8%) year olds (Fig. 4.9). In 1996 (post ME1), there was a significant decline in the age structure from the previous year (*KS statistic* = 0.68, *P < 0.05*), and catch samples mostly consisted of 1 (44.5%) and 2 (53.1%) year olds. In 1997, the 2 year old age class dominated (77.9%) catch samples. During 1998, samples mostly consisted of 1 (47.3%) and 2 year old age classes (35.9%). In 1999, after ME2 there were no declines in the age structure of samples from Spencer Gulf. The strong 1-year old age class present in 1998 progressed to be a strong 2-year old age class (69.2%). In 2000, a narrow range of age classes from 1 to 3 year olds were present and a strong (64.4%) 2 year old age class remained. The 2 year old class from the previous year had progressed to the 3 year old age class (27.5%). In 2001 a broad range of age classes between 1 and 4 years old were present in catch samples and 2 (56%) and 3 (37.8%) year old age classes continued to dominate.
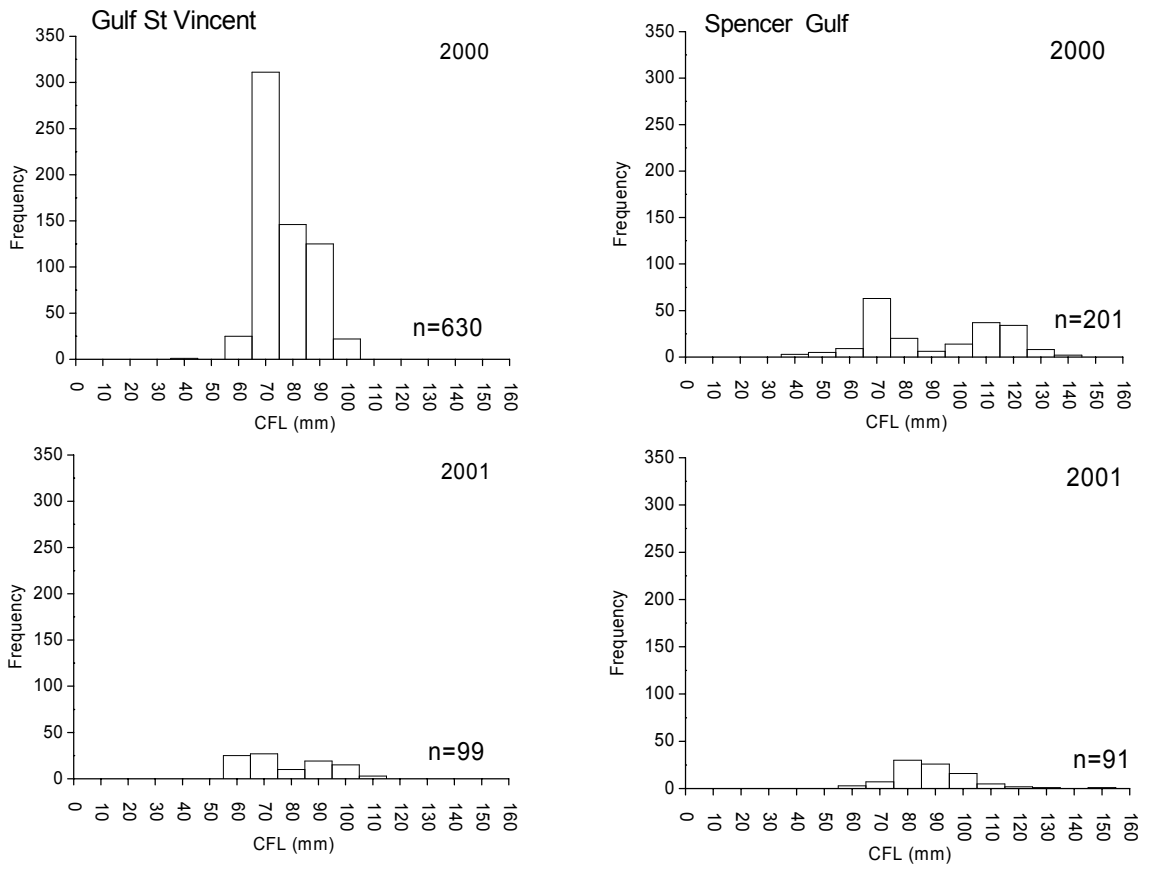
Figure 4.1. Length frequency distributions for juveniles from Spencer Gulf and Gulf St Vincent collected between 2000 and 2001 using fishery independent methods
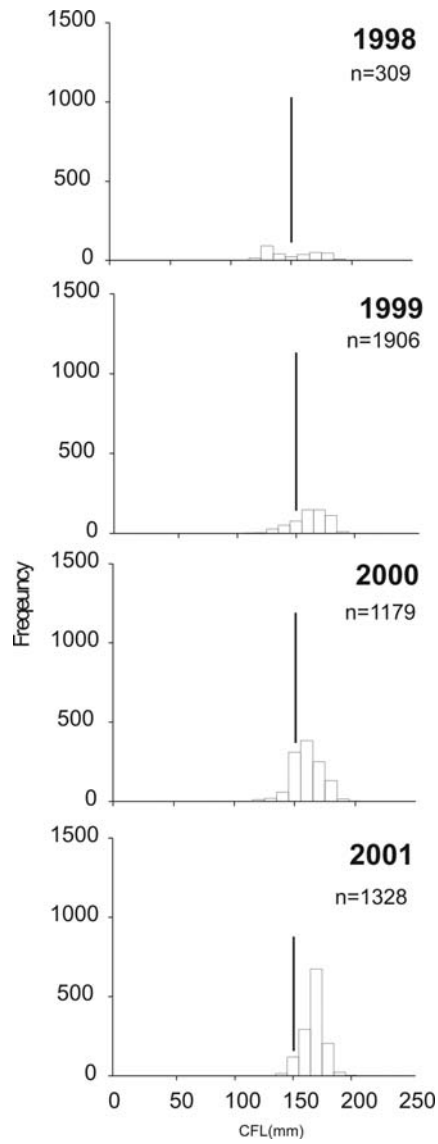
Figure 4.2. Length frequencies for fishery independent ageing samples collected in the eastern GAB between 1998 and 2001.
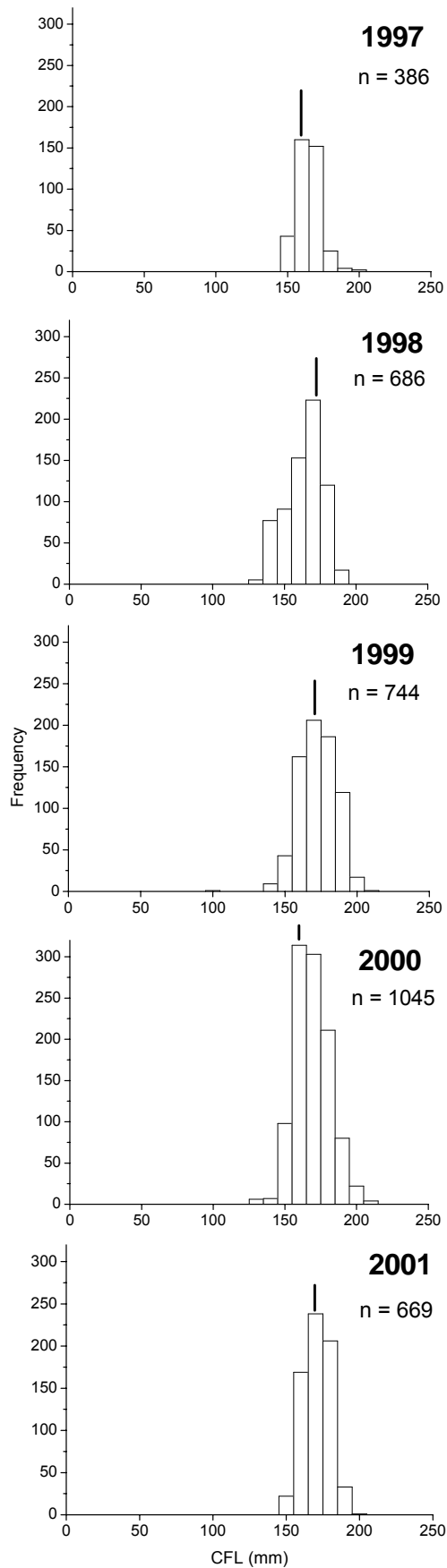
Figure 4.3. Length frequencies by year for fishery independent samples collected in the eastern GAB during the DEPM cruises between 1997 and 2001. The lines above the frequency distributions are to assist with interpreting the position of size modes.
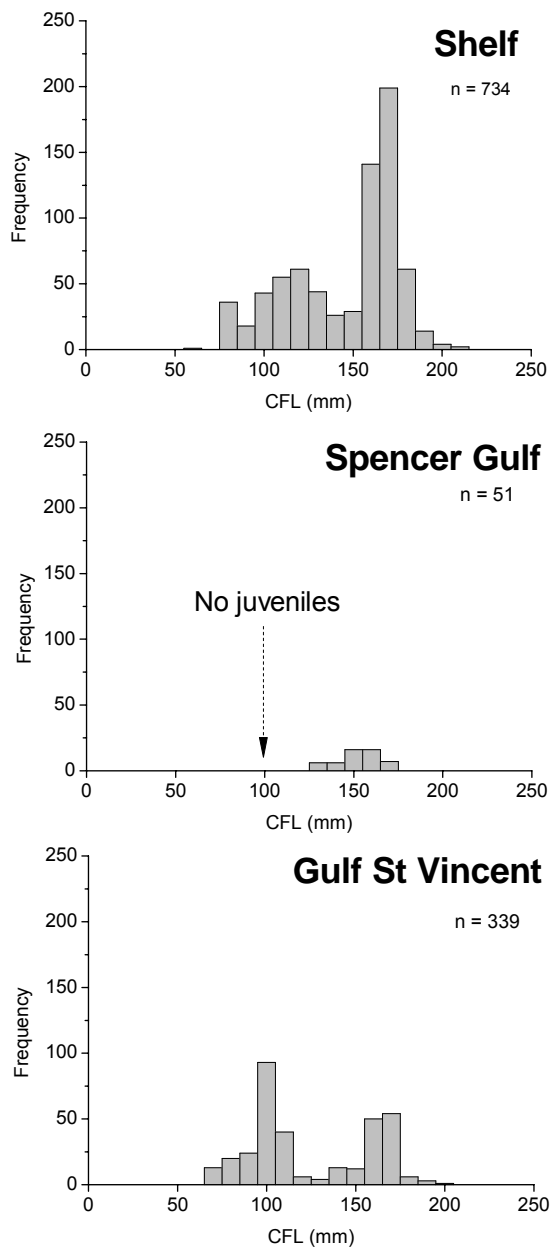
Figure 4.4. Length frequencies for juvenile and adult sardine collected in 1999 from the beach in shelf waters, Spencer Gulf and Gulf St Vincent during the second mass mortality event.
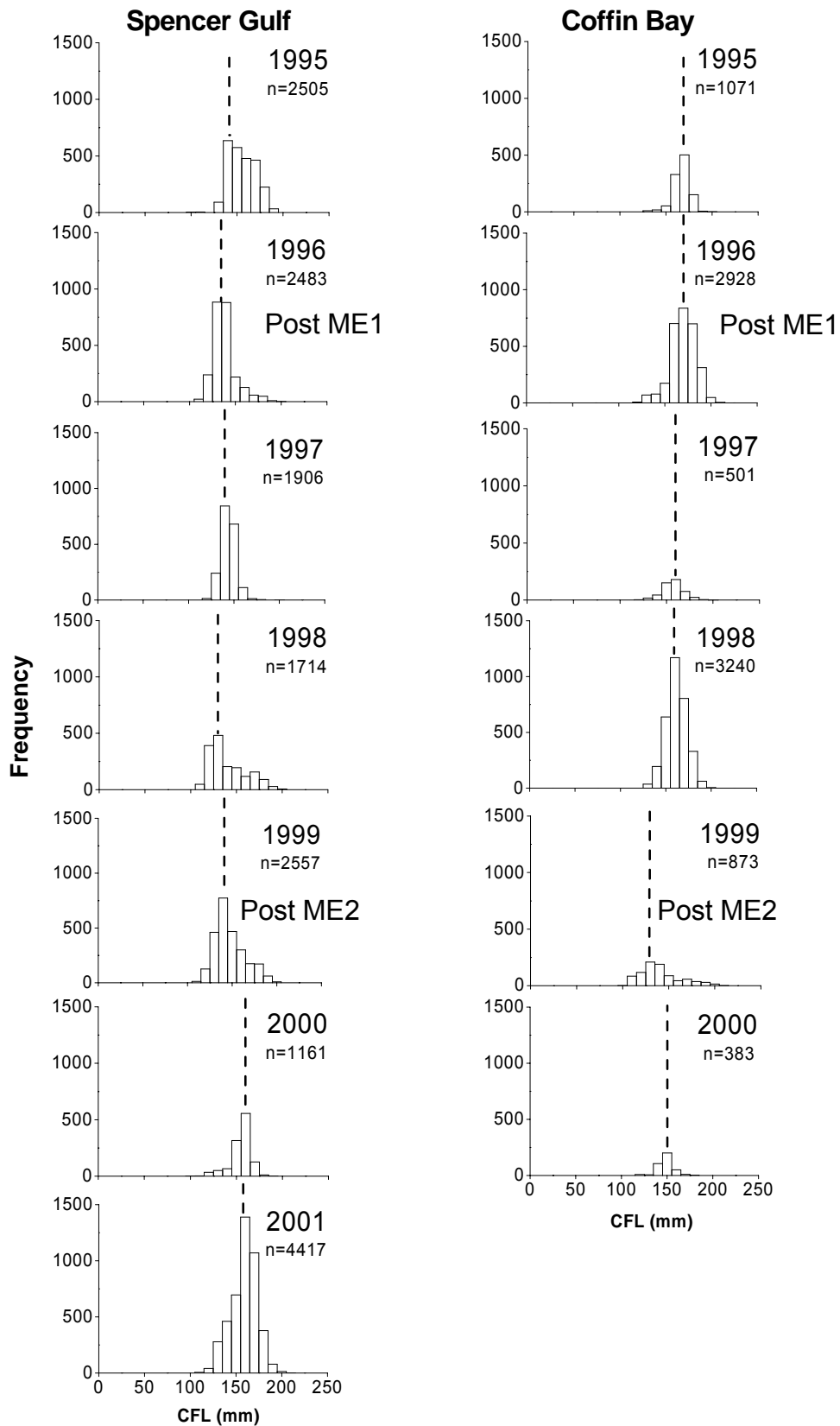
Figure 4.5. Length frequencies by year for samples collected from the commercial catch in Spencer Gulf and Coffin Bay between 1995 and 2001. The dotted lines are to assist with interpretation of the location of size modes.
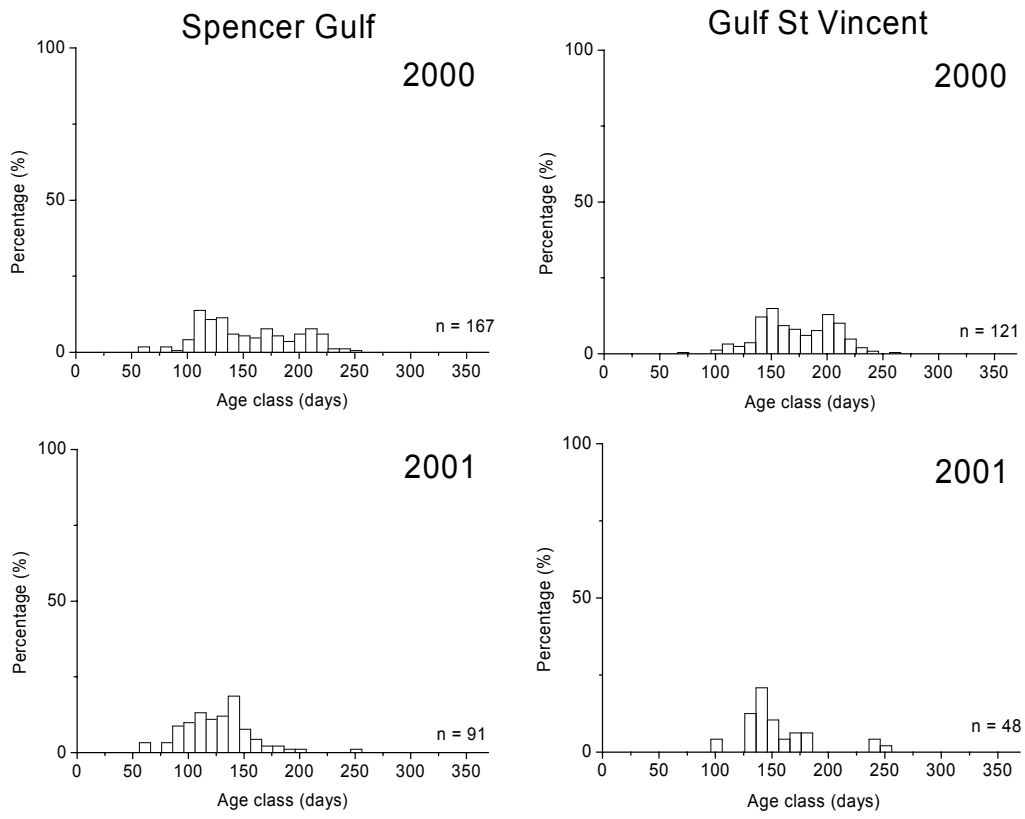
Figure 4.6. Age structure of juveniles collected in Spencer Gulf and Gulf St Vincent in 2000 and 2001.
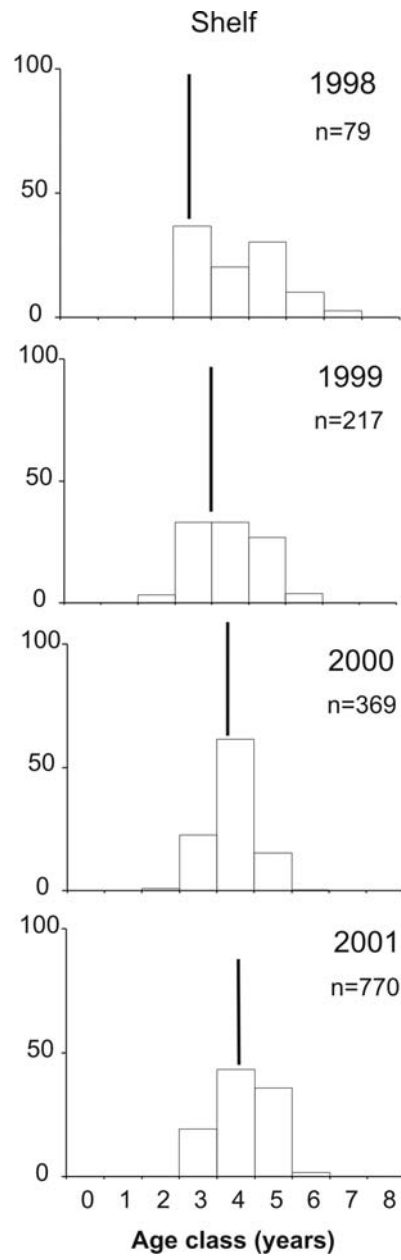
Figure 4.7. Age structures of sardine collected in shelf waters between 1998 and 2001 using fishery independent methods.
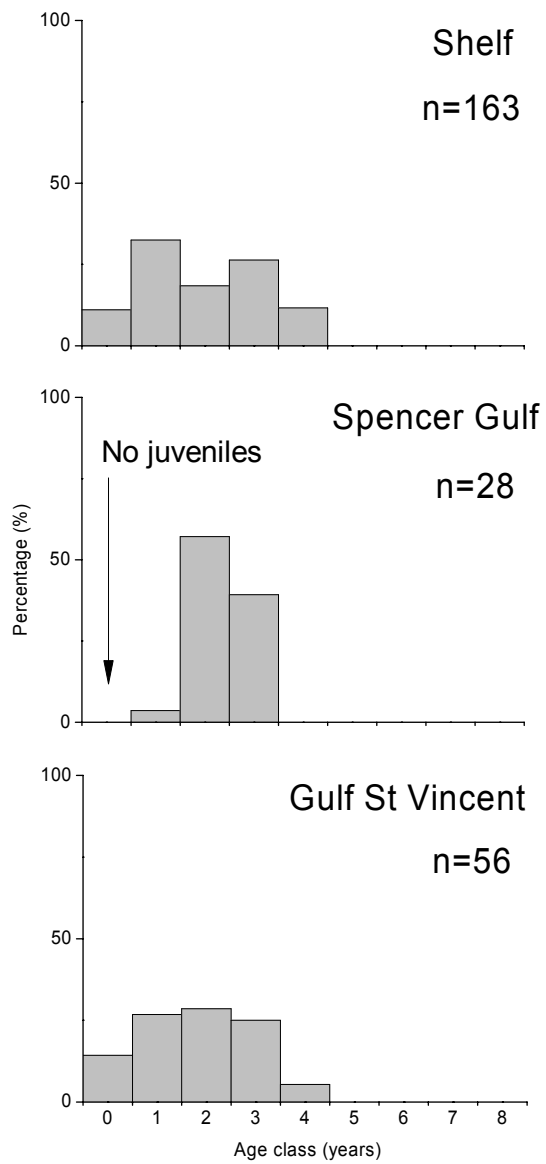
Figure 4.8. Age structure of sardine collected from the beach in shelf waters, Spencer Gulf and Gulf St Vincent during ME2.
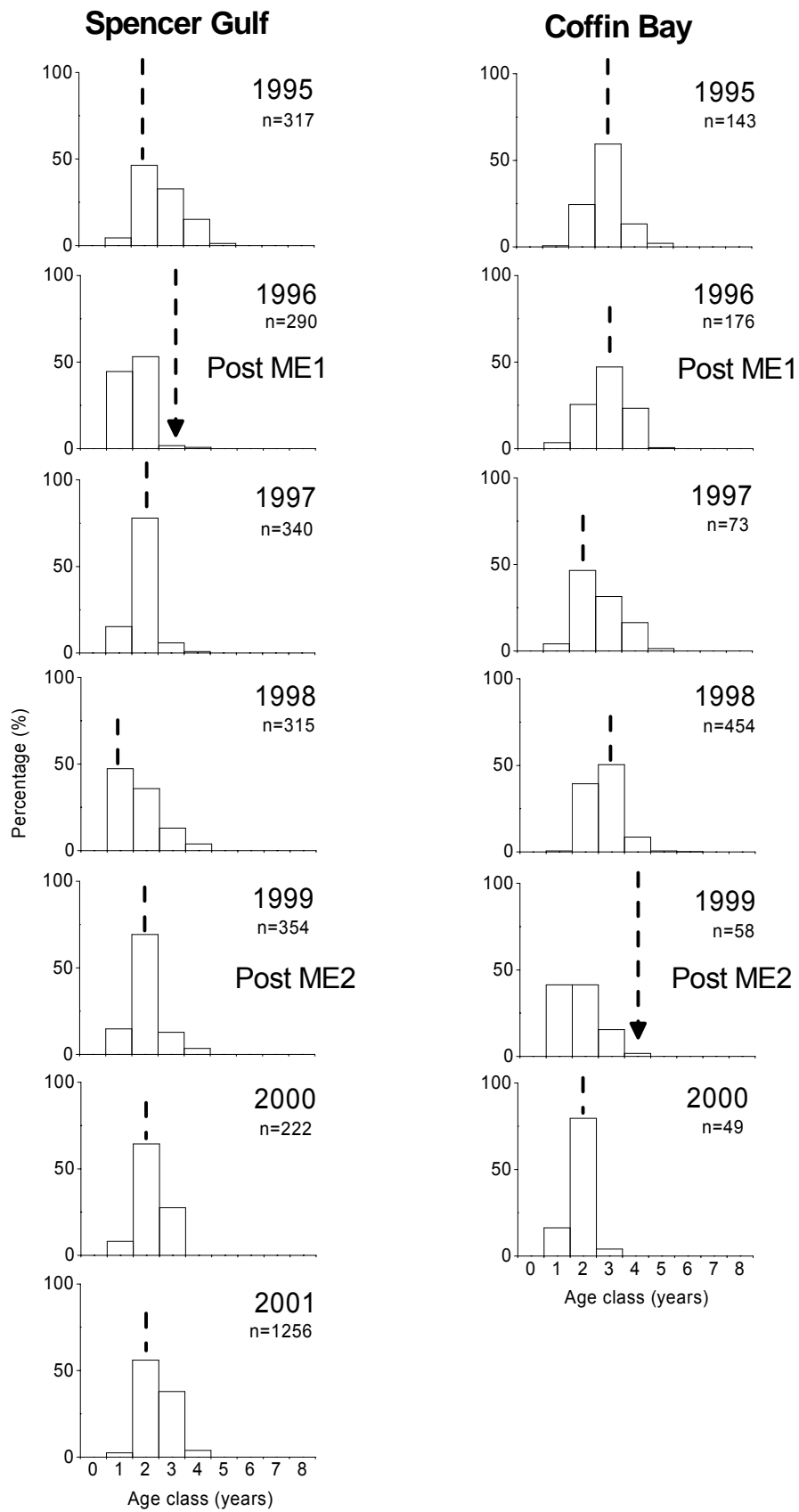
Figure 4.9. Age structure of commercial catch samples from Spencer Gulf and Coffin Bay between 1995 and 2001.

89

4.3. Discussion

This section was focused on an investigation of the size and age structure of South Australian sardine after the mass mortality events in 1995 and 1998 using both commercial and fishery independent data. Both mass mortality events reduced the spawning biomass of sardine in South Australia by approximately 75%. The 1995 mortality resulted in a decline in the spawning biomass from 165,000 to 37,000 tonnes and the 1998 event led to a decline of similar magnitude, from 146,000 to 36,000 tonnes (Ward *et al.* 2001a).

Following the second mass mortality event, the size and age structures of fishery independent samples collected in offshore shelf waters of the eastern Great Australian Bight and off Kangaroo Island were different to those of the commercial catch samples from Spencer Gulf and to a lesser extent, off Coffin Bay Peninsula. Larger (>160 mm, CFL), older (≥3 years) individuals dominated fishery independent samples and were likely to have contributed significantly to the recovery of the spawning biomass, as they were collected in areas rarely targeted by the South Australian Sardine Fishery (Ward *et al.* 2003a). Unfortunately, there was no robust fishery independent sampling regime in offshore waters prior to or during the first mass mortality event. This made it impossible to compare the impacts of both mortalities on the size and age structures of the broader sardine population.

Analysis of the size and age structures of the commercial samples suggested there were regional and temporal differences in the impacts of the two mortality events on South Australian sardine. However, these differences need to be interpreted carefully as they may, in part, reflect the different sampling methods used. Prior to the first mortality event the commercial catch samples in Spencer Gulf comprised a wide range of size and age classes, from 130 to 190 mm and 1 to 5 years olds. After the first mortality event, there was a significant decline in the presence of larger (>150 mm), older (>2 year old) size and age classes in commercial samples from Spencer Gulf. This reduction in larger and older fish was not repeated after the second mortality event, which originated in Spencer Gulf. Samples collected off Coffin Bay showed no significant declines in modal size or age after the first mortality, but the abundance of older, larger fish declined after the second mortality event. Unfortunately, a retraction of fishing effort away from Coffin Bay led to fewer commercial samples being collected from this region after the second mortality event.

Southern Spencer Gulf is an important nursery area for sardine larvae and juveniles, is hydrographically complex and is where most of the sardine fishery effort occurs (Fig. 4.10) (Bruce and Short 1990; Rogers *et al.* 2004). Small juveniles (0+ year olds) and early adults were more common near inshore islands and in the bays, whereas and larger, mature fish were found further offshore in the southern and central parts of the gulf (Rogers *et al.* 2004). Murray and Gaughan (2003) suggested that the partial separation of juvenile and adult aggregations in Western Australia may have protected some of the stock from exposure to herpesvirus during the second mass mortality. This study also suggested that if juveniles were not killed, spawning biomass rebuilding would take ~5 years, which concurs with the preliminary runs of the age structured model in chapter 5. Although sample sizes of dead fish collected during the second mortality event were low, no dead juveniles were found in Spencer Gulf.

There is currently no information available on the movement of sardine between shelf and gulf regions in South Australia. The spawning stock occupies a broad geographical range and the size and age structures of fishery independent samples collected in shelf waters indicated that the spatially limited, commercial size and age structures were clearly not representative of the broader population.
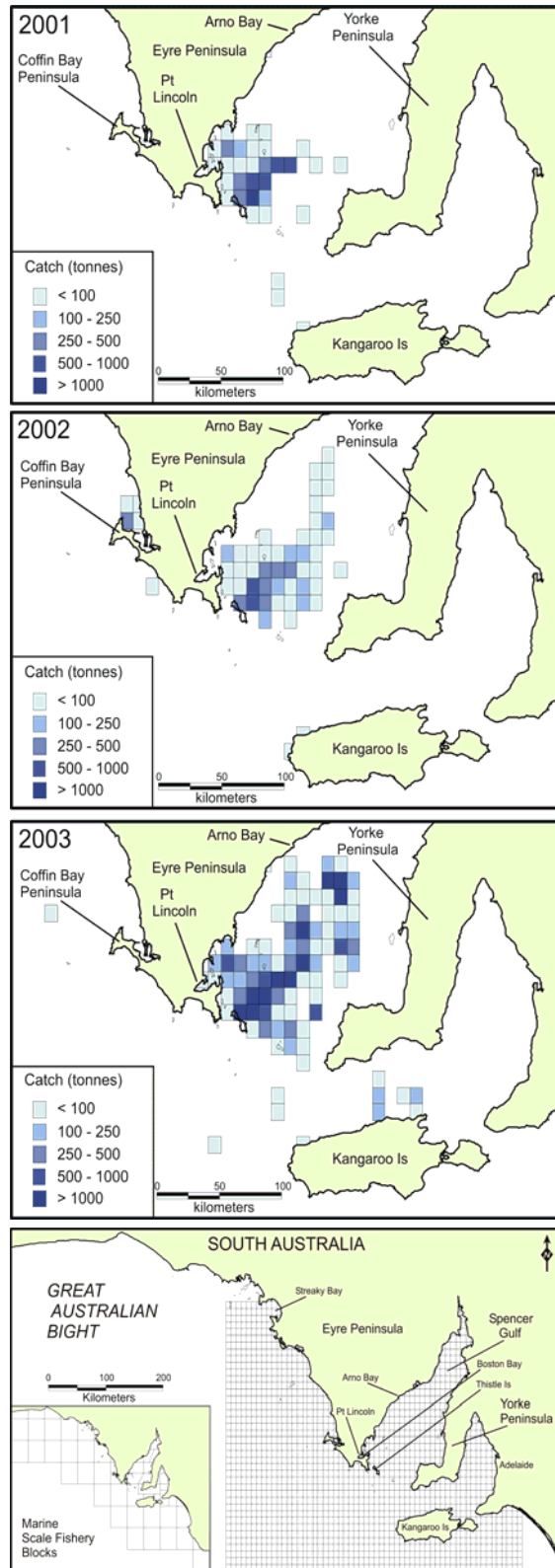
Figure 4.10. Spatial catch patterns for the South Australian Sardine Fishery during the recovery from the second mass mortality event. (Bottom left main) Grid of 6 x 6 minute squares shows the areas fished by the fishery since its inception in 1991 (Inset) Marine scale fishery block used to determine areas fished before GPS positions were recorded with each catch and effort record

5.0 IMPLEMENTATION OF AN AGE STRUCTURED STOCK ASSESSMENT MODEL FOR THE SOUTH AUSTRALIAN SARDINE FISHERY: CAN IT BE USED TO PREDICT SPAWNING BIOMASS IN THE FUTURE?

Objectives:

1. To assess the potential for using data on the abundance of recruits in catches as an index of recruitment.

2. To implement an age structured stock assessment model for the South Australian Sardine Fishery.

Juvenile sardine (0+ year olds) were not present in samples collected from commercial catches between 1995 and 2001. The 1 and 2 year old age classes represented the first recruits to the fishery and the 2 year old age class was the most common. The strength of the 2 year old age classes were not predicted by the strength of 1 year old age classes in the previous year, with the exceptions of in 1996 and 1998 when particularly strong 1 year old age classes were recruited to the fishery. Recruitment predicted by the model was poorly correlated with annual recruitment age class strength. This information all suggests commercial catch at age data is not a suitable index of annual recruitment strength.

Average and high recruitment levels were assigned between 2001 and 2003 in the preliminary runs of the age structured model. The average recruitment scenario resulted in the biomass predicted by the model being lower than the DEPM biomass estimates. The biomass predicted by the model under the low recruitment scenario was approximately 160,000 tonnes by 2006. Under the high recruitment scenario the model predicted the biomass would increase to between 250,000 and 300,000 tonnes by 2006.

Uncertainties surrounding natural mortality, recruitment and biases in the age samples from the commercial catch limited the suitability of this model for predicting future biomass. The age-structured model will be used to conduct a retrospective evaluation of the performance indicators, reference points and decision rules that have been established for the South Australian Sardine Fishery.

5.1 Introduction

No previous studies have investigated the links between abundance of recruitment age classes and spawning biomass of sardine in South Australian waters. Beckley and van der Lingen (1999) suggested fluctuations in the biomass of adult sardine off South Africa were related to the abundance of 2+ year olds in the population in the previous year. The opportunistic nature of recruitment estimates derived from commercial catch at age data and variability caused by sampling inadequacies, including poor spatial and temporal coverage can cause considerable uncertainty in the reliability of this information (Maunder and Deriso 2003). The age structured stock assessment model we have implemented was based on a model developed for the Western Australian sardine fishery. This model includes age structure data collected during fishery independent surveys in the eastern Great Australian Bight and additional data collected from the South Australian commercial catch between 1995 and 2001.

The specific aims of this section were to (1) investigate the relationship between DEPM based spawning biomass estimates and the proportions of 1 and 2 year old age classes recruited to the fishery, (2) determine if the strength of one year old age classes in commercial catch samples could be tracked consistently in the following year, and (3) determine if the age structured model can be used to reliably predict the spawning biomass in the future.

5.2 Methods

5.2.1 Age class progressions and recruitment

The Spencer Gulf commercial age structure dataset provided the most complete age structure dataset and was used to determine if a relationship existed between the 1 year old and 2 year old age classes in the following year. Spencer Gulf represents the centre of the historical range for the sardine fishery (Fig. 5.1). The 2 year old age classes were consistently the most abundant recruitment age class in catch samples and were used as the first strong recruitment age class in the preliminary runs of the model. Annual sardine spawning biomass estimates were sourced from Ward *et al.* (2004a).

5.2.2 Age structured stock assessment model

The age structured stock assessment model used in this study was written by Dr Peter Stephenson (Department of Fisheries Western Australia) and was based on a model written by Dr Norm Hall (Murdoch University) for Western Australian sardine. The data used in the model were as follows:

Catch and age composition

Monthly commercial catch data from the South Australian Sardine Fishery were available from 1992 to 2002 (Table 5.1). Age-composition data from commercial catches were available from 1995 to 2002 and from fishery independent research surveys from 1998 to 2002. The proportion in each age class and the number of sardine allocated ages is shown in Table 5.2 and 5.3. Weightings of between 0 and 1 were applied to age composition data during preliminary model runs.

Fishery independent spawning biomass estimation

Estimates of spawning biomass were obtained using the daily egg production method (DEPM). DEPM surveys were conducted in February and March between 1995 and 2002 (Fletcher *et al.* 1996) (Table 5.4) (Fig. 5.1). These estimates of spawning biomass were included in the age structure model. In the early years the coefficients of variation were large but lower in the later years. Weightings of between 0 and 1 were applied to the spawning biomass estimates during preliminary model runs. The weightings assigned to the spawning biomass estimates reflected confidence in these estimates relative to our confidence in the other parameters.

Change in abundance over time

The model was driven by the catches in monthly time steps with ages $a = 0$ to $a = 96$ months and instantaneous rate of natural mortality, M, assumed to be constant for all ages and estimated in the model. The sardine population was assumed to be in state of unfished equilibrium in 1992. As sardine spawn in March in South Australia, the initial recruitment θ_{Init} was estimated in the model for March 1992. The number of sardine (millions), $N_{a,t}^{s}$, of age a ($a = 0$ to $a = 96$ months) and sex s ($s = f$ and $s = m$) at the beginning of time-step $t=0$ (March 1992) was calculated from Equation 1.

$$
N_{a,0}^{s} =
\begin{cases}
0 & \text{if } \mathrm{mod}(a,12) \neq 0 \\
\rho_s \theta_{Init} & \text{if } a = 0 \\
N_{a-12,0}^{s} \exp(-M) & \text{if } \mathrm{mod}(a,12) = 0 \text{ and } 0 < a < 96 \\
N_{a-12,0}^{s} \exp(-M)/[1 - \exp(-M)] & \text{if } a = 96
\end{cases}
\tag{1}
$$

where ρ_s is the proportion of each sex with $\rho_f = 0.59$ and $\rho_m = 1 - \rho_f$ .and $\mathrm{mod}(a,12) = a - 12\,\mathrm{int}(a/12)$.

Recruitment

Sardine recruitment is highly variable and depends on environmental conditions and to a lesser extent, stock size. Yearly recruitment was determined from the Beverton and Holt stock-recruitment relationship with the parameters determined from the biomass in the initial state using a steepness parameter (Hilborn *et al.* 1994) fixed at $h = 0.7$. Low steepness factor values were assigned based on the assumption that recruitment is not highly reliant on stock size. To account for environmental variation, for the years 1992 to 2000, the Beverton and Holt recruitment was multiplied by a log-normally distributed recruitment deviation factor, which was estimated in the model. After 2000, there was no information in the age-composition data to calculate recruitment deviations and therefore environmental variation in years after 2000 were modelled by a log-normally random sample with standard deviations equal to that estimated for years 1992 to 2000. The recruits into the age 0 fish were represented by R_t, Equation 2:

$$R_t = \begin{cases} 0 & \text{if } m' \neq 3 \\ R_t & \text{if } m' = 3 \text{ and } y = 1992 \\ R_t \; e^{d_t} & \text{if } m' = 3 \text{ and } 1993 \leq y < 2000 \\ R_t \; e^{\varepsilon_t} & \text{if } m' = 3 \text{ and } y \geq 2000 \end{cases} \tag{2}$$

where m' is the month, R_t the recruitment in the fishery from the stock recruitment relationship, d_t is the recruitment deviation parameter, and ε_t is a random selection from $N(0, \sigma_t^2)$ where σ_t is the standard deviation of d_t. Weightings of between 0 and 1 were applied to recruitment deviations during preliminary model runs.

Abundance in successive time steps

Sardine numbers in successive time-steps after recruitment were reduced due to losses from natural mortality and fishing according to Equation 3:

$$N_{a,t}^s = \begin{cases} R_t & \text{if } a = 0 \text{ and } m' = 7 \\[2ex] N_{a-1,t-1}^s\left(1 - H_{t-1}V_{a-1}\right)\exp(-M/12) & \text{if } a > 0 \text{ and } a \neq 96 \\[2ex] \left[\begin{array}{l} N_{a-1,t-1}^s\left(1 - H_{t-1}V_{a-1}\right) \\ + N_{a+11,t-1}^s\left(1 - H_{t-1}V_{a+11}\right) \end{array}\right]\exp(-M/12) & \text{if } a = 96 \\[3ex] N_{a-1,t-1}^s\left(1 - H_{t-1}V_{a-1}\right)\left(1 - D_{1995}\right)\exp(-M/12) & \text{if } y = 1995 \text{ and } m' = 4 \\[2ex] N_{a-1,t-1}^s\left(1 - H_{t-1}V_{a-1}\right)\left(1 - D_{1999}\right)\exp(-M/12) & \text{if } y = 1998 \text{ and } m' = 8 \end{cases} \tag{3}$$

where R_t is the recruitment in each region, H_t is the harvest rate (proportion of the vulnerable sardines caught) for time-step t, V_a is the proportion of sardines of age a that are vulnerable. $D_{1995} = 0.7$ is the estimated proportion of fish age ≥ 3 that died as a result of the first mass mortality event in 1995 and $D_{1998} = 0.7$ is the estimated proportion of fish aged ≥ 1 that died as a result of the second mass mortality event in 1998 (Ward *et al.* 2001a).

Vulnerable biomass

Sardine in South Australia mostly first become vulnerable to fishery exploitation at the age of two years old. The vulnerability of sardine aged 2, 3, and 4 years is estimated in the model with 5-year-old fish being fully recruited. Vulnerability at age a (months) was assumed constant throughout the year while fish remained within the same age-class. Vulnerable biomass, $\hat{B}_t$ in tonnes, at the beginning of time-step t for sardine of sex s was calculated as:

$$\hat{B}_t = 1000 \sum_{s=m,f} \sum_{a=0}^{A} N_{a,t}^s V_a W_a^s \tag{4}$$

where W_a^s is the weight (kg) at age a and sex s and $W_a^s = 0.0000039939 \times (L_a^s)^{3.20}$.

Harvest rate

The monthly harvest rate, H_t^r, at each time step was calculated from the observed catch and the current estimate of vulnerable biomass, Equation 5.

$$H_t^r = \begin{cases} \dfrac{C_t}{\hat{B}_t} & \text{if } C_t^r < \hat{B}_t^r \\ 1.0 & \text{otherwise} \end{cases} \tag{5}$$

where C_t is the monthly catch and $\hat{B}_t$ is the current estimate of vulnerable biomass.

Penalty function

To ensure the model parameters produced estimates of vulnerable biomass sufficiently large to allow the removal of the observed catch, a penalty function, P_1 (Equation 6), was included in the objective function if the catch exceeded 95% of the vulnerable biomass in the region. These factors are necessary with catch-conditioned models.

$$P_1 = 100 \sum_{\substack{t=0 \\ C_t > 0.95 B_t}}^{T} \left(C_t - 0.95 \hat{B}_t \right)^2 \tag{6}$$

Growth

The growth of sardines is represented by the von Bertalanffy growth curve:

$$L_a^s = L_\infty^s \left\{ 1 - \exp\left[-K^s \left(\frac{a}{12} - t_0^s \right) \right] \right\} \tag{7}$$

where L_a^s is the fork length (mm) of a fish of age *a* months ($0 \le a \le A$) and sex s ($s = m$ for males, $s = f$ for females), and the values of the growth parameters are $L_\infty^f = 191.9$, $L_\infty^m = 183.4$ $K^f = 0.62$, $K^m = 0.57$ and $t_0^s = 1.0$.

Age at sexual maturity

The proportion of sardine that were sexually mature at age *a* (months) is given by:

$$p_a = \frac{1}{1 + \exp\left[-\ln(19)\left(\dfrac{\text{int}(a/12) - 3.02}{5.5 - 3.02}\right)\right]} \quad (8)$$

where the age at which 50% were mature was 3.02 years and the age at which 95% were mature was 5.5 years (Ward unpublished data).

Log-likelihood associated with DEPM biomass estimates

Estimates of spawning biomass, D_t, and their estimated coefficients of variation, CV_t, determined from the DEPM surveys, are presented in Table 5.4. The estimated mature biomass, $\hat{D}_t^s$, in tonnes, at the beginning of time-step *t* for sardine of sex *s*, was calculated as:

$$\hat{D}_t^s = 1000 \sum_{s=m,f} \sum_{a=0}^{A} N_{a,t}^s \, p_a \, W_a^s \quad (9)$$

Errors were assumed to be normally distributed and an estimate of the log-likelihood associated with the DEPM derived biomass estimates was obtained using Equation 10:

$$\lambda_1 = -\frac{\displaystyle\sum_{\substack{t \\ D_t \text{ exists}}} \left(D_t - \hat{D}_t\right)^2}{2\left(CV_t \, D_t\right)^2} - \ln(CV_t \, D_t) \quad (10)$$

Log-likelihood associated with age

The estimated proportion, $\hat{p}_{a,t}$, of vulnerable sardine within each age-class a, combining sexes, were calculated for the 2+ to 8+ age classes recruitment in May each year. It was assumed that the observed ages represented samples from a multinomial distribution, and thus the log-likelihood associated with age samples (ignoring constant terms) was determined using Equation 11:

$$\lambda_3 = \sum_{\substack{t \\ m'=5 \\ \text{Sample exists}}} N_t \left\{ \sum_{a=24}^{96} \left\{ p_{a,t} \log(\hat{p}_{a,t}) \right\} \right\}$$ (11)

where $p_{j,t}$ is the observed proportion at age and $\hat{p}_{j,t}$ is the model estimated proportion at age and N_t is the sample size.

.

Overall log-likelihood

The overall log-likelihood was the sum of log-likelihoods for the observed biomass estimates, age-composition data, recruitment deviations, as well as the penalty function associated with harvest rates (Equation 12).

$$\lambda = \lambda_1 + \lambda_2 - P_1$$ (12)

The objective function was maximised using the software package, AD Model Builder (Otter Research Ltd 1994). The population was projected forward from 2003 to 2006 using harvest rates based on the DEPM estimate of the stock size the previous year and decision rules for determining the Total Allowable Commercial Catch (TACC) were designed to keep the spawning biomass greater than 40% of the biomass in 1992.

A Bayesian procedure was used to determine estimates of uncertainty of the mature biomass, D. A joint posterior probability distribution was generated using the Markov Chain Monte Carlo (MCMC) procedure in AD model Builder. A subset of 2000 values from the joint posterior distribution was obtained by saving every 1000th value from 2,500,000 cycles of the MCMC and rejecting the first 500 generated during the "burn in" period. The mature biomass was determined using the sub-sample from the joint posterior distribution. After 2002, the yearly recruitment determined from the Stock Recruitment Relationship (SRR) was multiplied by the exponent of annual recruitment deviations randomly sampled from a log-normal distribution with mean zero and variance taken as that from the

historical annual recruitment deviations. The mode and 95% confidence intervals were determined from the marginal posterior distribution of spawning biomass.

Comparison of estimated recruitment and recruitment age class strength
Recruitment estimates determined from the age structured stock assessment model, were compared to the relative strength of 1 and 2 year old age classes in the commercial catch between 1995 and 2001. Degrees of linear association were compared using Pearson coefficients of correlation with 1 and 2 year time-lags assigned to the 1 and 2 year old age classes, respectively.

Table 5.1. Catches of sardine (tonnes) in South Australia

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
1992	2	18	34	42	10	33	62	39	3	28	121	73
1993	131	127	169	214	189	74	115	234	68	101	31	11
1994	224	453	330	227	214	369	436	185	77	76	250	400
1995	387	372	369	28	33	191	121	244	127	51	256	283
1996	163	663	544	387	633	187	66	191	110	83	259	247
1997	180	299	276	653	275	601	484	117	38	21	2	79
1998	151	552	687	1619	1267	789	483	312	272	140	0	165
1999	134	237	367	404	524	268	499	593	68	106	167	181
2000	47	328	448	607	382	531	278	189	97	34	194	370
2001	341	420	1291	1193	604	59	65	2	87	137	113	237
2002	815	637	1452	3339	3157	1104	1232	350	331	141	47	720

Table 5.2. Proportional age-composition from samples collected from commercial catches and the total number of fish allocated ages.

Year	2	3	4	5	6	7	8	n
1995	0.5252	0.3361	0.0987	0.0294	0.0105	0.0010	0.0010	476
1996	0.5685	0.2886	0.1137	0.0262	0.0029	0.0010	0.0010	343
1997	0.5040	0.3427	0.1129	0.0323	0.0081	0.0010	0.0010	248
1998	0.2607	0.4018	0.2339	0.0929	0.0107	0.0010	0.0010	560
1999	0.5685	0.3382	0.0641	0.0292	0.0010	0.0010	0.0010	343
2000	0.1751	0.4903	0.2918	0.0428	0.0010	0.0010	0.0010	257
2001	0.1427	0.4586	0.3191	0.0730	0.0066	0.0010	0.0010	1219
2002	0.1500	0.4500	0.3000	0.0600	0.0400	0.0010	0.0010	400
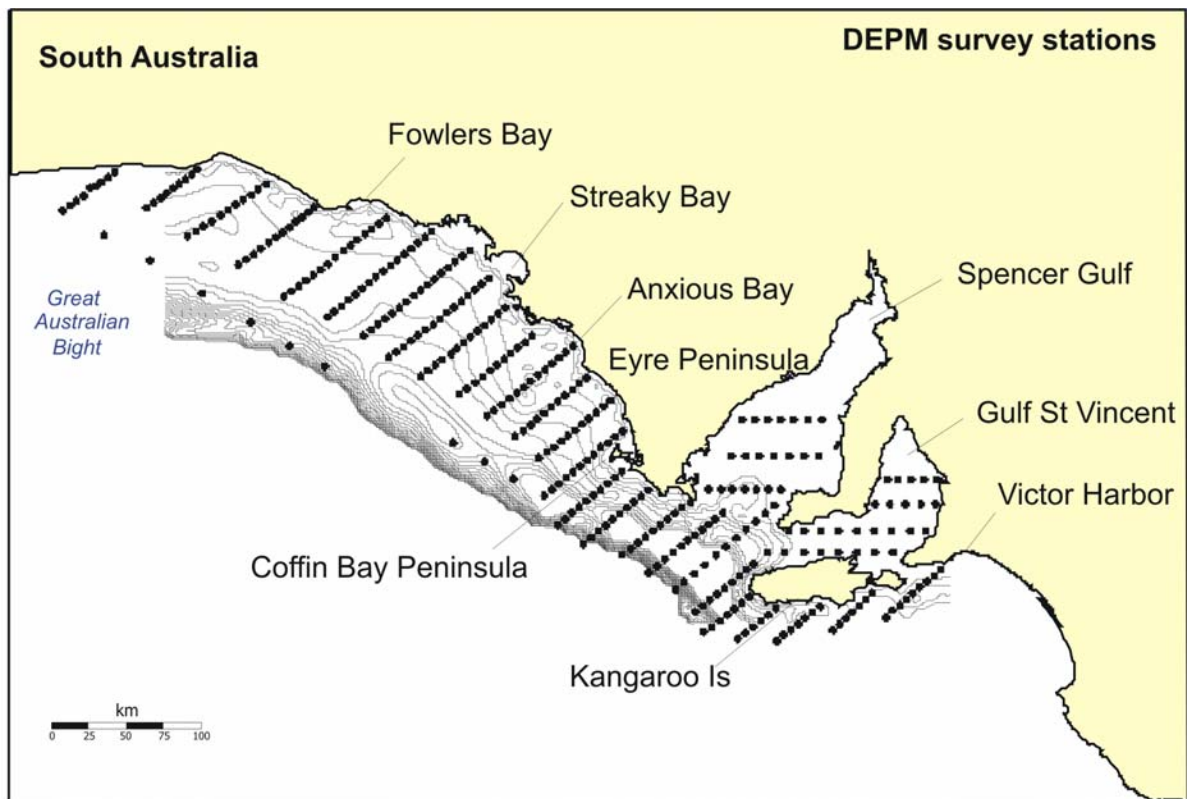
Figure. 5.1. The DEPM survey conducted annually in South Australian gulf and shelf waters. The black circles show the location of each station sampled for sardine eggs.

Table 5.3. Proportional age-composition from samples collected during fishery independent research surveys, together with the total number of fish allocated ages.

Year	2	3	4	5	6	7	8	n
1998	0.0000	0.4156	0.2078	0.2727	0.0909	0.0130	0.0000	77
1999	0.1806	0.2731	0.3750	0.1574	0.0139	0.0000	0.0000	216
2000	0.0707	0.4239	0.4266	0.0625	0.0163	0.0000	0.0000	368
2001	0.0000	0.0213	0.4440	0.3947	0.1227	0.0160	0.0013	750
2002	0.0000	0.0213	0.4440	0.3947	0.1227	0.0160	0.0013	750

Table 5.4. Estimates of sardine spawning biomass from the DEPM between 1995 and 2002.

Year	Month	Estimate	CV
1995	3	165,000	0.6
1996	3	37,000	0.5
1997	3	78,000	0.4
1998	3	146,000	0.28
1999	3	36,000	0.33
2000	3	91,000	0.37
2001	3	142,000	0.26
2002	3	240,600	0.42

5.3 Results

5.3.1 Age class progressions and recruitment

Fig. 5.2 shows that except in 1996 and 1998, the 2 year old age class was the dominant recruitment age class between 1995 and 2001. With the exceptions of 1997 and 1999, the strength of the 2 year old age classes was not predicted by the strength of 1 year old age classes in the previous year (Figs 5.2 and 5.3). Fig 5.2 shows the decline in the abundance of the 1 year old age classes between 1999 and 2001 coincided with increases in spawning biomass from 36,000 to 142,000 tonnes.

5.3.2. Age structured stock assessment model

Preliminary runs

We have presented two preliminary runs of the age-structured model using average and high recruitment scenarios (Fig. 5.4) between 2001 and 2003 (Fig. 5.6). Model estimates of initial recruitment, natural mortality and vulnerability and the standard deviations are presented in Table 5.5. Estimates of annual recruitment indicated strong recruitment in 1997 and 1998 followed by weaker recruitment in 1999 and 2000. The later estimate is only based on the abundance of 2 year old fish in 2002. After 2000, there was no information in the age structure data to estimate deviation from recruitment determined from the SRR. Preliminary mature biomass estimates (Figs 5.5. and 5.7) generally agreed with the DEPM estimates of spawning biomass.

At average recruitment levels between 2001 and 2003, the model predicted the biomass would diverge from the DEPM biomass estimate, after 2002 and reach approximately 160,000 tonnes by 2006. Under high recruitment between 2001 and 2003 (deviation factors of 2.0), the model followed a similar trajectory to the DEPM estimates and predicted the biomass would recover to between 250,000 and 300,000 tonnes by 2006. The high values of biomass determined using the DEPM between 2002 and 2003 (240,600 to 269,063 tonnes) suggested a stronger than average recruitment event occurred in one or more of the years between 2000 and 2002 (Ward *et al.* 2003b). The fishery independent and commercial age-composition data for 1997 to 2002 is shown with the age structure model estimated proportions in each age class (Fig. 5.8).

Comparison of model estimated recruitment and recruitment age class strength

Between 1995 and 2001 only 13% of the variability in recruitment estimated by the model was explained by differences in the strength of the 1 year old age classes (*Pearson's Correlation coefficient, $r^2 = 0.13$, P = 0.43, n = 7*) (Fig 5.9). Similarly, only 14% of the change in recruitment estimated by the model was explained by variation in the strength of the 2 year old age classes (*Pearson's Correlation coefficient, $r^2 = 0.14$, P = 0.40, n = 6*) (Fig 5.9).
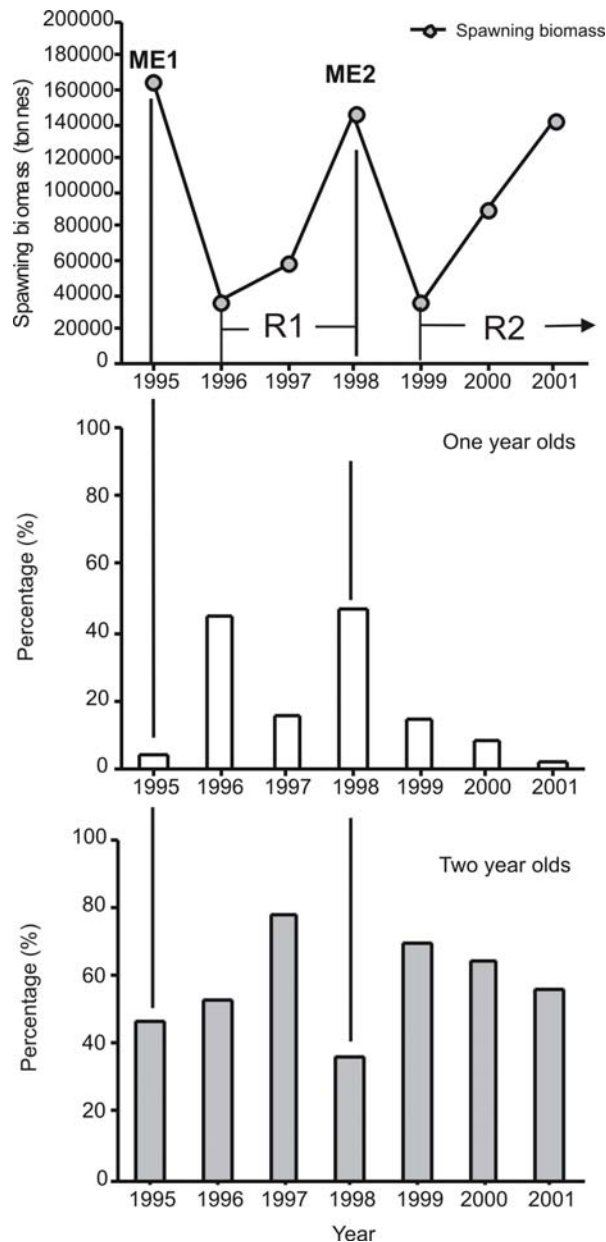
Figure 5.2. Annual changes in spawning biomass in response to mass mortality events in 1995 (ME1) and 1998 (ME2) and during the recovery periods (R1 and R2) (top) Abundance of 1 and 2 year old age classes in commercial catch samples during the same period.
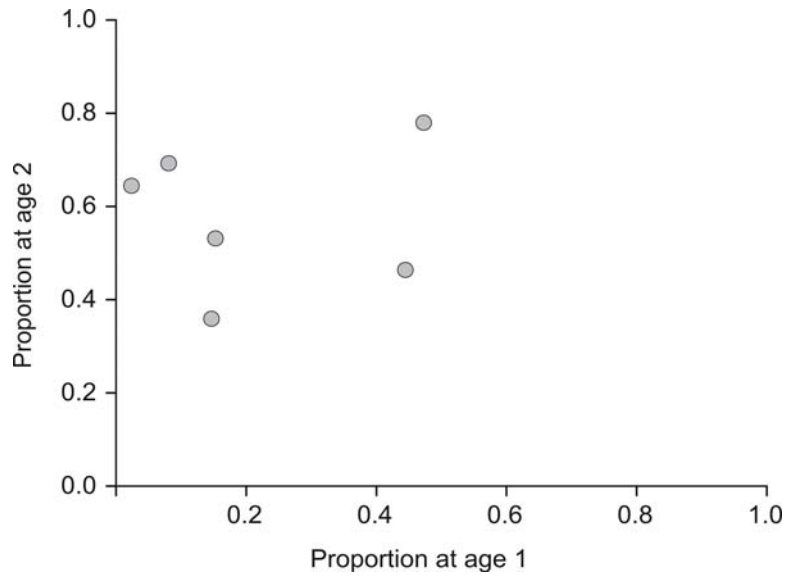
Figure 5.3. Relationship between the proportion of age class 1 and age class 2 sardine in the following year in commercial catch samples.

Table 5.5. Estimates of initial recruitment (θ_{Init}), vulnerability at age (V_2, V_3, V_4) and their standard deviations

Model parameter	Estimate	Standard deviation
θ_{Init}	44,479	5,087
V_2	0.038	0.006
V_3	0.209	0.025
V_4	0.621	0.051
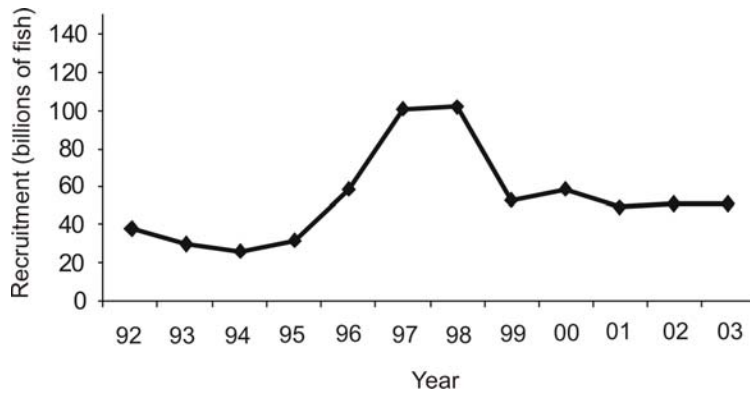
Figure 5.4. Sardine recruitment estimated in the age-structured model from 1992 to 2000 with average levels of recruitment in 2001 to 2003.
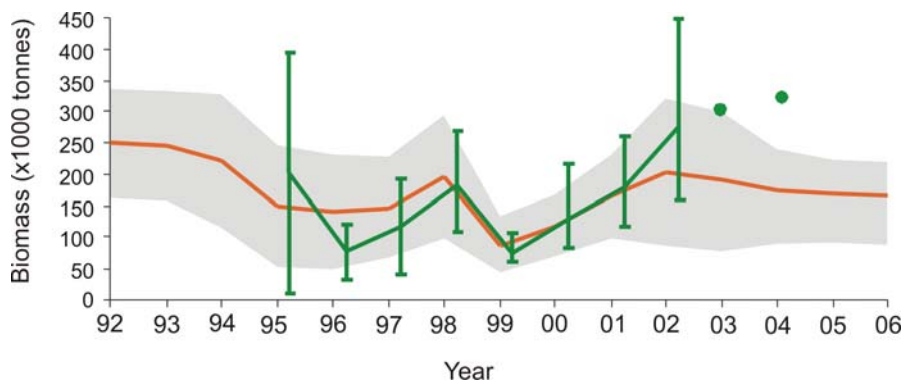
Figure 5.5. The model estimated mature biomass in orange (95% C.I.s in grey) and the DEPM spawning biomass estimates (95% C.I.s) in green. Recruitment is estimated from 1992 to 2000 and average recruitment was allocated from 2001 to 2003.
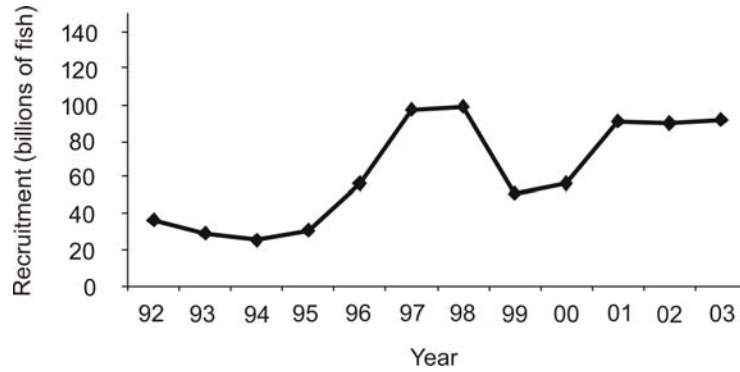
Figure 5.6. Recruitment estimated in the age-structured model from 1992 to 2000 with high recruitment in 2001 to 2003.
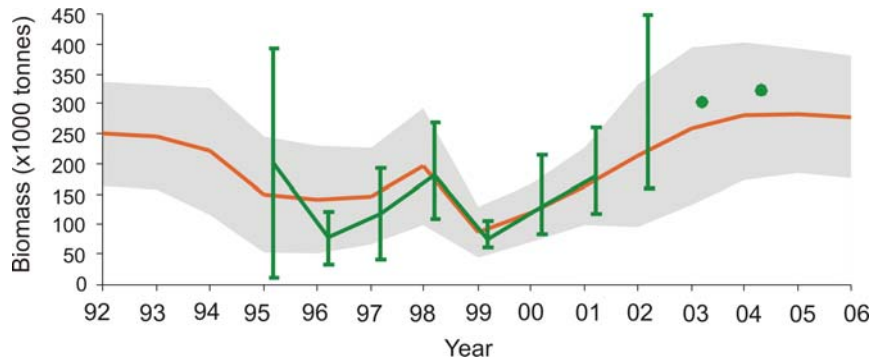
Figure 5.7. The model estimated mature biomass (with 95% C.I.s) in orange and the DEPM spawning biomass estimates (with 95% confidence intervals) in green. Recruitment is estimated from 1992 to 2000 and high recruitment was allocated from 2001 to 2003.
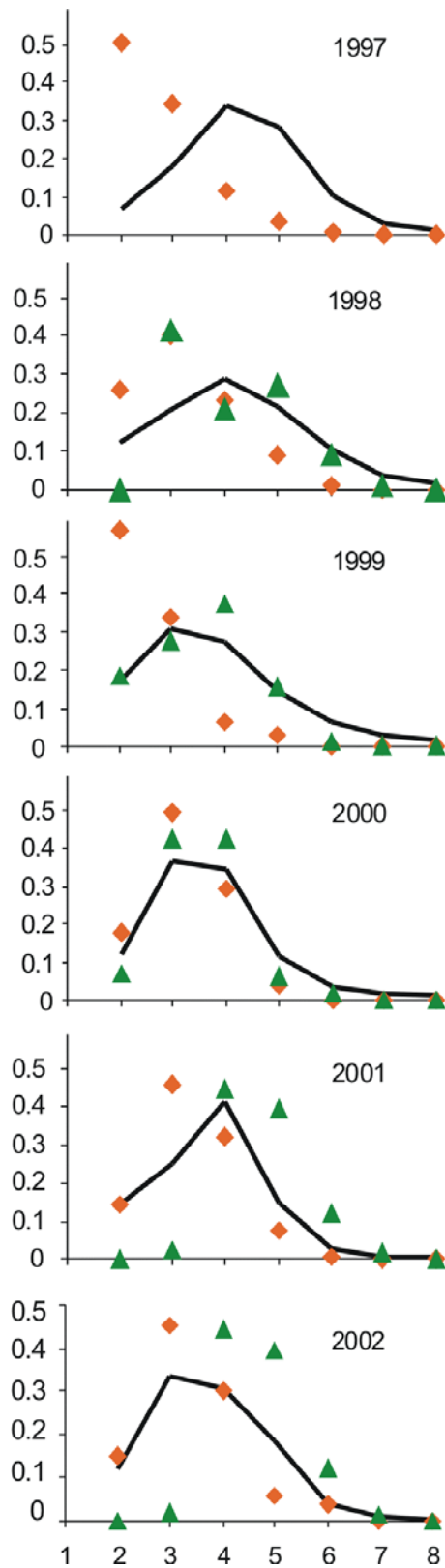
Figure 5.8. Proportional age-composition data from fishery independent research surveys (green), commercial catches (orange) and model estimates (black lines).
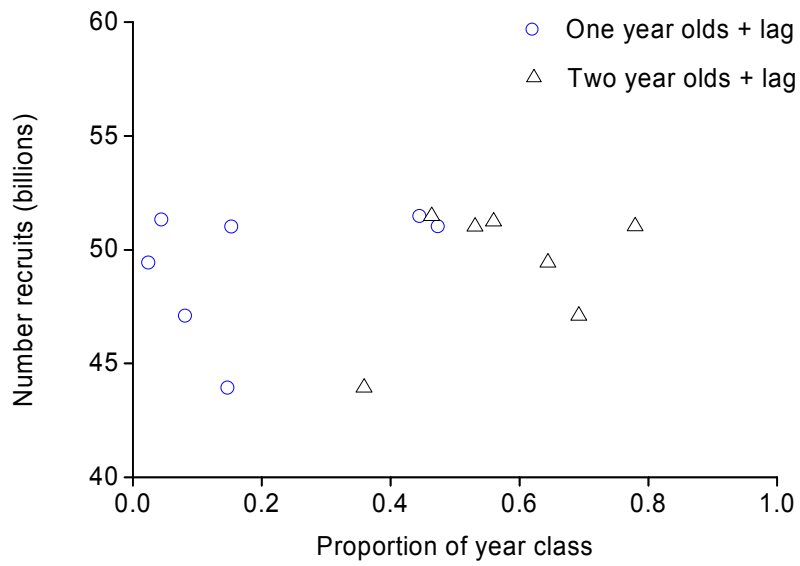
Figure 5.9 Relationship between 1 and 2 year old age class strength and recruitment estimated by the age structured stock assessment model with R ratio = 0.2, ages weighted at 0.01 and DEPM wt = 1 (full weighting).

5.4 Discussion

5.4.1 Age class progressions and biomass

The 2 year old age classes were consistently the first strong recruitment age class to support the fishery catches and were used as the recruitment age class. With the exception of two years the 1 year old age class comprised <20% of samples and 0+ year old fish were not present in catch samples. The strength of 2 year old age classes was not predicted by the strength of 1 year old age classes in the previous year unless a particularly strong 1 year old age class was present in the fishery. This only occurred in 1997 and 1999 when the fishery was recovering from the two mass mortality events. The strength of the 1 year old age classes in commercial samples decreased as the spawning biomass increased and the 2 year old age classes were prominent in samples during the recovery of the biomass from the two mass mortality events in 1995 and 1998. These strong recruitment age classes may have contributed to the rapid recovery of the stock. In summary, our analysis suggests commercial catch at age data are of limited use as an indicator of inter-annual variation in recruitment. Other suitable methods for determining fishery independent indices of recruitment may be needed if this information is required to manage the fishery in the future.

5.4.2 Age structured stock assessment model

Preliminary runs of the age structured stock assessment model predicted that the biomass would recover to between 250,000 and 300,000 tonnes by 2006 under high recruitment conditions. This was similar to the DEPM estimates, which showed a rapid recovery occurred following the last mass mortality event in 1998/1999 (Ward *et al.* 2004). The recovery of the spawning stock in South Australia was supported by the presence of strong 2 and 3 year old age classes that persisted in catch samples even after the second mortality event killed ~75% of the spawning stock. Between 1998 and 1999, the age composition predicted by the model was similar to the fishery independent samples and diverged from the commercial age structures. There was greater agreement between the model estimates and the observed age structure data between 2000 and 2001.

Uncertainties surrounding key parameters limited the usefulness of the age structured model as a tool for managing the South Australian Sardine Fishery. In particular, the lack of data on natural mortality rates, recruitment variability and biases in the age samples from the commercial catch limited the suitability of this model for predicting future biomass. Hence, annual DEPM estimates will remain the basis for managing the South Australian Sardine Fishery into the foreseeable future. However, the age structured model will be used to conduct a retrospective management strategy evaluation (MSE) of the

performance indicators, reference points and decision rules that have been established for the fishery (Shanks 2004).

6. GENERAL DISCUSSION

6.1 Reliable age determination

We implemented a series of simple methods to assign approximate ages to otoliths with poor readabilities using age-otolith weight relationships for otoliths with higher readabilities. This was similar to the approach outlined by Worthington *et al.* (1995) and represented a significant improvement in our methodologies. The main disadvantage of translucent zone counting methods was that only ~20% of otoliths were accepted due to poor readability and rejected otoliths represented a significant cost, in terms of lost laboratory time. It is important to note that the linear regressions between age and otolith weight should be updated annually to account for any temporal and spatial variability in these relationships. Cost-benefit analysis showed that using otolith weight-age relationships to construct age structures for sardine was both time and cost efficient. The time saved using age-otolith weight methods is especially relevant for fisheries laboratories that are responsible for producing several fishery assessment reports each year.

Although the annual deposition of opaque and translucent zones in otoliths from adult sardine was not validated directly via otolith marking in tank-held specimens, other indirect evidence showed that these structures reflected a measurable time scale. This evidence included the continuum in increasing otolith weight from juvenile to adult stages that paralleled formation of alternating opaque and translucent zones. Preliminary edge analyses showed translucent edges in otoliths from adult sardine were deposited over an extended period from early summer until mid-winter and deposition of opaque edges peaked during spring. This concurs with findings for sardine in California where opaque zones in sardine otoliths mostly formed in late spring (Barnes and Foreman 1994). The potential for error in this method due to subjectivity in classification of edge types and variability in light refraction between otoliths was acknowledged and controlled by not including any otoliths that were difficult to classify (Campana 2001). Edge analysis has now been incorporated into our standard otolith processing methodologies to allow further investigation of the seasonal patterns of zone deposition.

6.2 Otolith weight-age relationships

Rates of otolith weight deposition were higher (0.86 mg. in yr 1) during juvenile stages than during adulthood (0.23 mg.yr^{-1}). This change in the otolith weight-age relationship

may reflect physiological changes that occur as energy that is mostly used for growth during the juvenile stage is redistributed toward reproductive output during the onset of sexual maturity. The quantity of otolith weight deposited per year in adult sardine during this study was identical to findings in Western Australia (Fletcher and Blight 1996).

6.3 Problems associated with age determination

Estimates of ageing precision for otoliths from adult sardine (APE = 9.75-19.51%) were similar to findings during a similar study in California (APE = 16%, Butler *et al.* 1996). These analyses have been incorporated into our annual fishery assessment process. Inconsistencies in the position of the first translucent zone in some sardine otoliths led to confusion between readers and variability in age estimates that may be significant in such a short-lived and commercially exploited species. This illustrates the importance of otolith reader experience and the need for clear and consistent guidelines regarding the characteristics that underpin each level of the subjective readability index. This is critical when two separate agencies age the same otolith collection, as was the case during the preliminary stages of this study.

Age-specific marginal increment analysis was unsuitable for validating the seasonal deposition of translucent zones in sardine otoliths due to the difficulties involved in clearly defining the margins between successive zones. Unlike in other temperate species, there was a lack of seasonal oscillation in the development of the marginal increment in the 2 or 3 year old age classes. During a similar study, Gaughan and Mitchell (2000) also found that marginal increment analysis was unsuitable for validating the seasonal timing of zone deposition in otoliths from subtropical sardine *Sardinella lemuru.*

We gained a better understanding of the timing of deposition of the first translucent zone using daily increment counts in otoliths from juveniles. Waldron (1998) found the first translucent zone approximated 1 year of age, but may represent 13-17 months of age in sardine from South African waters. This issue was considered and accounted for when developing the algorithm for converting the translucent zone counts to a biologically meaningful age based on an assumed birth-date. It is important to note that the assumptions of a birth-date may have the negative implication of introducing an extra level of variability and uncertainty to the age dataset. The algorithm for adjusting the translucent zone counts will be refined when more information on the seasonality of otolith zone formation becomes available.

6.4 Growth rates and productivity

This was the first comprehensive study of the age and growth patterns of sardine from all life history stages in southern Australian waters. Sardine in South Australia achieve slightly larger maximum sizes (250 mm, TL) to those from other regions of southern Australia, but do not grow as large or as fast as those in the highly productive upwelling systems off southern Africa and the Americas.

The maximum and mean growth rates for sardine larvae recorded during this study in both upwelling and non-upwelling areas of the eastern Great Australian Bight were higher than those previously reported for southern Australia. The mean growth rate (0.56 mm.day^{-1}) for larvae in upwelling regions in the eastern Great Australian Bight was comparable to those observed in the productive boundary current systems off southern Africa and America but lower than for larvae off Japan (Castillio *et al.* 1985; Thomas 1986; Wantanabe and Kuroki 1997).

This study includes the first comprehensive investigation of the age and growth patterns and relationships between age and otolith weight for juvenile sardine in southern Australia. Small juveniles (40-70 mm, CFL) were particularly elusive and samples were mostly obtained from the gulfs. Average growth rates for juvenile sardine in southern Spencer Gulf (0.44 mm.day^{-1}) were lower than for those in South African waters (0.55-0.60 mm.day^{-1}) (Beckley and van der Lingen 1999). Instantaneous growth rates determined from the Laird-Gompertz growth curves for larvae and juveniles (0.59 mm.day^{-1}) in South Australia were also lower than for juvenile sardine in California (0.71 mm.day^{-1}) (Quinonez-Velazquez 2000).

Von Bertalanffy growth coefficients (k = 0.33-1.05 yr^{-1}) and asymptotic sizes (161-219 mm) for sardine in South Australia were higher than estimates for sardine in Western Australia (k = 0.58-0.81 yr^{-1}, L_∞= 164-174 mm) and lower than estimates in South African (k = 1.50 yr^{-1}, L_∞ = 306 mm) and southern Californian waters (k = 1.19 yr^{-1}, L_∞ = 205 mm) (Fletcher and Blight 1996; Butler *et al.* 1996; Beckley and van der Lingen 1999). Spatial and temporal differences in *VB* parameters for sardine within South Australian waters need to be treated with caution as differences in sample size and the distribution of age data can have a significant impact on the magnitude of these parameter estimates. Maximum likelihood methods of Kimura (1980) used to test differences between *VB* curves are statistically 'sensitive' and may not represent biologically relevant differences in growth patterns as discussed by Manning (2004).

6.5 Mortality events – effects on size and age structure

Sardine mass mortalities in 1995 and 1998 provided a rare opportunity to monitor changes in size and age structure following the events using fishery independent and commercial catch samples. Size and age structures of fishery independent samples collected in offshore shelf regions of the eastern Great Australian Bight and off Kangaroo Island after the second mortality diverged from those of the commercial catch samples from Spencer Gulf. Larger (>160 mm, CFL) older (≥3 years) sardine were dominant in offshore shelf regions during the recovery from the second mortality event. In contrast, analysis of samples from the commercial catch suggested the mortalities may have had negative impacts on the size and age structure of sardine and that the impacts were regionally and temporally divergent. Unfortunately, inadequacies in the sampling regimes made it difficult to make robust, spatial and temporal comparisons of size and age structures in Spencer Gulf and Coffin Bay after both mass mortalities.

Few juvenile sardine (<110 mm, CFL, 0+ year olds) were killed during the first mass mortality event that originated off Eyre Peninsula (Ward *et al.* 2001a). Modelling of the recovery of sardine populations in Western Australia suggested that if juveniles were not killed, spawning biomass rebuilding would only take ~5 years (Murray and Gaughan 2003). Size and age structure showed that during the second mortality event, juveniles were killed in Gulf St Vincent and in shelf waters, yet none were found dead in a small number of samples collected in Spencer Gulf. The lack of an impact on the size and age structure of commercial samples in Spencer Gulf following the second mass mortality suggests that significant quantities of adult sardine may have survived in this region, which may partly explain the rapid recovery of the spawning biomass in South Australian waters since 1999 (Ward *et al.* 2003a, b).

6.6 Commercial and independent samples – Do they represent the South Australian sardine population?

Egg abundance data collected during the DEPM spawning biomass surveys suggest the South Australian sardine population is spatially dynamic and the distribution of adults varies considerably between years. Therefore, age and growth parameters based on spatially limited, commercial size and age structure datasets are unlikely to be representative of the wider population. No information is currently available on sardine movement or the degrees of intermixing of sardine between regions. This information gap represents a major limitation when analysing these datasets and discussing possible implications of the findings. This study also highlighted the need for a direct comparison of

fishery independent and commercial sampling methods, as it is possible that net selectivity and differences in gear types may have biased size and age structure comparisons.

6.7 Age structured model – uncertainties

Use of the age structured stock assessment model for South Australian sardine has been problematic. Age structure data collected by commercial and fishery independent sampling were spatially limited and did not represent the age structure of the broader population. Age structures used in this model were from a population that had been affected by two large-scale, mass mortality events and recruitment estimates were based on assumed stock-recruitment relationships and the relative strengths of 2 year old age classes. Age structured stock assessment models may become useful for management of this fishery if reliable recruitment indicators can be developed and representative age structure data can be obtained for the South Australian sardine population. Unfortunately these objectives are likely to be difficult and costly to achieve. However, the age structured model will be used to evaluate the decision rules, performance indicators and reference points that are currently used to manage the fishery.

6.8 Recruitment

Schools of juveniles were difficult to locate during the initial stages of this project. The nursery areas we identified included semi-protected areas near small rocky islands in southern Spencer Gulf and sheltered bays.

Commercial sardine fishery catch at age data were of limited use as an indicator of recruitment. The lack of pre-recruitment (0+ year old) age classes in catches between 1995 and 2001 may have been due to the partial separation of juvenile habitat from the main areas of operation by the fishery. Our analysis showed the relationship between 1 and 2 year old age classes in the following year was poor and the strength of 2 year old year classes was not predicted by the strength of 1 year old age classes in the previous year unless particularly strong 1 year old age classes were recruited to the fishery. The proportion of 1 year old age classes in the catch decreased as the spawning biomass increased, which suggests density dependent effects and changes in environmental conditions may have influenced recruitment during the later part of the recovery from the second mass mortality event.

6.9 Improvements - refining biomass estimates

Estimates of sardine abundance obtained using acoustic techniques would provide useful additional information for management of the fishery. Acoustic surveys could be run within the main fishing areas in southern Spencer Gulf and have the potential to address pertinent questions, which include localised depletion, movement patterns, relationships with local scale oceanographic features and inter-annual recruitment variability.

Onboard continuous underway fish egg samplers (CUFES) collect sardine eggs while the research vessel is steaming along transects during the DEPM survey. CUFES are used by research agencies in South Africa and California to reduce the levels of uncertainty in spawning biomass estimates. Incorporating CUFES into the annual DEPM surveys has the potential to improve the precision of estimates of spawning area and egg production that are used to calculate spawning biomass in South Australia (van der Lingen 1998; Lo *et al.* 2001).

This study showed the age structured stock assessment model was not an appropriate stock assessment tool for the South Australian Sardine Fishery but was suitable for conducting an MSE of the decision rules for the fishery.

7. CONCLUSIONS

This report represents the first comprehensive analysis of the age and growth patterns of sardine during all life history stages in southern Australian waters and shows that using age-otolith weight relationships to construct age structures is both suitable and cost effective. The comparatively high growth rates observed for larvae, juvenile and adult sardine add to the growing body of evidence that shows that southern Australian coastal waters are not universally unproductive.

Investigation of size and age structures of sardine after the two mass mortalities showed there were spatial and temporal differences in the impacts of the two events. However, these differences need to be interpreted cautiously because the commercial samples were clearly not representative of the broader South Australian sardine population and the fishery independent samples were mostly collected in the eastern Great Australian Bight.

In the absence of reliable recruitment estimates and representative age structure data the age structured stock assessment model is unsuitable for managing the South Australian Sardine Fishery but may be suitable for evaluating the potential outcomes of different harvest rate strategies. DEPM spawning biomass estimates will continue to provide the basis for the management of the South Australian Sardine Fishery.

8. BENEFITS AND ADOPTION

This report provides:

- The first comprehensive analysis of the age and growth patterns of larval, juvenile and adult sardine in southern Australian waters. This is a significant improvement to our knowledge base on the longevity and growth patterns of sardine and provides a strong foundation for future research. These findings will benefit other state agencies that monitor the size, growth patterns and age structure of sardine from commercial catches, e.g. Western Australia and Victoria.

- Life history information necessary to establish an ecosystem-based model for the South Australian Sardine Fishery.

- Benefits to other Australian and international fisheries research agencies by outlining suitable methodologies for application of age-otolith weight relationships to determine age structures for other commercially important species, which have otoliths that are difficult to interpret.

- An assessment of an age structured stock assessment model for the South Australian Sardine Fishery in the absence of reliable recruitment information and representative age structure data.

Dissemination of results

- Southern Fisheries magazine articles by T.M. Ward.

- Preliminary age structure results were used to establish decision rules for setting the TACC for the South Australian Sardine Fishery. (PIRSA Fisheries).

- Peer reviewed journal articles based on chapters of this report are in preparation and review.

- Age and growth information was used in three sardine stock assessment reports submitted to PIRSA Fisheries in the period between 2000 and 2003.

- The 2004 Sardine Fishery Assessment report includes preliminary runs of the age structured stock assessment model.

9. FURTHER DEVELOPMENT

- Collect fisheries independent samples of sardine from the main fishing area in southern Spencer Gulf to allow comparison of size and age structures with samples from the commercial catch.

- Continue scaled down (10 aged, 50 otoliths weighed, all measured) monitoring of size and age structure from monthly commercial catch samples.

- Recalibrate age-otolith weight relationships annually to continue monitoring of the age structure as part of the annual sardine stock assessments.

- Directly compare size and age structure of samples collected using commercial and fishery independent methods to determine if sampling biases, such as mesh size selectivity and efficiency influence data used in the age structured stock assessment model.

- Investigate movement patterns of sardine in and out of southern Spencer Gulf to determine the extent of localised depletion in the main fishing area.

- Investigate the timing of deposition of the first translucent zone in juvenile/sub-adult sardine.

- Validate the seasonal timing of deposition of translucent zones in adult sardine using otolith marking of tank-held specimens.

REFERENCES

Arrenius, F. and Hansson, S. (1993). Food consumption of larval, young and adult herring and sprat in the Baltic Sea. *Marine Ecology Progress Series* **96**: 125-137.

Barnes, J. T. and Foreman, T.J. (1994). Recent evidence for the formation of annual growth increments in the otoliths of young Pacific sardines (*Sardinops sagax*). *California Fish and Game* **80(1)**: 29-35.

Beamish, R. and Fournier, D. A. (1981). A method for comparing the precision of a set of age determinations. *Journal of the Fisheries Research Board of Canada* **36**: 1395-1400.

Beckley, L. E. and van der Lingen, C. D. (1999). Biology, fishery and management of pilchards (*Sardinops sagax*) in southern African waters. *Marine and Freshwater Research* **50**: 955-978.

Blaxter, J. H. and Hunter, J. R. (1982). The Biology of Clupeoid Fishes. *Advances in Marine Biology* **20**: 1-223.

Brown, P. C. (1984). Primary production at two contrasting near shore sites in the southern Benguela upwelling region, 1977-1979. *South African Journal of Marine Science* **2**: 205-215.

Bruce, B. D. and Short, D. A. (1990). Observations on the distribution of larval fish in relation to a frontal zone at the mouth of Spencer Gulf, South Australia. *Bureau of Rural Resource Proceedings* **15**: 124-137.

Bunce, A. (2004). Improving fisheries sustainability: using seabirds to manage marine resources. 2004 ASFB Conference: Fisheries Ecosystem Symposium. 19-24 September, Adelaide, South Australia.

Butler, J. L., Granados, G. M. L., Tomas Barnes, J., Yaremko, M., and Macewicz, B. J. (1996). Age composition, growth, and maturation of the Pacific sardine (*Sardinops sagax*) during 1994. *CalCOFI Rep.* **37**: 152-159.

Campana, S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology.* **59**: 197-242.

Cardinale, M. and Arrhenius, F. (2004). Using otolith weight to estimate the age of haddock (*Melanogrammus aelefinus*): a tree model application. *J. Appl. Ichthyol.* **20**. 470-475.

Castillo, G., Aguilera, E., Herrera, G., Bernal, P. A., Butler, J. L., Chong, J., Gonzalez, H., Oyarzun, C. and Veloso, C. (1985). Larval growth rates of the Pacific sardine *Sardinops sagax* off central Chile, determined by counts of daily rings in otoliths. *Biologia Pesquera* **14**: 3 - 10.

Cochrane, K. L. (1999). Review of the Western Australian Pilchard Fishery. No. 129. 28pp.

Escribano, R. and Hidalgo, P. (2000). Spatial distribution of copepods in the north of the Humboldt Current region off Chile during coastal upwelling. *Journal of the Marine Biological Association of the United Kingdom* **80**: 283-290.

Ewing, G. P., Welsford, D. C., Jordan, A. R., and Buxton, C. (2003). Validation of age and growth estimates using thin otolith sections from the purple wrasse, *Notolabrus fucicola. Marine and Freshwater Research* **54**: 985-993.

Fishbase (2004). FishBase Website. www.fishbase.org/search.cfm.

Fletcher, W. J. (1991). A test of the relationship between otolith weight and age for the pilchard *Sardinops neopilchardus. Canadian Journal of Fisheries and Aquatic Science* **48**: 35-38.

Fletcher, W. J., Tregonning. R. J. and Sant, G. J. (1994). Interseasonal variation in the transport of pilchard eggs and larvae off southern Western Australia. *Marine Ecology Progress Series* **111**: 209 - 224.

Fletcher, W. J. (1995). Application of the otolith weight-age relationship for the pilchard, *Sardinops sagax neopilchardus. Canadian Journal of Fisheries and Aquatic Science* **52**: 657-664.

Fletcher, W. J. and Blight, S. J. (1996a). Validity of using translucent zones of otoliths to age the pilchard *Sardinops sagax neopilchardus* from Albany, Western Australia. *Marine and Freshwater Research* **47**: 617-624.

Fletcher, W. J., Hayes, E. A., Tregonning, R. J., and Blight, S. J. (1996b). Use of the daily egg production method to estimate the stock size of Western Australian sardines (*Sardinops sagax*). *Marine and Freshwater Research* **47**: 819-825.

Fowler, A. J. and Doherty, P. J. (1992). Validation of annual growth increments in the otoliths of two species of damselfish from the southern Great Barrier Reef. *Australian Journal of Marine and Freshwater Research* **43**: 1057-1068.

Fowler, A. J. and Short, D. A. (1998). Validation of age determination from otoliths of the King George whiting *Silliginodes punctata* (Perciformes). *Marine Biology* **130**: 577-587.

Francis, C. and Campana, S. (2004). Estimating age from otolith measurements: A review and a new approach. (Presentation/Abstract). Third International Symposium on Fish Otolith Research and Application. pg 97.

Gaughan, D. J., Mitchell, R. W. D., (2000). The biology and stock assessment of the tropical sardine, *Sardinella lemuru*, off the mid-west coast of Western Australia. Final Report to FRDC. Fisheries Western Australia: North Beach. No. 119. 135 pp.

Gaughan, D. J., Baudains, G. A., Mitchell, R. W. D., and Leary, T. I. (2001a). Pilchard (*Sardinops sagax*) nursery areas and recruitment process assessment between different regions in southern Western Australia. Western Australia Fisheries Report **131**: 1-44.

Gaughan, D. J., Fletcher, W. J., and White, K. V. (2001b). Growth rate of larval *Sardinops sagax* from ecosystems with different levels of productivity. *Marine Biology* **139**: 831-837.

Green, C., Robertson, S. and Golub, K. K. (2002) Age and growth of orange roughy *Hoplostethus atlanticus* from the southern Tasman Rise. MAFRI Report. Queenscliff, Victoria. 18 pp.

Griffin, D. A., Thompson, P. A., Bax, N. J., and Hallegraeff, G. M. (1997). The 1995 mass mortality of pilchards: no role found for physical or biological oceanographic factors in Australia. *Marine and Freshwater Research* **48**: 27-58.

Hayashi, A., Yamashita, Y., Kawaguchi, K., and Ishii, T (1989). Rearing method and daily otolith ring of Japanese sardine larvae. *Nippon Suisan Gakkaishi* **55**: 997-1000.

Hilborn, R., Pikitch, E. K., and McAllister, M. K. (1994). A Bayesian estimation and decision analysis for an age-structured model using biomass survey data. *Fish. Res* **19**: 17-30.

Hoedt, F. E. (2002) Growth in eight species of tropical anchovy determined from primary otolith increments. *Marine and Freshwater Research,* **53***: 859-867.*

Hyatt, A. D, Hine, P. M. Whittington, D. A., and Bax, N. J. (1997). Mass mortality in the pilchard (*Sardinops sagax neoplichardus*) in Australia and New Zealand in 1995. II Identification of the herpesvirus in the gill epithelium. *Diseases of Aquatic Organisms* **28**: 17-29.

Hyndes, G. A., Lonerangan, N. R. and Potter, I. C (1992). Influence of sectioning otoliths on marginal increment trends and age and growth estimates for the flathead *Platycephalus speculator*. *Fishery Bulletin* **90**: 276-284.

Kämpf, J., Doubell, M., Griffin, D., Matthews, R. L. and Ward, T. M. (2004). Evidence of a Large Seasonal Coastal Upwelling System along the Southern Shelf of Australia. *Journal of Geophysical Research-Letters* (in press).

Kerstan, M. (2000). Estimation of precise ages from the marginal increment widths of differently growing sardine (*Sardinops sagax*) otoliths. *Fisheries Research* **46:** 207-225.

Kimura, D. K. (1980). Likelihood methods for the von Bertalanffy growth curve. *Fishery Bulletin* **77**: 765-776.

Lewis, R. K. (1981). Seasonal upwelling along the southeastern coastline of South Australia. *Australian Journal of Marine and Freshwater Research* **32**: 843 - 54.

Mackie, D. W. (1995). A management plan for the experimental sardine fishery. *Australian Fisheries Management Series* **13**: 1-19.

Lo, C. H. N., Hunter, J. R. and Charter, R. C. (2001) Use of a continuous underway fish egg sampler for ichthyoplankton surveys: application to the estimation of daily egg production of Pacific sardine (*Sardinops sagax*). *Fish Bull.* 99 554-571.

Mann, K. H. and Lazier. J. R. N. (1996). Dynamics of Marine Ecosystems. London, Blackwell Science. 394 pp.

Manning, M. J. (2004). Comparing Growth Curves: Statistical significance is not the same as biological significance. (Presentation/Abstract). Third International Symposium on Fish Otolith Research and Application. p 107.

Maunder, M. N. and Deriso, R. B. (2003). Estimation of recruitment in catch at age methods. *Canadian Journal of Fisheries and Aquatic Science* **60:** 1204-1216.

McClatchie and Ward, T.M. (accepted). Alongshore variation in upwelling intensity in the eastern Great Australian Bight. *Journal of Geophysical Research-Oceans.*

Middleton, J. F. and Cirano, M. (2002). A northern boundary current along Australia's southern shelves: the Flinders Current. *Journal of Geophysical Research-Oceans* **107**: 3129.

Misund, O. A., Coetzee, J. C., Freon, P., Gardener, M., Olsen, K., Svellingen, I. and Hampton, I. (2003). Schooling behaviour of sardine *Sardinops sagax* in False Bay, South Africa. *African Journal of Marine Science* **25**: 185-193.

Motoda, S., Kawamura, T. and Taniguchi, A. (1978). Differences in productivities between the Great Australian Bight and the Gulf of Carpentaria. *Marine Biology* **46**: 93-99.

Murray, A. G. and Gaughan, D. J. (2003). Using an age-structured model to simulate the recovery of the Australian pilchard (*Sardinops sagax*) population following epidemic mass mortality. *Fisheries Research* **60**: 415-426.

Murrray, G.M., O'Callaghan, M.O., and Jones, B. (2003). A model of spatially evolving herpesvirus epidemics causing mass mortality in Australian pilchard *Sardinops sagax. Diseases of Aquatic Organisms* **54**: 1-14.

Nakata, K., Zenitani, H. and Inagake, D. (1995). Differences in food availability for Japanese sardine larvae between the frontal region and the waters on the offshore side of Kuroshio. *Fisheries-Oceanography* **4**: 68 - 79.

Neira, F. J., Miskiewicz, A. G., and Trnski, T. (1998). Larvae of Temperate Australian Fishes. Laboratory Guide for Larval Fish Identification. University of Western Australia Press. 474pp.

Pearce, A., Helleren, S. and Marinelli, M. (2000). Review of productivity levels of Western Australia coastal and estuarine waters for mariculture planning purposes. Fisheries Western Australia Research Report No. 123. Fisheries Western Australia. Perth.

Peterson, W. T., Arcos, D. F., McManus, G. B., Dam, H., Bellantoni, D., Johnson, T. and Tiselius, P. (1988). The Nearshore Zone during Coastal Upwelling: Daily Variability and Coupling between Primary and Secondary Production off Central Chile. *Progress in Oceanography* **20**: 1-40.

Quinonez-Velazquez, C., Nevarez-Martínez, M. O., and Gluyas-Millán, M. G. (2000). Growth and hatching dates of juvenile pacific sardine *Sardinops caeruleus* in the Gulf of California. *Fisheries Research* **48**: 99-106.

Re, P. (1984). Evidence of daily and hourly growth in pilchard larvae based on otolith growth increments, *Sardina pilchardus* (Walbaum 1792). *Cybium* **8**: 33-38.

Rogers, P. J., Geddes, M., and Ward, T. M. (2003). Blue sprat *Spratelloides robustus* (Clupeidae: Dussumerieriinae): a temperate clupeoid with a tropical life history strategy. *Marine Biology* **142**: 809-824.

Rogers, P.J., Stephenson, P., McClatchie, S., Dimmlich, W.F., McLeay, L.J., and Ward.,T.M. (2004). Sardine (*Sardinops sagax*). Stock Assessment. Report to Primary Industries and Resources South Australia. South Australian Research and Development Institute (Aquatic Sciences).

Schahinger, R. (1987). Structure of coastal upwelling events observed off the south-east coast of South Australia. *Australian Journal of Marine and Freshwater Research* **38**: 439-459.

Schwartzlose, R. A., Alheit, J., Bakun, A., Baumgartner, T. R., Cloete, R., Crawford, R. J. M., Fletcher, W. J., Green-Ruiz, Y., Hagen, E., Kawasaki, T., Lluch-Belda, D., Lluch-Cota, S. E., MacCall, A. D., Matsuura, Y., Nevarez-Martinez, M.O., Parrish, R.H., Roy, C., Serra, R., Shust, K.V., Ward, M.N. and Zuzunagza, J.Z. (1999). Worldwide large scale fluctuations of sardine and anchovy populations. *South African Journal of Marine Science* **21**: 289-347.

Shanks, S. (2004). Ecological Assessment of the South Australian Pilchard Fishery. South Australian Fisheries Management Series. Primary Industries and Resources South Australia. Adelaide; South Australia. 51pp.

Small, L. F. and Menzies. D. W. (1981). Patterns of primary productivity and biomass in a coastal upwelling region. *Deep Sea Research* **28**: 123-149.

Stephenson, P. (2004). . Use of otolith weight in the determination of stock size. Third International Symposium on Fish Otolith Research and Application. Abstract, p. 40.

Thomas, R. M. (1986). Growth of larval pelagic fish in the south-east Atlantic from daily otolith rings in 1982/83 and 1983/84. *South African Journal of Marine Science* **4**: 61-77.

Van der Lingen, C. D., Checkley, JR, D., Barange, M., Hutchings, L. and Osgood, K. (1998). Assessing the abundance and distribution of eggs of sardine, *Sardinops sagax*, and round herring, *Etrumeus whiteheadi*, on the western Agulhas Bank, South Africa, using a continuous underway fish egg sampler. Fisheries Oceanography, **7:1:** 35-47.

Verheye, H. M., Hutchings, L., Huggett, J. A. and Painting, S. J. (1992). Mesozooplankton dynamics in the Benguela ecosystem, with emphasis on the herbivorous copepods. South *African Journal of Marine Science* **12**: 561-584.

Waldron, M. (1998). Annual ring validation of the South African sardine *Sardinops sagax* using daily growth increments. *South African Journal of Marine Science* **19:** 425-430.

Ward, T. M., Hoedt, F. E., McLeay, L., Dimmlich, W. F., Kinloch, M., Jackson, G., McGarvey, R., Rogers, P. J., and Jones, K. (2001a). Effects of the 1995 and 1998 mass mortality events on the spawning biomass of sardine, *Sardinops sagax*, in South Australian waters. *ICES Journal of Marine Science* **58:** 865-875.

Ward, T. M., Hoedt, F., McLeay, L. J., Dimmlich, W. F., Jackson, G., Rogers, P. J. and Jones. K. (2001b). Have recent mass mortalities of the sardine *Sardinops sagax* facilitated an expansion in the distribution and abundance of the anchovy *Engraulis australis* in South Australia? *Marine Ecology Progress Series* **220**: 241-251.

Ward, T. M. and Staunton-Smith. J. (2002). Comparison of the spawning patterns and fisheries biology of the sardine (*Sardinops sagax*) in temperate South Australia and sub-tropical southern Queensland. *Fisheries Research* **56**: 37-49.

Ward, T. M., Rogers, P. J., McLeay, L., Schmarr, D., and Dimmlich, W. F. (2003a). Sardine (*Sardinops sagax*). Stock Assessment. Report to Primary Industries and Resources South Australia. South Australian Research and Development Institute (Aquatic Sciences). **RD03/0198.** 72pp.

Ward, T. M., McLeay, L., Rogers, P. J., and Dimmlich, W. F. (2003b). Spawning biomass of sardine (*Sardinops sagax*) in South Australia in 2003. Report to Primary Industries and Resources South Australia. South Australian Research and Development Institute (Aquatic Sciences) **RD03/0195.** 32pp.

Ward, T. M., McLeay, L. J., and McClatchie, S. (2004). Spawning Biomass of Pilchard (*Sardinops sagax*) in South Australia in 2004. Report to Primary Industries and

Resources South Australia. South Australian Research and Development Institute (Aquatic Sciences). **RD04/0153.** 33 pp.

Watanabe, Y. and Kuroki, T. (1997). Asymptotic growth trajectories of larval pilchard (*Sardinops melanostictus*) in the coastal waters off western Japan. *Marine Biology* Berlin. **127**: 369-378.

Worthington, D. G., Doherty, P. J., and Fowler, A. J. (1995). Variation in the relationship between otolith weight and age: implications for the estimation of age of two tropical damselfish (*Pomocentrus moluccensis* and *P. wardi*). *Canadian Journal of Fisheries and Aquatic Science* **52**: 233-242.

Xiao, Y. (1996). How does somatic growth rate affect otolith size in fishes? *Canadian Journal of Fisheries and Aquatic Science* **53**: 1675-1682.

APPENDIX 1

Intellectual Property and Valuable Information

During the course of this project no applications for patents were made. This publication is protected by copyright. Apart from any use as permitted under the *Copyright Act* 1968, no part may be reproduced without written permission.

APPENDIX 2.

Principal research staff

Name	Position	Responsibilities
Dr. Tim Ward	Principal Investigator and senior scientist SARDI Aquatic Sciences	Staff supervision, managed budget. Reported on project results. Prepared project milestones and final report.
Paul Rogers	Research Scientist/technical officer SARDI Aquatic Sciences Adelaide University	Preparation of final report Laboratory and fieldwork.
Dr. Peter. Stephenson	Fisheries Modeller (Western Australian Fisheries)	Developed age structured stock assessment model for sardine. (Ch. 5).
David Schmarr	Technical officer SARDI Aquatic Sciences	Laboratory and fieldwork. Prepared figures in Ch. 4. Aged some juveniles and all adults. Developed decimal age algorithm (Ch. 2).
Wetjens Dimmlich	Technical officer/ student/ research scientist SARDI Aquatic Sciences Adelaide University	Developed sardine fisheries database and collected samples on research cruises.
Lachlan McLeay	Technical officer SARDI Aquatic Sciences	Laboratory and fieldwork. Collected biological samples during the 1998/9 mortality event.
Nathan Strong	Technical officer/student SARDI Aquatic Sciences Adelaide University	Laboratory and fieldwork. Student-Larval sardine project.
Megan Westlake	Technical officer SARDI Aquatic Sciences	Laboratory and fieldwork. Collected biological samples during the 1998/9 mortality event.