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Spawning biomass of sardine, *Sardinops sagax*, in waters off South Australia in 2014

Ward, T.M., Ivey, A.R. and Carroll, J.D.

SARDI Publication No. F2007/000566-6
SARDI Research Report Series No. 807

SARDI Aquatics Sciences
PO Box 120 Henley Beach SA 5022

October 2014

Report to PIRSA Fisheries and Aquaculture

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

SOUTH AUSTRALIA

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This publication may be cited as:
Ward, T.M., Ivey, A.R. and Carroll, J.D. (2014). Spawning biomass of sardine, *Sardinops sagax,* in waters off South Australia in 2014. Report to PIRSA Fisheries and Aquaculture. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2007/000566-6. SARDI Research Report Series No. 807. 33pp.

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Printed in Adelaide: October 2014

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Date: 31 October 2014

Distribution: PIRSA Fisheries and Aquaculture, SAASC Library, University of Adelaide Library, Parliamentary Library, State Library and National Library

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

We thank the master and crew of the *RV Ngerin*, Chris Small, Jason Nicol and Andrew Sellick for their assistance during the research surveys. Mr. Chris Leigh (University of Adelaide Anatomy Department) conducted the histological analyses of sardine ovaries. Matt Lloyd, Owen Burnell, Lorenzo Andreacchio, Graham Hooper, and Bruce Jackson (SARDI Aquatic Sciences) provided technical assistance during the surveys. Alex Dobrovolskis helped dissect the adult samples. Dr Rick McGarvey provided advice about the egg production bootstrap routine. Dr Mike Steer and Dr Stephen Mayfield reviewed and provided valuable comments on a draft of the report. The report was approved for publication by Professor Gavin Begg.

PREFACE

The Daily Egg Production Method (DEPM) has been used to assess the stock status of sardine, *Sardinops sagax*, in South Australian waters since 1995. The estimate of spawning biomass obtained using this method is the key biological performance indicator for the South Australian Sardine Fishery (SASF). The present report provides an estimate of the spawning biomass of sardine in waters off South Australia in February-April 2014.

EXECUTIVE SUMMARY

This report provides an estimate of the spawning biomass of sardine, *Sardinops sagax,* in waters off South Australia in 2014.

Surveys were conducted from the *RV Ngerin* between February and April 2014. The total survey area was 122,437 km^2.

Sea surface temperatures (SSTs) ranged from 14.7 to 22.1°C. Low SSTs and elevated concentrations of chlorophyll-*a* were recorded in coastal waters of the western Eyre Peninsula, reflecting the occurrence of seasonal upwelling during the surveys.

A total of 7,955 live sardine eggs were collected from 355 sites. The majority of eggs were collected from shelf waters of the eastern and central Great Australian Bight.

The spawning area (*A)* in 2014 of 71,859 km^2 was the highest recorded off South Australia.

Mean daily egg production (P_0) was 85.0 $eggs.day^{-1}.m^{-2}$ (95% CI = 60.9 – 120.2 $eggs.day^{-1}.m^{-2}$).

Nine samples comprising 1,486 mature fish were collected at three inshore locations; no adult samples were collected from offshore waters where most spawning occurred.

Estimates of mean adult reproductive parameters were: female weight, *W* = 47.4 g (95% CI = 41.7 – 53.0); batch fecundity, *F* = 17,133 hydrated oocytes (95% CI = 14,431 – 19,903); sex ratio, *R* = 0.57 (95% CI = 0.53 – 0.61); spawning fraction, *S* = 0.040 (95% CI = 0.028 – 0.055).

These estimates of reproductive parameters are unlikely to be representative of adult sardines occurring in offshore waters, which are often larger and spawn more frequently than those found inshore. The absence of reliable estimates of spawning fraction is of particular concern as this parameter has a strong influence on estimates of spawning biomass.

The large spawning area observed during this study provides strong evidence that the spawning biomass in 2014 was large. However, the lack of adult samples from offshore sites prevented reliable estimation of population size. The spawning biomass estimate obtained using the 2014 value of spawning fraction (0.04) was >700,000 t, which is unlikely. Using the maximum and mean spawning fractions for 1998 to 2013 (0.12 and 0.18) provides spawning biomass estimates of ~166,000 and ~240,000 t, respectively. However, as adult samples have not been collected from offshore waters of South Australia the suitability of these values is unknown.

There is an urgent need to establish a reliable method for sampling adult sardines in offshore waters of South Australia to ensure that future estimates of spawning biomass are robust.

1. INTRODUCTION

1.1 Daily Egg Production Method

The Daily Egg Production Method (DEPM) was developed for stock assessment of the northern anchovy, *Engraulis mordax* (Parker 1980; Lasker 1985), and has been applied to at least 18 species of small pelagic fishes worldwide (Stratoudakis *et al.* 2006; Neira and Lyle 2008; Dimmlich *et al.* 2009; Ward *et al.* 2009). The method is widely used because it is often the most practical option available for stock assessment of small pelagic species.

The DEPM relies on the premise that the biomass of spawning adults can be calculated by dividing the mean number of pelagic eggs produced per day throughout the spawning area (i.e. total daily egg production) by the mean number of eggs produced per unit *mass* of adult fish (i.e. mean daily fecundity; Lasker 1985). Total daily egg production is the product of mean daily egg production (P_0) and total spawning area (A). Mean daily fecundity is calculated by dividing the product of mean sex ratio (by weight, R), mean batch fecundity (number of oocytes in a batch, F), mean spawning fraction (proportion of mature females spawning each day/night, S) and mean female weight (W). Spawning biomass (SB) is calculated according to the equation:

$$SB = P_0.A / (R.F.S / W).$$

Equation 1

The DEPM can be applied to fishes that spawn multiple batches of pelagic eggs over an extended spawning season (e.g. Parker 1980). Data used to estimate DEPM parameters are typically obtained during fishery-independent surveys involving vertical plankton tows at sites located at regular intervals along parallel cross-shelf transects. Adult samples are often taken opportunistically during the survey and may be complemented by samples collected concurrently from commercial vessels (Stratoudakis *et al.* 2006). The key assumptions of the DEPM are that: 1) surveys are conducted during the main (preferably peak) spawning season; 2) the entire spawning area is sampled; 3) eggs are sampled without loss and identified without error; 4) levels of egg production and mortality are consistent across the spawning area; and 5)

representative samples of spawning adults are collected during the survey period (Parker 1980; Alheit 1993; Hunter and Lo 1997; Stratoudakis *et al.* 2006).

The DEPM is used widely but a range of challenges have been encountered and estimates of spawning biomass are generally considered to be accurate (unbiased) but relatively imprecise (e.g. Alheit 1993; Hunter and Lo 1997; Stratoudakis *et al.* 2006). There are considerable uncertainties associated with the estimation of P_0 and S in particular (Fletcher *et al.* 1996; McGarvey and Kinloch 2001; Ward *et al.* 2001a, b; Gaughan *et al.* 2004). For example, P_0 has been determined using a variety of statistical approaches. Ward *et al.* (2011) showed that these approaches provide very different estimates of P_0 and suggested that the log-linear model of Piquelle and Stauffer (1985) should be used because it fits strongly over-dispersed sardine egg density data better and provides more logically consistent and precautionary estimates of P_0 than the exponential mortality model and most generalized linear models. Bernal *et al.* (2011) suggested using an "all years" estimate of mortality to estimate egg production which reduces the number of degrees of freedom in each yearly regression but loses information about inter-annual variations in mortality. FRDC Project 2014/026 is currently evaluating a variety of methods for estimating egg production.

S is often the most difficult DEPM parameter to estimate for clupeoids. Obtaining representative samples of adults can be difficult because during the spawning period spawning females are over-represented in ephemeral spawning aggregations and under-represented in the remainder of the population (Stratoudakis *et al.* 2006). Much of the uncertainty surrounding estimates of S is associated with determining whether imminent or recent spawners or both should be used in calculations. However, the size and reproductive characteristics of clupeoids can also vary spatially and temporally and it is critical that the design of the adult sampling program adequately addresses these issues.

1.2 Application of the DEPM off South Australia

The DEPM has been used to estimate the spawning biomass of sardine, *Sardinops sagax*, in South Australian waters since 1995 (Ward *et al.* 1998; 20011a). Application of this method has facilitated the rapid and sustainable development of the South Australian Sardine Fishery (SASF), despite the effects of two mass mortality events that both killed over 70% of the adult population of sardine in waters off South Australia (e.g. Ward *et al.* 2001a; 2011a).

The need to establish an effective method for sampling adult sardine in offshore waters of South Australia has been identified as a high priority in several previous spawning biomass reports (e.g. Ward et al. 2013) and was a key finding of an international workshop on small pelagic fisheries held in Adelaide in July 2014 (Ward, unpublished data).

1.3 Aim and Objectives

This report provides an estimate of the spawning biomass of sardine in gulf and shelf waters of SA during February-April 2014. The objectives of the report are:

1. To describe the distribution and abundance of sardine eggs in relation to environmental variables;
2. To estimate DEPM parameters (*A*, *P*, *W*, *R*, *F*, *S*);
3. To use the DEPM to estimate the spawning biomass in 2014;
4. To evaluate the uncertainty associated with this assessment and make recommendations regarding future research needs.

2. METHODS

2.1 Study Area and Biophysical Variables

2.1.1 Study area

Two surveys were conducted aboard the *RV Ngerin* in shelf and gulf waters of South Australia between February and April 2014. Plankton samples were collected at 355 sites on 34 transects between Victor Harbor and Head of Bight (Fig. 1). Of these 355 samples, 15 were additional to the pre-determined survey. In these cases, additional samples were taken on the seaward end of transects when sardine eggs were observed in the Continuous Underway Fish Egg Sampler (CUFES, Fig. 1).

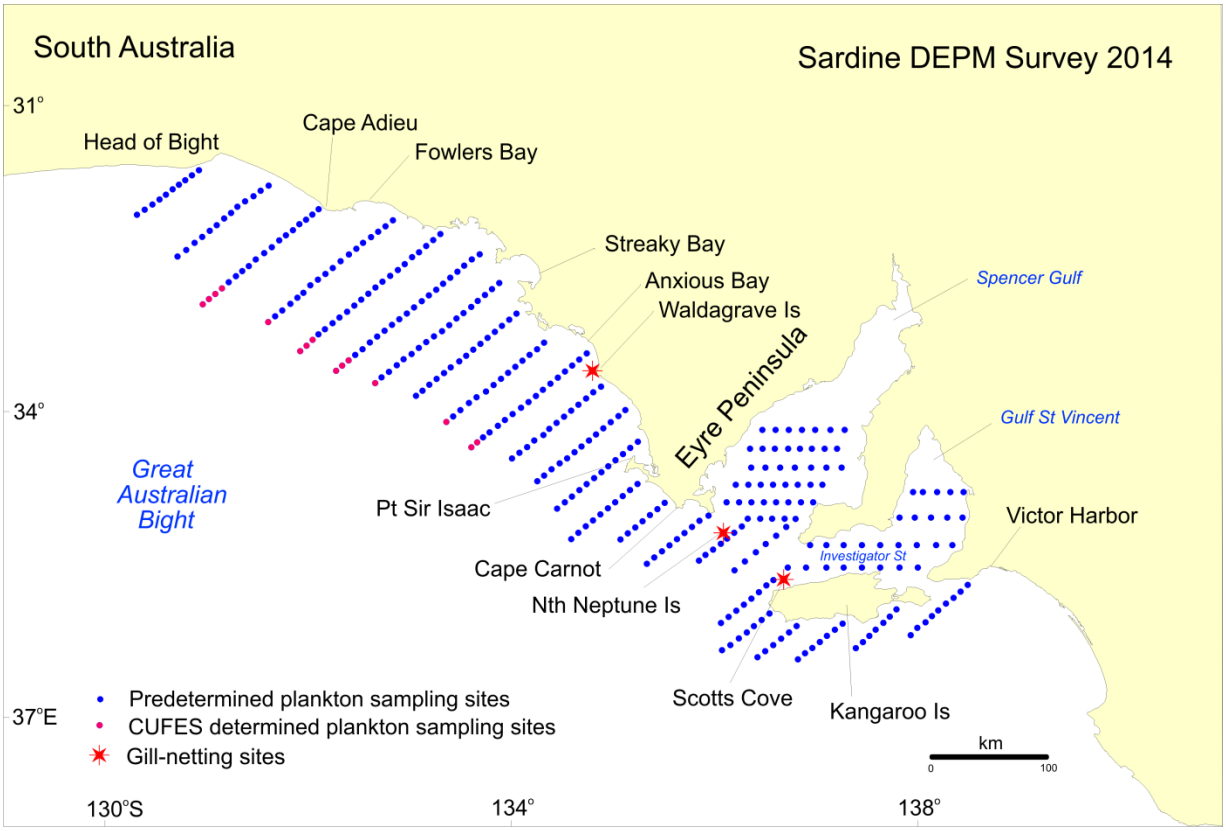

Figure 1. Map of South Australia showing sites where plankton and adult samples were collected during the 2014 DEPM surveys.

2.1.2 Water temperature and primary production

At each site (Fig. 1), a *Sea-Bird* Conductivity-Temperature-Depth (CTD) recorder fitted with a fluorometer was lowered to a depth of 70 m, or to 10 m from the bottom in waters less than 80 m deep. Estimates of water temperature and fluorescence at a depth of 3 m were extracted from each profile. Where CTD temperature was absent a correction factor was applied to the on-board temperature measurement (average difference between CTD and on-board temperature). Fluorescence is an indicator of primary production and gives an un-calibrated measure of chlorophyll-*a* concentration ($\mu g.L^{-1}$). Spatial plots of SST and chlorophyll-*a* concentration were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

2.1.3 Secondary production – zooplankton abundance

An index of zooplankton abundance at each site was estimated by dividing the displacement volume of zooplankton (ml) collected during plankton tows by the total volume of water filtered (m^3). Spatial plots of zooplankton abundance were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

2.2 Daily Egg Production and Spawning Area

2.2.1 Plankton sampling

Plankton samples were collected at each site using paired Californian Vertical Egg Tow (CalVET) plankton nets. Each CalVET net had an internal diameter of 0.3 m, 330 µm mesh and plastic cod-ends. During each tow the CalVET nets were deployed to within 10 m of the seabed at depths <80 m or to a depth of 70 m at depths >80 m and retrieved vertically at a speed of ~1 $m.s^{-1}$. General Oceanics™ 2030 flow-meters and factory calibration coefficients were used to estimate the distance travelled by the net during each tow. Where there was a discrepancy of more than 500 units between flow-meters, the relationship between wire length released and flow-meter units was used to determine which was correct and that value repeated. Upon retrieval of the nets the samples from each of the two cod-ends were washed into a sample container. Plankton samples were fixed using 5% buffered formaldehyde and seawater.

2.2.2 Laboratory analysis

Sardine eggs and larvae were identified in each sample using published descriptions (White and Fletcher 1996; Neira *et al.* 1998). Eggs in each sample were counted, staged and assigned approximate ages based on descriptions and temperature-development keys in White and Fletcher (1996).

2.2.3 Egg density

The number of eggs of each day class under one square metre of water (P_t) was estimated at each site according to Equation 2:

$$P_t = \frac{C.D}{V} \qquad \text{Equation 2}$$

where, C is the number of eggs of each age in each sample, V is the volume filtered (m^3), and D is the depth (m) to which the net was deployed (Smith and Richardson 1977). Plots of egg distribution and abundance were prepared using Surfer® (Ver. 8).

2.2.4 Spawning time and density weightings

The development time of sardine eggs is dependent on water temperature (Picquelle and Stauffer 1985). Kernel density methods were used to estimate the modal time of egg abundance for three categories of SST (Ward *et al.* 2011a). A peak spawning time of 2:00 am was established based on the assumption that Stage 2 eggs are approximately 3-4 hours old. In waters <19.0^{o}C, 19.0-20.0^{o}C and >20.0^{o}C, Stages 1-6, 1-7 and 1-8 were less than 24 hours old, respectively, and Stage 7-12, 8-12 and 9-12 eggs were 24-48 hours old. Ages were assigned to day-1 eggs (i.e. 0 – 24 hours old) by subtracting the estimated spawning time from the sampling time. Ages of day-2 eggs were assigned similarly, but an additional 24 hours were added to their ages. To prevent miss assignment to day class for young eggs sampled in the two hours prior to the 2:00 am spawning time, young eggs taken between 12 and 2 am were assigned an

age of one minute. Densities of day-1 and day-2 eggs were weighted according to the relative size of the area from which they were taken.

2.2.5 Spawning area

The Voronoi natural neighbour (VNN) method (Watson 1981) was applied using the statistical package 'R' (Baddeley and Turner 2005; R Development Core Team 2014) to generate a polygon around each sampling site with the boundary as the midpoint equidistant between each sampling site (Fig. 2). The area represented by each site (km^2) was then determined. The spawning area (*A*) was defined as the total area of grids where live sardine eggs were found.

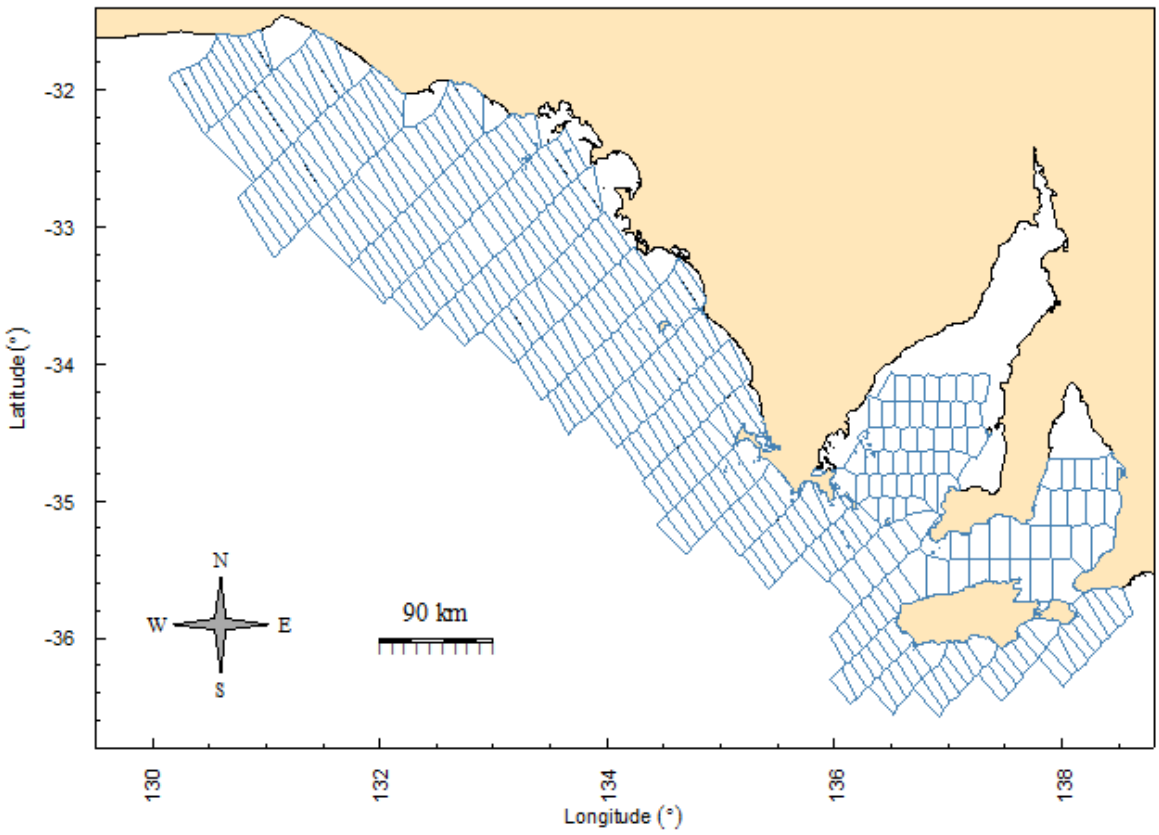

Figure 2. Voronoi nearest neighbour polygons generated in R, used to estimate the total spawning area in 2014.

2.2.6 Daily egg production (P_0) and egg mortality

Mean daily egg production (P_b) was calculated by fitting the linear version of the exponential egg mortality model to estimates of egg age and density at each site (Picquelle and Stauffer 1985). To allow the inclusion of data from sites where either day 1 or day 2 eggs were absent, one egg was added to the counts of both day 1 and day 2 eggs at every positive site. The linear version of the exponential egg mortality model is:

$$lnP_b = \ln(P_i) - Zt \qquad \text{Equation 3}$$

where, P_i is the density of eggs of age t at site i and Z is the instantaneous rate of egg mortality.

Estimates of P_b obtained using the linear version of the exponential mortality model have a strong negative bias, therefore a bias correction factor was applied following the equation of Picquelle and Stauffer (1985):

$$P_0 = e^{(\ln P_b + \sigma^2/2)} \qquad \text{Equation 4}$$

where, σ^2 is the variance of the estimate of biased mean daily egg production (P_b).

2.3 Adult Reproductive Parameters

2.3.1 Sampling methods

Each afternoon when the *RV Ngerin* was in areas where sardine schools were known to aggregate and conditions were suitable for gillnetting (i.e. adequately protected from the swell), searching was undertaken using a dual frequency echo sounder (*Furuno* - 60 and 180 KHz)

(Fig. 1). The *RV Ngerin* was then anchored where several schools were observed. Samples of adults were collected using a gillnet comprising three panels, each with a different multi-filament nylon mesh size (*double diamond*: 210/4 ply meshes – 25, 28 and 32 mm). Surface and sub-surface lights (500 W) were illuminated near the net after it was set. Net soak times varied from 15 minutes to 3 hours depending on the number of fish caught. After the net was retrieved, fish were removed and dissected immediately. All sardines collected were counted and sexed. Mature and immature males and females were frozen. Mature females were fixed in 5% buffered formaldehyde solution. Calculations of female weight, sex ratio, batch fecundity and spawning fraction were based on samples collected from Scotts Cove in Investigator Strait, North Neptune Island in southern Spencer Gulf and Waldegrave Island in the eastern Great Australian Bight (GAB).

2.3.2 Female weight (W)

Mature females from each sample were removed from formalin and weighed (± 0.01 g). Fixation in formalin has a negligible effect on fish weight (Lasker 1985). The mean weight of mature females in the population was calculated from the average of sample means weighted by proportional sample size:

$$W = \overline{\left[\overline{W_i} * \frac{n_i}{N} \right]} \qquad \text{Equation 5}$$

where, $\overline{W_i}$ is the mean female weight of each sample *i*; *n* is the number of fish in each sample and *N* is the total number of fish collected in all samples.

2.3.3 Male weight

Mature males in each sample were thawed and weighed (± 0.01 g).

2.3.4 Sex ratio (R)

The mean sex ratio of mature individuals in the population was calculated from the average of sample means weighted by sample size:

$$R = \overline{\left[\overline{R_i} * \frac{n_i}{N} \right]} \qquad \text{Equation 6}$$

where, *n* is the number of fish in each sample, *N* is the total number of fish collected in all samples and $\overline{R_i}$ is the mean sex ratio of each sample calculated from the equation:

$$\overline{R_i} = \frac{F}{(F+M)} \qquad \text{Equation 7}$$

where, *F* and *M* are the respective total weights of mature females and males in each sample *i*.

2.3.5 Batch fecundity (F)

Batch fecundity was estimated from ovaries containing hydrated oocytes using the methods of Hunter *et al.* (1985). Both ovaries were weighed and the number of hydrated oocytes in three ovarian sub-sections were counted and weighed. The total batch fecundity for each female was calculated by multiplying the mean number of oocytes per gram of ovary segment by the total weight of the ovaries. The relationship between female weight (ovaries removed) and batch fecundity was determined by linear regression analysis and used to estimate the batch fecundities of mature females in all samples.

2.3.6 Spawning fraction (S)

Ovaries of mature females were sectioned and stained with haematoxylin and eosin. Several sections from each ovary were examined to determine the presence/absence of post-ovulatory follicles (POFs). POFs were aged according to the criteria developed by Hunter and Goldberg

(1980) and Hunter and Macewicz (1985). The spawning fraction of each sample was estimated as the mean proportion of females with hydrated oocytes plus day-0 POFs (*d*0) (assumed to be spawning or have spawned on the night of capture), day-1 POFs (*d*1) (assumed to have spawned the previous night) and day-2 POFs (*d*2) (assumed to have spawned two nights prior). The mean spawning fraction of the population was then calculated from the average of sample means weighted by proportional sample size.

$$S = \overline{\left[\overline{S_i} * \frac{n_i}{N} \right]} \qquad \text{Equation 8}$$

where, *n* is the number of fish in each sample, *N* is the total number of fish collected in all samples and $\overline{S_i}$ is the mean spawning fraction of each sample calculated from the equation:

$$\overline{S_i} = \frac{[(d0 + d1 + d2POFs)/3]}{n_i} \qquad \text{Equation 9}$$

where, *d*0, *d*1 and *d*2 POFs are the number of mature females with POFs in each sample and n_i is the total number of females within a sample.

2.4 Spawning Biomass and Bootstrapping Procedures

2.4.1 Spawning biomass estimates

Spawning biomass was calculated according to Equation 1 using the estimate of P_0 obtained via the log-linear model and adult reproductive parameters collected during the 2014 survey.

2.4.2 Bootstrapping procedures and confidence intervals

To account for the covariance of adult parameters within individual samples, confidence intervals for all four adult parameters were calculated using a two stage bootstrap with 100,000 bootstrap iterations (Efron and Tibshirani 1993). For each iteration, the individual samples were resampled with replacement to obtain the bootstrapped samples. For each of the bootstrapped samples, the fish were resampled with replacement to generate a complete survey. The adult parameters *W*, *S* and *R* were calculated from the bootstrapped survey using the method described above. Batch fecundity (*F*) was calculated from the mean gonad-free weight using the batch relationship obtained by bootstrapping with replacement from females with hydrated oocytes. For each bootstrap iteration the value $W / R.F.S$ was used in the calculation of bootstrapped confidence intervals for spawning biomass. The 95% confidence intervals of spawning biomass were estimated by calculating the spawning biomass 100,000 times from A and the 100,000 bootstrapped estimates of P_0 and $W / R.F.S$ using the percentile method. Parameter estimates were calculated independently in Excel 2010 and R 3.1.0 (for quality assurance) with confidence intervals estimated with R 3.1.0.

2.5 Sensitivity Analysis

Sensitivity analyses were conducted to assess the effects on estimates of spawning biomass of variations in the values obtained for each parameter in 2014 and between 1998 and 2013.

3. RESULTS

3.1. Biophysical Variables

3.1.1 Sea surface temperature

Sea surface temperatures (SSTs) ranged from to 14.7 to 22.1°C (Fig. 3) between February and April 2014. High SSTs (>19°C) were recorded in Spencer Gulf, Gulf St Vincent, south of Kangaroo Island and throughout the central GAB. A plume of cool water (<18°C) extended from western Kangaroo Island to Streaky Bay with particularly cold water (<16°C) against the coast on western Eyre Peninsula.

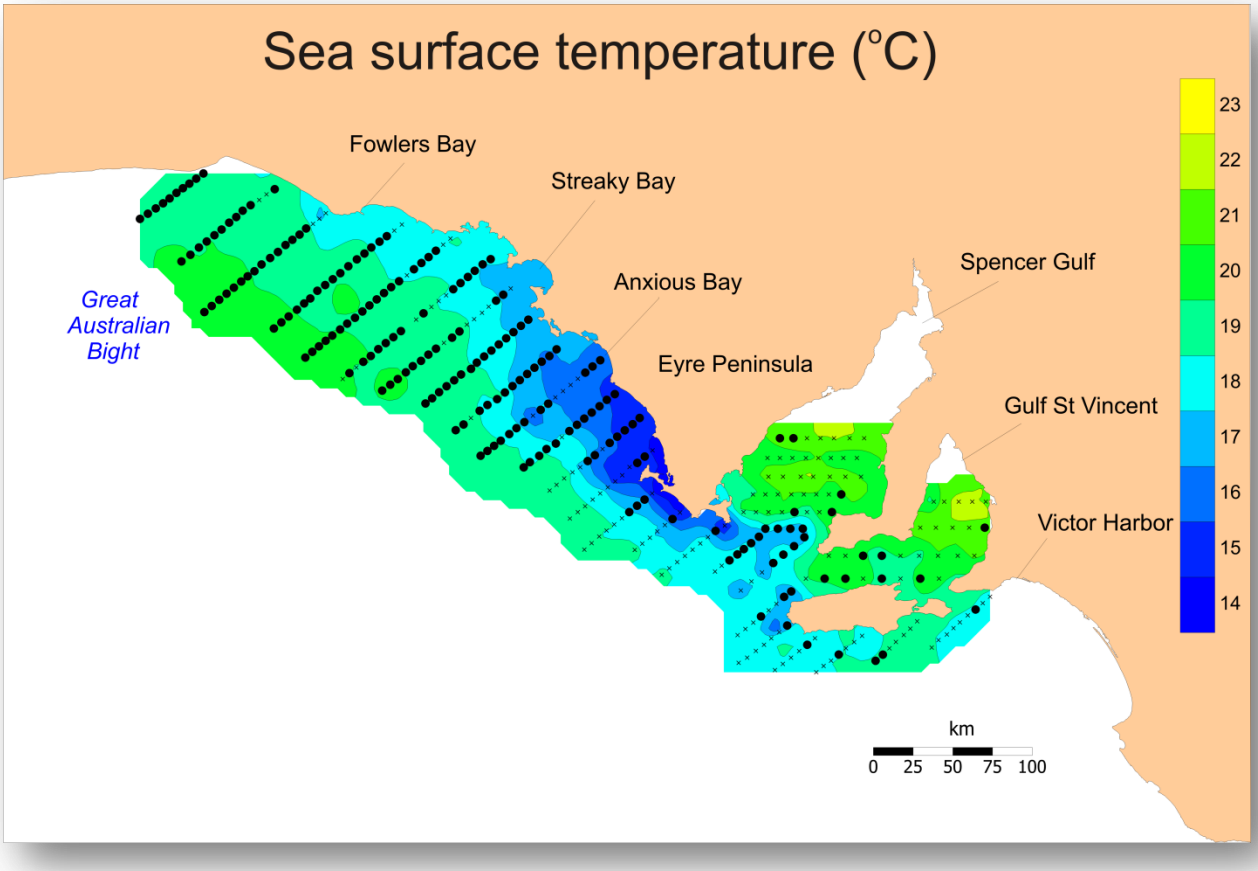

Figure 3. Sea surface temperature profile across the 2014 February – April survey, showing sites where sardine eggs were collected (●).

3.1.2 Fluorescence (chlorophyll-a)

Chlorophyll-*a* concentration at each site ranged between 0.0004 and 0.883 µg.L^{-1} (Fig. 4) between February and April 2014. The highest values were recorded off Pt Sir Isaac and Cape Adieu and in southern Spencer Gulf and Anxious Bay. The remainder of coastal and shelf waters mainly had chlorophyll-*a* concentrations ranging between 0.01 and 0.1 µg.L^{-1}.

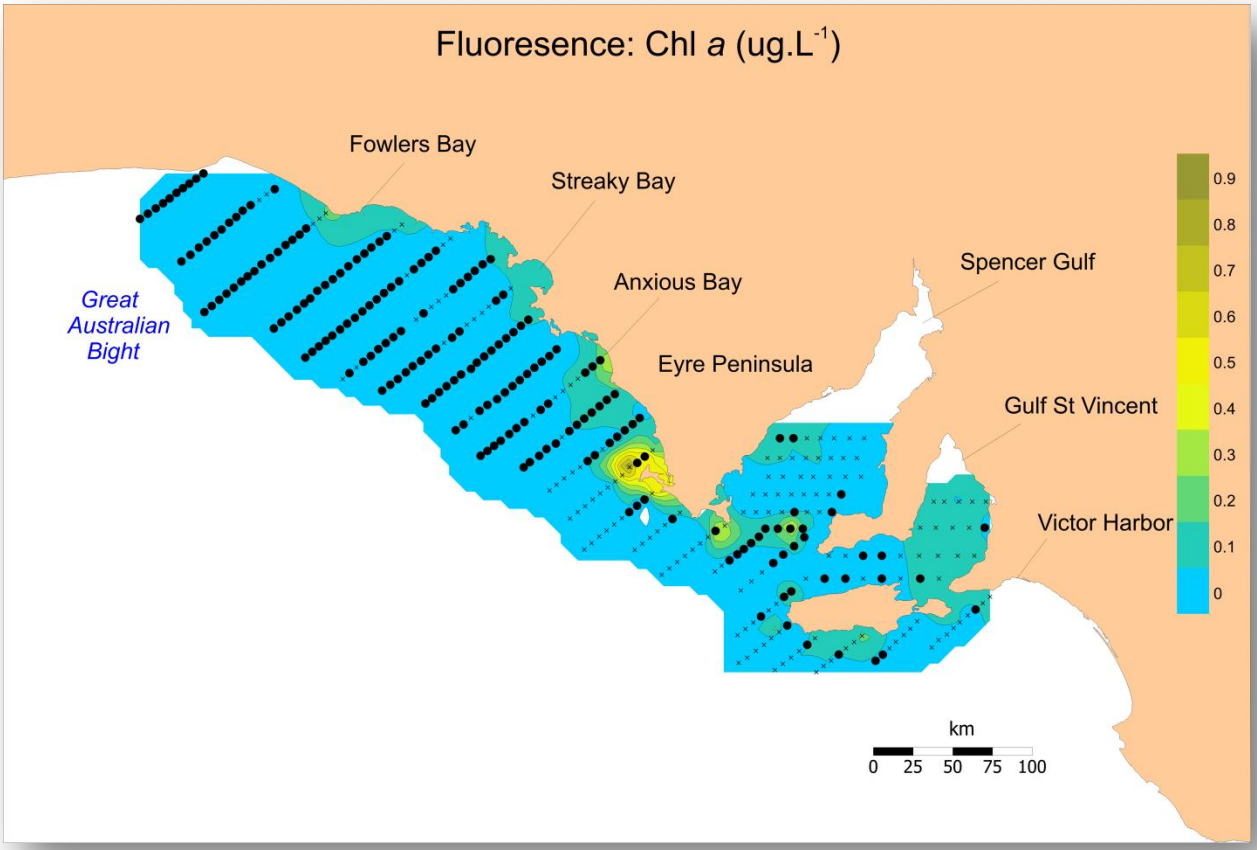

Figure 4. Surface concentration of chlorophyll-a inferred from fluorescence readings across the 2014, February - April survey area, showing sites where sardine eggs were collected (●).

3.1.3 Zooplankton abundance

Total densities of zooplankton ranged between 0 and 9.8 ml.m^{-3} (Fig. 5) between February and April 2014. The highest densities occurred in the mouth of Spencer Gulf, Investigator Straight, outside Streaky Bay and at the Head of the Bight.

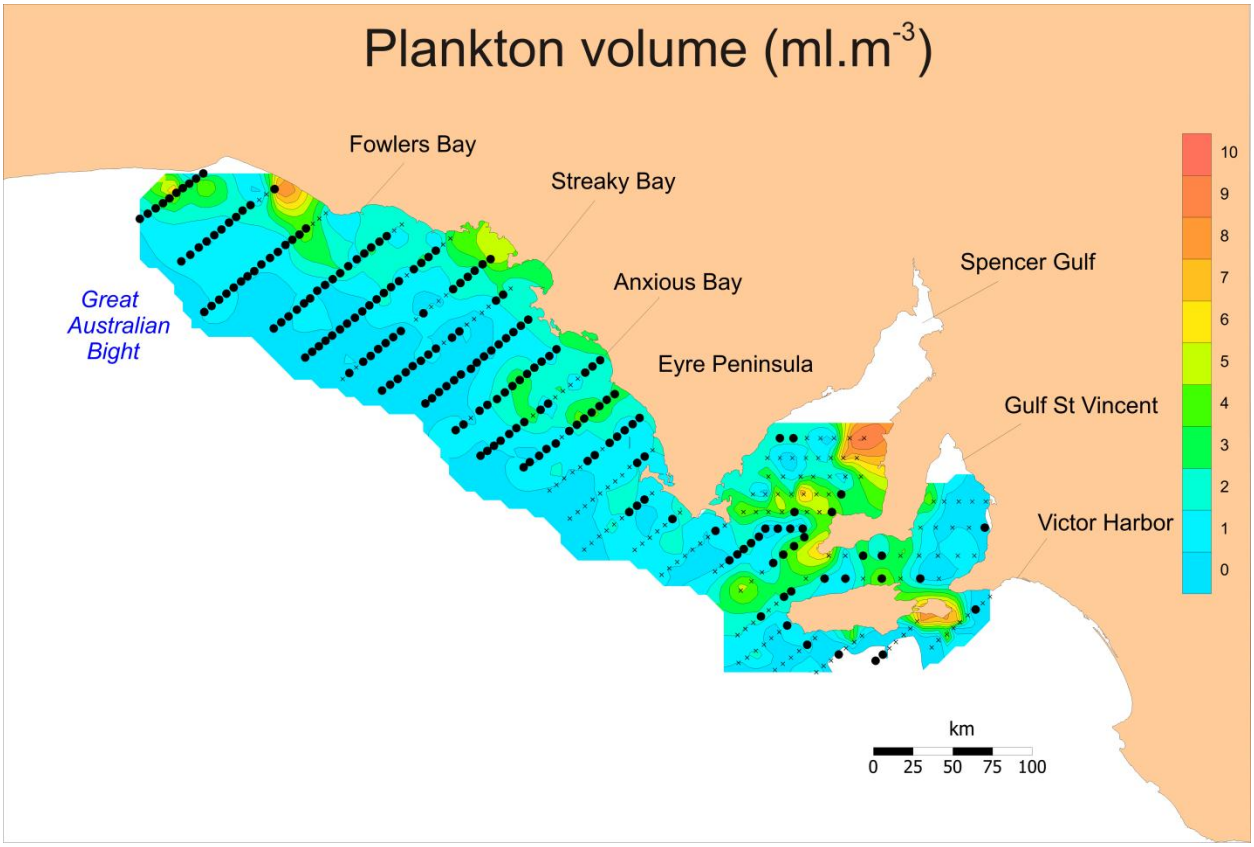

Figure 5. Distribution and abundance (ml.m^{-3}) of zooplankton across the 2014, February - April survey area, showing sites where sardine eggs were collected (●).

3.2 Distribution and Abundance of Eggs and Larvae

3.2.1 Distribution and abundance of eggs

A total of 7,955 live sardine eggs were collected at 196 of 355 (55.2%) sites on 34 transects between the Head of Bight and Victor Harbor between February and April 2014 (Fig. 6). The sites with the highest egg densities were located in the mouth of Spencer Gulf, north of Coffin Bay Peninsula and in mid/outer shelf waters of the eastern and central GAB. The highest egg density recorded was 21,569 eggs.m^{-2}.

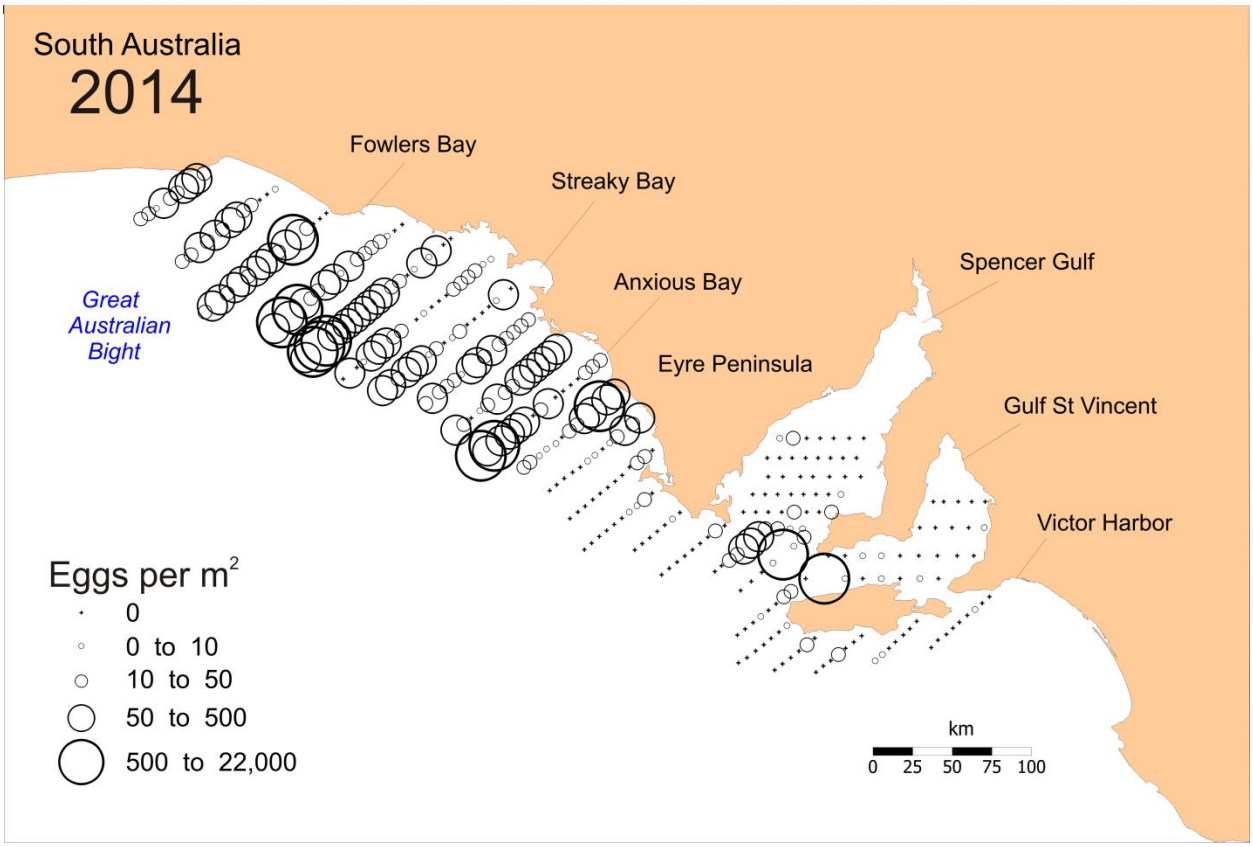

Figure 6. Spatial patterns of live sardine egg distribution and abundance between February and April 2014.

3.3 Spawning Area

The estimated spawning area for the entire survey area was 71,859 km^2, comprising 58.7% of the total area sampled (122,437 km^2, Table 1). The presence of eggs in CUFES samples was used as a basis for extending some transects (Fig. 1). An additional 15 sites were sampled in 2014, representing an additional 5,601 km^2; 13 of these samples contained live sardine eggs and contributed 4,983 km^2 to the spawning area.

Table 1. Mean daily egg production (P_0, log-linear model) and spawning area (*A)*. Table shows the variance (σ^2) term used in estimate.

	Area sampled (km ^{2})	Spawning area *A* (km ^{2})	Percentage of area sampled	$\sigma^2 P_b$	P_0 (eggs.d^{-1}.m^{-2})
Total survey	122,437	71,859	58.7	1.51	85.0

3.4 Daily Egg Production (P_0)

The estimate of mean daily egg production, P_0 obtained using the linear version (Eq. 3) of the exponential egg mortality (recommended by Ward *et al.* 2011a) was 85.0 eggs.day^{-1}.m^{-2} (95% CI = 60.9 – 120.2, Fig. 7, Table 1,5).

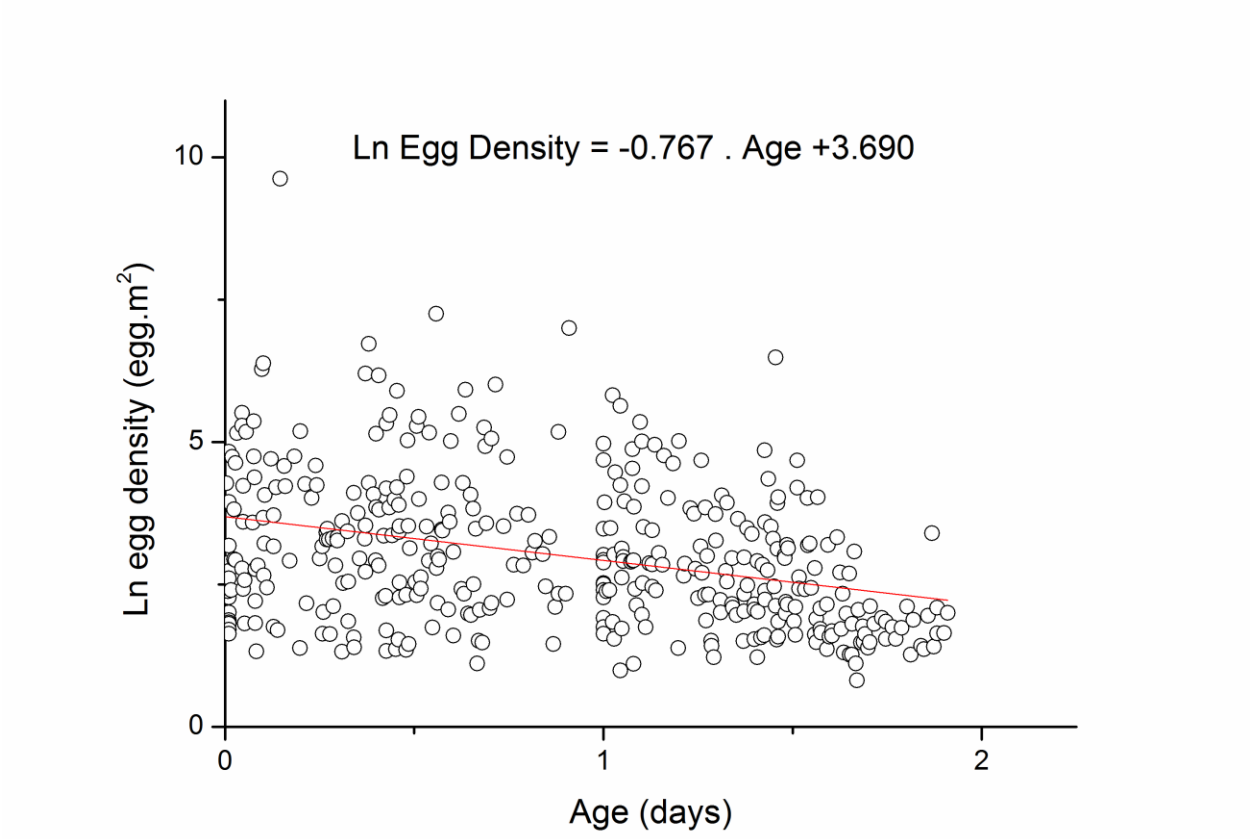

Figure 7. Linear regressions between ln-transformed sardine egg density (eggs.m^{-2}) and age (days) data in 2014.

3.5 Adult Reproductive Parameters

A total of 13 samples comprising 1,512 mature sardines were collected at Scotts Cove, Waldegrave Island and North Neptune Island (Table 2). Estimates of the adult female reproductive parameters used in calculations of spawning biomass are provided in Tables 3, 4 and 5. The means and ranges of adult parameters in samples collected between 1998 and 2013 and the bootstrapped 95% confidence intervals are shown in Table 5.

Table 2. Sampling details for adult sardine collected during the 2014 DEPM surveys.

Date	Location	Survey	N samples	*n* fish
24/02/2014	Scotts Cove	1	3	464
04/03/2014	Waldegrave Island	1	4	503
25/03/2014	Scotts Cove	2	4	459
05/04/2014	Neptune Island	2	1	60
		Total	12	1,486

3.5.1 Mean female weight

The mean weight of mature females in samples collected in 2014 ranged from 34.6 to 60.4 g (Table 3). The weighted mean weight of mature females in 2014 was 47.4 g (95% CI = 41.7 – 53.0, Table 3, 5). The mean weight of mature females collected between 1998 and 2013 was 58.3 (45.2 – 78.7).

3.5.2 Sex ratio

The sex ratio calculated from the 2014 survey was 0.57 (95% CI = 0.52 – 0.61) (Table 4, 5). The mean sex ratio between 1998 and 2013 was 0.53 and ranged between 0.36 and 0.68 (Table 5).

Table 3. Number of sardine in samples by sex and estimates of female weight, *W* and sex ratio, *R* (proportion of females by weight) for samples collected in 2014. Values in bottom row are sums (*) and weighted means (#).

Sample	Location	Date	Male	Female	Mean Male Weight	Mean Female Weight (*W*)	Sex Ratio by weight (*R*)
1	Scotts Cove	24/02/2014	56	55	47.9	53.4	0.52
2	Scotts Cove	24/02/2014	75	95	45.4	50.4	0.58
3	Scotts Cove	24/02/2014	79	104	49.0	49.2	0.57
4	Waldegrave Is.	04/03/2014	29	15	35.7	35.2	0.34
5	Waldegrave Is.	04/03/2014	67	81	34.5	36.4	0.56
6	Waldegrave Is.	04/03/2014	77	90	35.7	36.4	0.54
7	Waldegrave Is.	04/03/2014	53	91	36.9	37.9	0.64
8	Scotts Cove	25/03/2014	60	93	57.1	62.3	0.63
9	Scotts Cove	25/03/2014	23	23	60.1	54.7	0.48
10	Scotts Cove	25/03/2014	39	50	59.5	55.5	0.54
11	Scotts Cove	25/03/2014	80	91	55.9	54.2	0.52
12	N. Neptune Is.	05/04/2014	16	44	32.4	36.4	0.76
			669*	**843***	**46.1#**	**47.4#**	**0.57#**

3.5.3 Batch fecundity

Batch fecundity ranged from 4,922 to 34,831 hydrated oocytes for the 32 female sardines with ovaries containing hydrated oocytes examined in 2014. Based on the relationship (Batch Fecundity = 458.3 x Gonad Free Female Weight – 3,902, R^2 = 0.819, Fig. 8) and the mean gonad free female weight (46.1 g) for all samples collected in 2014, mean batch fecundity was 17,133 oocytes per batch (95% CI = 14,431 – 19,930; Table 5). The mean batch fecundity for samples collected between 1998 and 2013 was 17,250 oocytes per batch (10,904 – 24,790, Table 5).

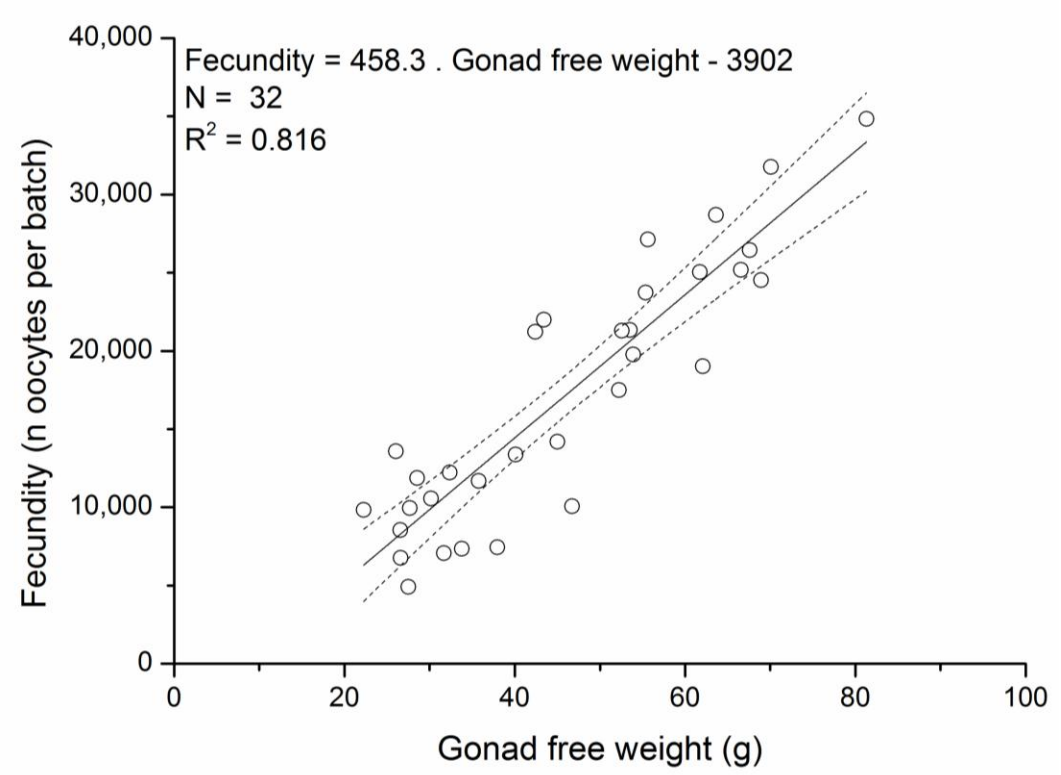

Figure 8. Relationship between gonad-free weight and batch fecundity in 2014 (dotted line = 95% CI).

3.5.4 Spawning fraction

Of the 832 ovaries examined, 47 had hydrated oocytes and/or day-0 POFs, 21 had day-1 POFs and 33 day-2 POFs (Table 4). The spawning fraction of females in each sample ranged from

0.018 to 0.098. The weighted mean spawning fraction for all 2014 data was 0.040 (95% CI = 0.028 – 0.055). For 1998 – 2013, the mean spawning fraction was 0.122 and ranged between 0.044 and 0.179 (Table 5).

Table 4. Number of female sardine in samples and estimates of spawning fraction (*S)* for samples collected in 2014. Values in bottom row are sums* and weighted means#.

Sample	Location	Date	POF 0	POF 1	POF 2	Total	Spawning Fraction (S)
1	Scotts Cove	24/02/2014	13	0	0	55	0.079
2	Scotts Cove	24/02/2014	7	1	3	95	0.039
3	Scotts Cove	24/02/2014	8	2	2	104	0.038
4	Waldegrave Is.	04/03/2014	0	0	1	15	0.022
5	Waldegrave Is.	04/03/2014	1	1	4	81	0.025
6	Waldegrave Is.	04/03/2014	1	2	8	90	0.041
7	Waldegrave Is.	04/03/2014	2	6	6	91	0.051
8	Scotts Cove	25/03/2014	4	1	1	93	0.022
9	Scotts Cove	25/03/2014	1	1	2	23	0.058
10	Scotts Cove	25/03/2014	1	1	2	50	0.027
11	Scotts Cove	25/03/2014	1	4	0	91	0.018
12	N. Neptune Is.	05/04/2014	8	2	3	44	0.098
			47*	**21***	**32***	**832***	**0.040#**

Table 5. Parameters used in the calculations of spawning biomass. Values for 2014 and the mean, minimum and maximum for 1998 to 2013 are presented (for spawning area 2004 is excluded as the survey was not completed in this year).

Parameter	2014 (95% CI)	Mean 1998-2013 (min-max)
Egg Production (P_o, eggs.day^{-1}.m^{-2})	85.0 (60.9 – 120.2)	71.8 (38.1 – 120.9)
Sex Ratio (R)	0.57 (0.53 – 0.61)	0.53 (0.36 – 0.68)
Fecundity (F, eggs.female^{-1})	17,133 (14,431 – 19,930)	17,250 (10,904 – 24,790)
Spawning Fraction (S)	0.040 (0.028 – 0.055)	0.122 (0.044 – 0.179)
Female Weight (W, g)	47.4 (41.7 – 53.0)	58.3 (45.2 – 78.7)
Spawning Area (A, km^2)	71,859	38,350 (14,867 – 53,553)

3.6 Sensitivity Analysis

The sensitivity analyses show where parameter estimates from the 2014 surveys lie in comparison to the range of values recorded between 1998 and 2013 and their influence on estimates of spawning biomass (Fig. 9). Estimates of two parameters for 2014 lie outside the previous ranges and strongly influence the estimates of spawning biomass. These are spawning area (*A*) which is positively correlated with spawning biomass and spawning fraction (*S*) which has an inverse (negative) effect.

The spawning area (*A*) for 2014 was the largest observed since surveys began in 1998 (Fig. 9). The presence of sardine eggs over such a large area is a robust finding and the estimate of spawning area for 2014 should be used to calculate spawning biomass. Conversely, the estimate of spawning fraction (*S*) for 2014 was the lowest recorded. Samples used to estimate spawning fraction were taken outside the main spawning area and are unlikely to be representative of adults that spawned the majority of eggs. For this reason, the 2014 estimate of spawning fraction should not be used to calculate the spawning biomass in 2014. Replacing

this parameter with the maximum and mean spawning fraction obtained between 1998 and 2013 provides alternative estimates of the spawning biomass in 2014.

Mean female weight (W) for 2014 was below the all year mean which had a negative influence on spawning biomass. However this parameter is correlated with fecundity (*F*) and this relationship offsets the effect on the estimate spawning biomass. Estimates of egg production (P_0), sex ratio (*R*) and fecundity (*F*) were similar to the mean value from previous surveys.

3.7 Spawning Biomass

The estimate of spawning biomass calculated using all data from 2014 (i.e. including spawning fraction, *S*) was 742,360 t (95% CI = 462,582 – 1,229,495, Fig. 10). The spawning biomass calculated using the highest and mean spawning fractions for 1998-2013 were 166,157 t (95% CI = 116,970 – 238,316) and 243,925 t (95% CI = 171,717 – 349,858), respectively.

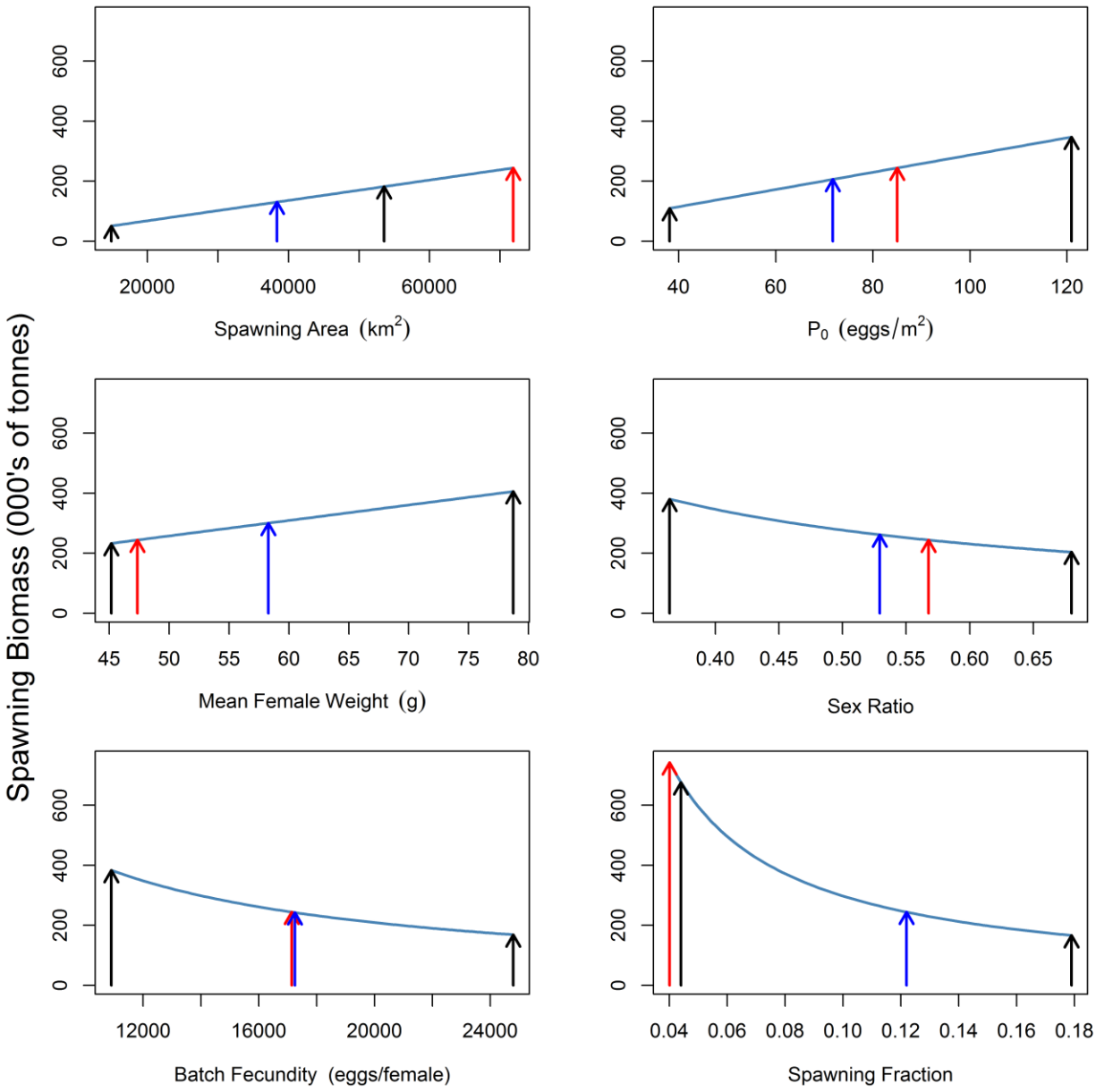

Figure 9. Sensitivity plots showing where 2014 parameter estimates (red arrow) lie in comparison to the range of values recorded between 1998 and 2013 and their influence on estimates of spawning biomass. Black arrows are the minimum and maximum values for 1998-2013. The blue arrow is the mean. Note that the influence of each 2014 estimate on spawning biomass was tested using all other 2014 parameters, except that mean spawning fraction was used when testing other parameters rather than the value of spawning fraction obtained for 2014.

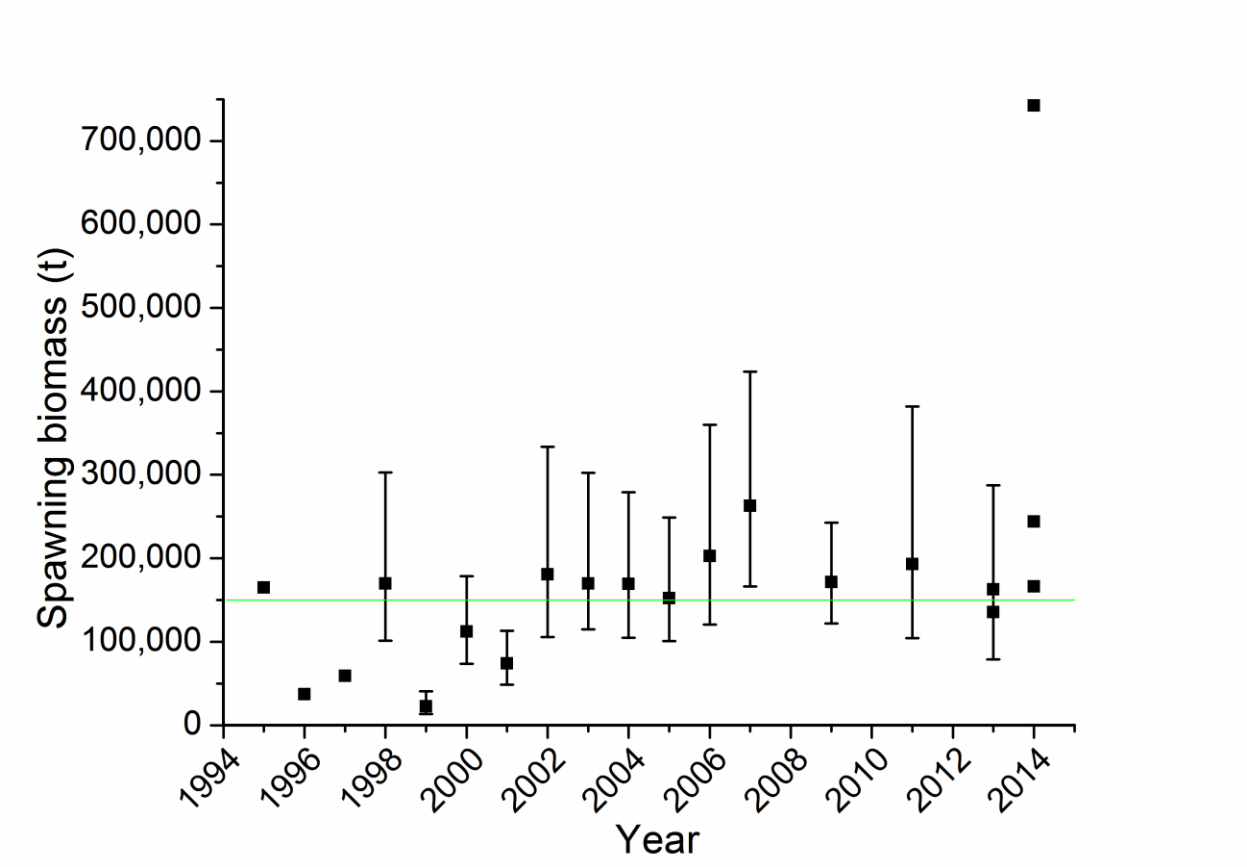

Figure 10. Spawning biomass estimates for sardine in South Australian waters from 1995 to 2014, including the 2014 estimate calculated using the observed spawning fraction and the mean and maximum spawning fraction recorded between 1998 and 2013. Error bars are 95% confidence intervals. The green line indicates the 150,000 t reference point.

4. DISCUSSION

4.1 Biophysical Variables and Egg Distribution

The low SSTs (<18^{o}C) and elevated concentrations of chlorophyll-*a* (>0.3 µg.L^{-1}) recorded in inshore waters off southern Eyre Peninsula during the surveys showed that strong upwelling occurred in the eastern GAB during February and March 2014 (e.g. McClatchie *et al.* 2006). Plankton densities were variable across the survey area and are difficult to interpret due to variations in the taxonomic composition of zooplankton among water masses.

As is the case in most DEPM surveys, egg samples collected in 2014 (total of 7,955 live eggs) were strongly over-dispersed with a few samples containing a very high number of eggs and almost half the samples (45%) containing no live eggs (Ward *et al.* 2011a).

The abundance of sardine eggs was low at sites located in Gulf St Vincent, eastern Investigator Strait and Spencer Gulf north of Wedge Island. High egg abundances were recorded at a few sites in southern Spencer Gulf and western Investigator Strait. As was the case in 2013, which was a weak upwelling year (Ward *et al.* 2013), high egg densities were recorded in 2014 over a large area in shelf waters of the eastern and central GAB.

A total of 15 additional sites were sampled on the seaward end of seven transects in the GAB, based on the presence of sardine eggs in the CUFES sample from the last fixed site on that transect. The high densities of sardine eggs observed on the seaward end of transects in both 2013 and 2014, suggest that the current sampling design may need to be extended out to the shelf break. Adaptive sampling based on CUFES samples should also be continued.

4.2 Spawning Area

The estimate of spawning area for 2014 (71,859 km^{2}; Table 1, 5) is the highest recorded in a DEPM survey conducted off South Australia. The large spawning area was due to the widespread occurrence of eggs in shelf waters of the GAB. Egg abundance east of Cape Carnot was relatively low compared to previous years (Ward *et al.* 2012).

4.3 Egg Production

The estimate of egg production (P_0) obtained in 2014 using the linear version of the exponential egg mortality model, which was the method recommended for sardine for SA by Ward *et al.* (2011a), was 85.0 eggs. day^{-1}.m^{-2} (95% CI = 60.9 – 120.2), which is similar to the mean value observed since 1998 of 71.8 eggs. day^{-1}.m^{-2}.

4.4 Adult Sampling

During the 2014 survey, 12 samples of adult sardines containing 843 females were collected from Scotts Cove, North Neptune Island and Waldegrave Island. No adult samples were obtained west of Waldegrave Island or from offshore waters where the majority of eggs were collected. Three lines of evidence that suggest that adult samples collected in 2014 may not be representative of adult sardines that occurred further offshore. 1) Previous studies have shown that sardine size tends to increase with distance from shore (Rogers and Ward 2007); estimates of mean female weight obtained from inshore sites in 2014 were relatively low compared to previous years (Table 5). 2) Spawning frequency of sardine has been shown to be positively correlated with fish size (Ganias *et al.* 2003); the estimate of mean female weight for 2014 was low compared to previous years and the estimate of spawning fraction was the lowest recorded for South Australia (Table 5; Fig. 9). 3) Spawning frequency of clupeoids has been shown to be correlated with water temperature (Takasuka *et al.* 2005; Uriarte *et al.* 2012); adult sampling sites where these relatively small fish were located were in or adjacent to areas of cool upwelled water (<18^{o};, Fig. 1, 3), whereas SSTs at offshore sites were higher (>19^{o}C).

4.5 Spawning Biomass

The large spawning area observed during this study provides strong evidence that the spawning biomass in 2014 was large. However, the lack of adult samples from offshore sites prevented reliable estimation of population size. The absence of a reliable estimate of spawning fraction is of particular concern because variation in this parameter has a strong influence on estimates of spawning biomass (Fig. 9, 10).

The spawning biomass estimate obtained using the 2014 value of spawning fraction (0.04) was >700,000 t, which is unlikely (Fig. 10). Using the maximum (0.18) and mean (0.12) spawning fractions for 1998 to 2013 provide estimates of spawning biomass of ~166,000 and ~240,000 t,

respectively. However, as adult samples have not been collected from offshore waters of South Australia the suitability of these values for calculating estimates of spawning biomass is unknown. Due to the lack of representative samples of adult sardines from offshore waters a robust estimate of the spawning biomass of sardine in waters off South Australia cannot be provided for 2014.

4.6 Future Research Needs

The absence of a robust estimate of the spawning biomass for 2014 emphasises the urgent need to establish a reliable method for sampling adult sardines in offshore waters. This need has been identified in previous reports (e.g. Ward *et al.* 2013) and was highlighted as a priority for South Australia at the recent international workshop on small pelagic fisheries held in Adelaide in July 2014 (Ward, unpublished data).

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