Spawning biomass of sardine, *Sardinops sagax* in waters off South Australia in 2009

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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 provided in the (now) biennial spawning biomass report is the key biological performance indicator for the South Australian Sardine Fishery. This report uses the DEPM to provide an estimate of the spawning biomass of sardine in waters off South Australian in February-March 2009.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

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EXECUTIVE SUMMARY

1. This report provides an estimate of the spawning biomass of sardine, *Sardinops sagax* in South Australian waters in 2009.

2. Data were obtained from research surveys conducted from the *RV Ngerin* during February and March 2009. The total survey area covered was 114,746 km^2.

3. Sea surface temperatures (SSTs) during the surveys ranged from 16.9 to 22.4°C and were lowest in inshore waters off western Eyre Peninsula. SSTs in 2009 were generally higher than in previous years, whereas chlorophyll-*a* concentrations and zooplankton densities were relatively low. These finding provide evidence that 2009 was a relatively weak upwelling year.

4. A total of 2582 sardine eggs was collected from 340 stations. High densities of eggs were recorded in southern Spencer Gulf, Investigator Strait and south of Kangaroo Island. Lower densities of egg were collected in coastal waters of the southern Eyre Peninsula and in shelf waters west of Cape Finnis.

5. A total of 19 samples comprising 3767 mature fish was collected at sampling locations in Investigator Strait, southern Spencer Gulf and the eastern Great Australian Bight.

6. The total spawning area (*A)* in 2009 was 53,553 km^2, which is the highest recorded in DEPM surveys in South Australian waters.

7. Mean daily egg production (P_0) calculated using the log-linear version of the egg mortality model, was 63.2 $eggs.day^{-1}.m^{-2}$ (95% CI = 46.1 - 87.1 $eggs.day^{-1}.m^{-2}$).

8. Estimates of mean adult reproductive parameters in 2009 were: female weight, *W* = 59.9 g (95% CI = 55.5 - 65.8); batch fecundity, *F* = 18,187 (95% CI = 16,313 - 20,457) hydrated oocytes, sex ratio, *R* = 0.36 (95% CI = 0.29 – 0.44) and spawning fraction, *S* = 0.156 (95% CI = 0.114 – 0.196).

9. The low estimate of R and high estimate of S samples suggest that spawning aggregations were over-represented in samples collected in 2009. Estimates of these two parameters obtained from samples collected between 1998 and 2007 were used to estimate spawning biomass for 2009: sex ratio, R = 0.53 (95% CI = 0.49 – 0.56) and spawning fraction, *S* = 0.123 (95% CI = 0.109 – 0.138).

10. The best estimate of spawning biomass for 2009 was 171,531 t (95% CI = 122,100 - 242,479), which lies within the target range of spawning biomasses of 150,000 - 300,000 t. A TACC for 2010 of 30,000 t would equate to an exploitation rate of approximately 17.5% of the spawning biomass in 2009.

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 et al. 2008; Dimmlich et al. 2009; Ward et al. 2009a). The method is widely used because it is often the most practical option available for stock assessment of small pelagic species. In many circumstances the only real alternative to the DEPM is acoustic surveys, which often produce biased estimates of biomass and require more sophisticated and expensive infrastructure, higher levels of technical support and expertise, and a longer developmental phase than the DEPM. A good example of these constraints comes from South Africa, where estimates of anchovy spawner biomass obtained via the DEPM over a decade were used to scale-up negatively-biased acoustic estimates while methods were being developed for *in situ* estimation of acoustic target strength.

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) and mean spawning fraction (proportion of mature females spawning each day/night, S) by mean female weight (W). Spawning biomass (SB) is calculated according to the equation:

$$SB = P_0.A / (R.F.S / W). \qquad \text{Equation 1}$$

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).

Although the DEPM is used widely, a range of problems 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). This imprecision is mainly due to uncertainties associated with the estimation of total daily egg production, i.e. P_0 and A. (Fletcher et al. 1996; McGarvey and Kinloch 2001; Ward et al. 2001a; Gaughan et al. 2004). A range of analytical methods have been used to calculate these parameters and these have the potential to significantly affect estimates of SB. For example, egg age has been estimated using a range of models that combine information on daily spawning synchronicity and mean egg developmental rates in relevant temperature ranges (e.g. Lo 1985; Piquelle and Stauffer 1985; Ibaibarriaga 2007). Perhaps most importantly, P_0 has been determined by fitting the exponential decay model to estimates of the mean age of daily cohorts and their density in each sample using non-linear regression, a log-linear model of ln-transformed data (e.g. Piquelle and Stauffer 1985;), generalised linear models (GLM) with appropriate link functions and generalised additive models (GAM) (ICES 2004). The delta method (Seber 1982) and parametric (Borchers et al. 1997) and non-parametric bootstraps (Jackson and Chen 2001; ICES 2004) have been used to estimate confidence intervals of P_0. A has been estimated by dividing the survey into stratified grids using a subjective 'manual' method (Lasker 1985) and objectively using nearest neighbour methods (Watson 1981; Ward et al. 2009a). Confidence intervals for A have been estimated using GAMs (Stratoudakis et al. 2003).

Many DEPM studies have been impeded by difficulties associated with obtaining representative samples of adults to estimate reproductive parameters (see Stratoudakis et al. 2006). Estimating R and W is relatively straightforward if representative samples can be collected. F has been estimated by calculating the relationship between fish weight (ovary-free) and batch fecundity for females with gonads containing hydrated ooocytes using either linear regression (Picquelle and Stauffer 1985) or a gamma or negative binomial GLM with an identity link function (ICES 2004); this relationship has then been applied to the mean gonad free weight of all mature female fish. S is often the most difficult DEPM parameter to estimate for clupeoids. Obtaining representative samples of adults is 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.

At least two reviews have concluded that the DEPM may be better tailored to anchovies (*Engraulis* spp.) than to sardine, *Sardinops sagax* (Alheit 1993; Stratoudakis et al. 2006). The main argument used to support this assertion is that because a higher proportion of anchovies are actively

spawning during the peak spawning season, daily fecundity can be estimated more precisely for anchovy than sardine (e.g. Alheit 1993; Stratoudakis et al. 2006). Despite these apparent limitations of the DEPM for stock assessment of sardine, the method is a critical component of the assessment of this species in several locations. For example, the DEPM has been used for stock assessment of *S. sagax* off the west coast of North America (e.g. Lo et al. 2005) and the western and southern coasts of Australia (Fletcher et al. 1996; Gaughan et al. 2004; Ward et al. 2009b). However, there are important differences between locations in the manner in which egg and adult samples are collected, data are analysed and in how estimates of spawning biomass are used to support fisheries management. A Continuous Underway Fish Egg Sampler has been used routinely in surveys off California but not Australia (e.g. Lo et al. 2001). In addition, adult samples have usually been collected by mid-water trawling off California, purse-seining off Western Australia (Gaughan et al. 2004) and gill-netting off South Australia (Ward et al. 2009b). Furthermore, estimates of *SB* obtained from DEPM surveys are used directly for fisheries management in South Australia, but are incorporated into age-structured stock assessment models in California and Western Australia.

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 (SA) waters since 1995 (Ward et al. 1998, 2009b). 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 SA waters (e.g. Ward et al. 2001a, 2008). The current harvest strategy indicates that a baseline TACC of 30,000 t will be maintained while the latest estimate of spawning biomass remains between 150,000 and 300,000, which corresponds to exploitation rates of 20% and 10%, respectively. A recent review that reanalysed data collected since 1998 using the various statistical methods identified the optimal approach for applying the DEPM in SA waters (Ward et al. 2009b). That review, and a study that investigated the potential for utilising a CUFES in future studies (Ward and Ivey 2009), also identified options for improving the methods currently used to estimate key DEPM parameters.

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-March 2009. The objectives of the report are:

1. To describe the distribution and abundance of sardine eggs in relation to environmental factors;
2. To estimate DEPM parameters (*A*, *P*, *W*, *R*, *F*, *S*);
3. To use the DEPM to estimate the spawning biomass in 2009

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 March 2009. Plankton samples were collected at 340 stations on 34 transects between Victor Harbor and Head of Bight (Fig. 1).

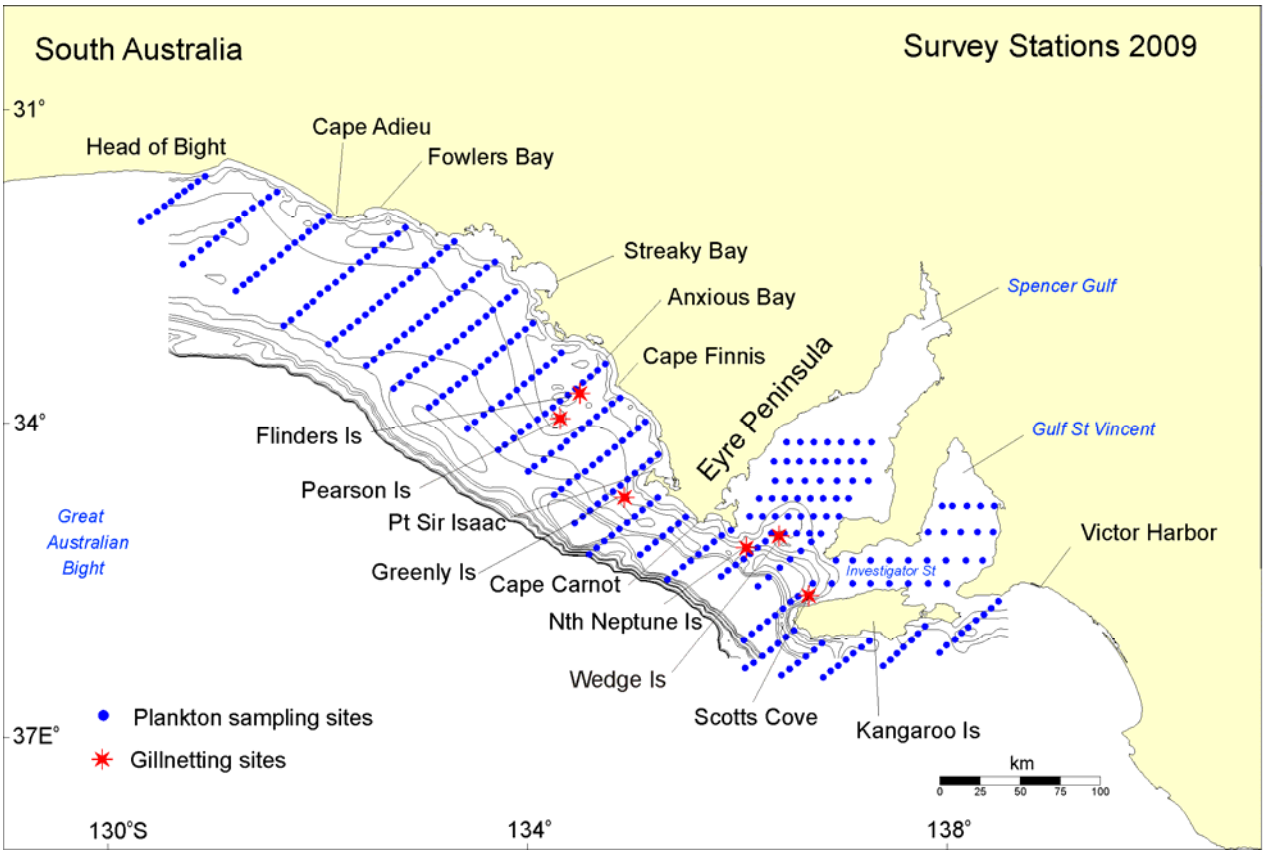

Figure 1. Map of South Australia showing stations where plankton and adult samples were collected during the 2009 DEPM surveys.

2.1.2 Water temperature and primary production

At each station (Fig. 1), a *Sea-Bird* Conductivity-Temperature-Depth (CTD) recorder fitted with a fluorometer was lowered to a depth of 70 metres, or to 10 metres 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. 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 station was estimated by dividing the volume of zooplankton (ml) collected during plankton tows by the total volume of water filtered (m^3). The large fraction of zooplankton (>1 mm) comprised mostly gelatinous taxa (salps, scyphozoans) and krill *Nyctiphanes australis.* The small fraction of zooplankton (<1 mm) comprised mostly copepods and cladocerans. 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 station 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. 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 stage 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 Mapinfo© (Ver. 8)

2.2.4 Spawning time and density weightings

The development time of sardine eggs is known to be 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 recorded in South Australian waters (Ward et al. 2009b). A peak spawning time of 2:00 am was assumed 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, respectively. 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. 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) in Mapinfo© (Vers. 8) was used 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 station (km^2) was then determined. The spawning area (*A*) was defined as the total area of grids where live sardine eggs were found.

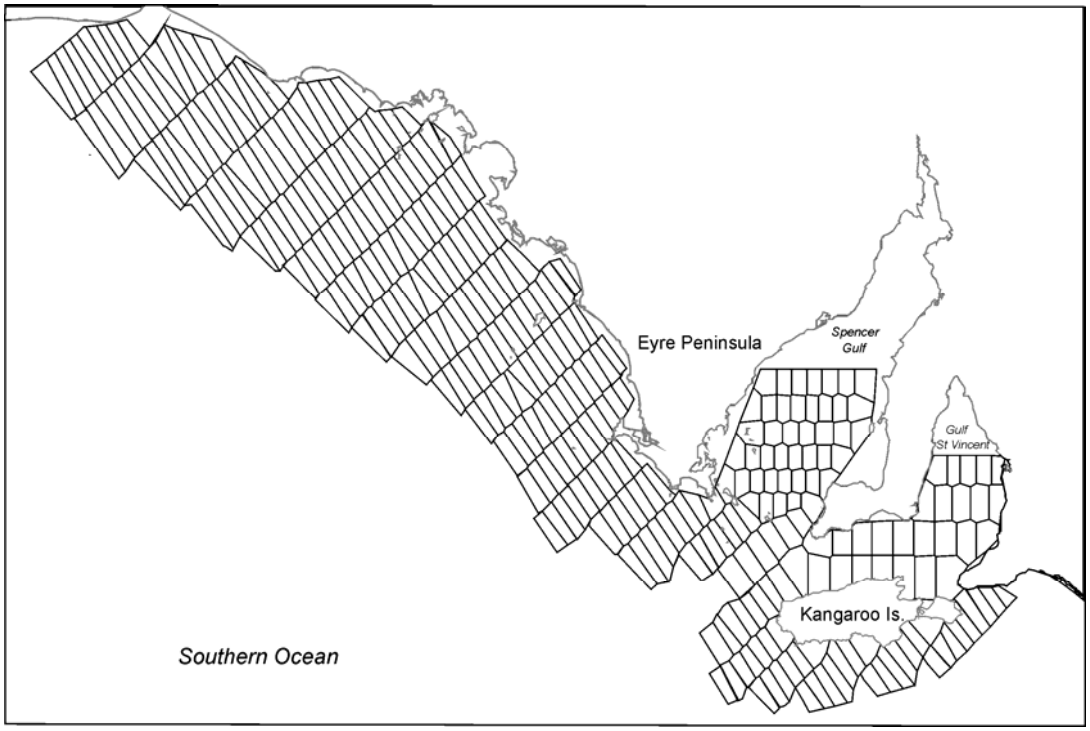

Figure 2. Voronoi nearest neighbour polygons generated in Mapinfo® (vers.8) used to estimate the total spawning area in 2009.

2.2.6 Daily egg production (P_0) and egg mortality

Biased 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 station (Picquelle and Stauffer 1985). To allow the inclusion of data from stations 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 station. 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).

2.2.7 Additional egg production and mortality models

To investigate the effects of P_0 on estimates of spawning biomass four additional models were fitted to the egg age and density data. Non-linear least squares regression with starting values of P_0 and Z were 100 and 0.5, respectively, were used to solve the exponential egg mortality model of Lasker (1985):

$$P_t = P_0 e^{-zt}, \qquad \text{Equation 5}$$

where P_t is density of eggs of age t and Z is the instantaneous rate of daily egg mortality.

P_0 was also estimated using three generalised linear models (GLMs). For observed egg densities (*Pi*), the generalised linear models were of the form:

$$g(u) = P_{0} - zt,$$ Equation 6

where u is the link function from an exponential family distribution such that $u_i \equiv E(P_i)$. One GLM (Gaussian) assumed a Gaussian distribution with a log link function, another GLM (Quasi) assumed a Quasi distribution with a log link function and variance proportional to the mean and the third GLM (Quasi^2) assumed a Quasi distribution with a log-link function and variance proportional to the mean squared. For more details on the GLMs used see Ward et al. (2009b).

2.3 Adult Reproductive Parameters

2.3.1 Sampling methods

Each afternoon, areas where sardine schools were known to aggregate were searched 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. Mature and immature males and females were counted. Mature females were fixed in 5% buffered formaldehyde solution. Immature females and males were frozen. 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 Greenly and Pearson Island in the eastern Great Australian Bight.

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:

$$W = \overline{\left[\overline{W_i} * \frac{n_i}{N} \right]}$$ Equation 7

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:

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

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:

$$\overline{R_i} = \frac{F}{(F + M)} \qquad \text{Equation 9}$$

where *F* and *M* are the respective total weights of mature females and males in each sample *i*. The mean sex ratio was also calculated for all years data from 1998 – 2007.

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. The batch fecundity for all data from 1998 – 2007 was also calculated.

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.

$$S = \overline{\left[\overline{S_i} * \frac{n_i}{N} \right]} \qquad \text{Equation 10}$$

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:

$$\overline{S_i} = \frac{[(d0 + d1 + d2POFs)/3]}{n_i} \qquad \text{Equation 11}$$

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. We also calculated spawning fraction for reproductive samples collected between 1998 – 2007.

2.4 Spawning Biomass and bootstrapping procedures

2.4.1 Spawning biomass estimates

Estimates of spawning biomass were also calculated using the estimate of P_0 obtained via the log-linear model and from adult reproductive parameters collected during:

- the 2009 survey;
- the 2009 survey to estimate mean female weight (*W*) and batch fecundity (*F*), and from 1998 to 2007 to estimate sex ratio (*R*) and spawning fraction (*S*);
- surveys between 1998 and 2007.

Estimates of spawning biomass were calculated using the estimates of P_0 obtained using the five egg production models described in sections 2.2.6 and 2.2.7, and the best estimates of adult parameters (i.e. mean female weight (*W*) and batch fecundity (*F*) estimated from adult samples collected in 2009, and sex ratio (*R*) and spawning fraction (*S*) estimates calculated from data collected between 1998 and 2007).

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 2003 and R 2.9.2 with confidence intervals estimated with R 2.9.2.

3. RESULTS

3.1. Biophysical variables

3.1.1 Sea surface temperature

Sea surface temperatures (SSTs) ranged from to 16.9 to 22.4°C (Fig. 3). Low SSTs (< 18°C) were recorded in inshore water along the western coast of Eyre Peninsula. High SSTs (> 19°C) were recorded in Spencer Gulf, Gulf St Vincent, across the mid-outer shelf waters of the eastern Great Australian Bight and throughout the central Great Australian Bight.

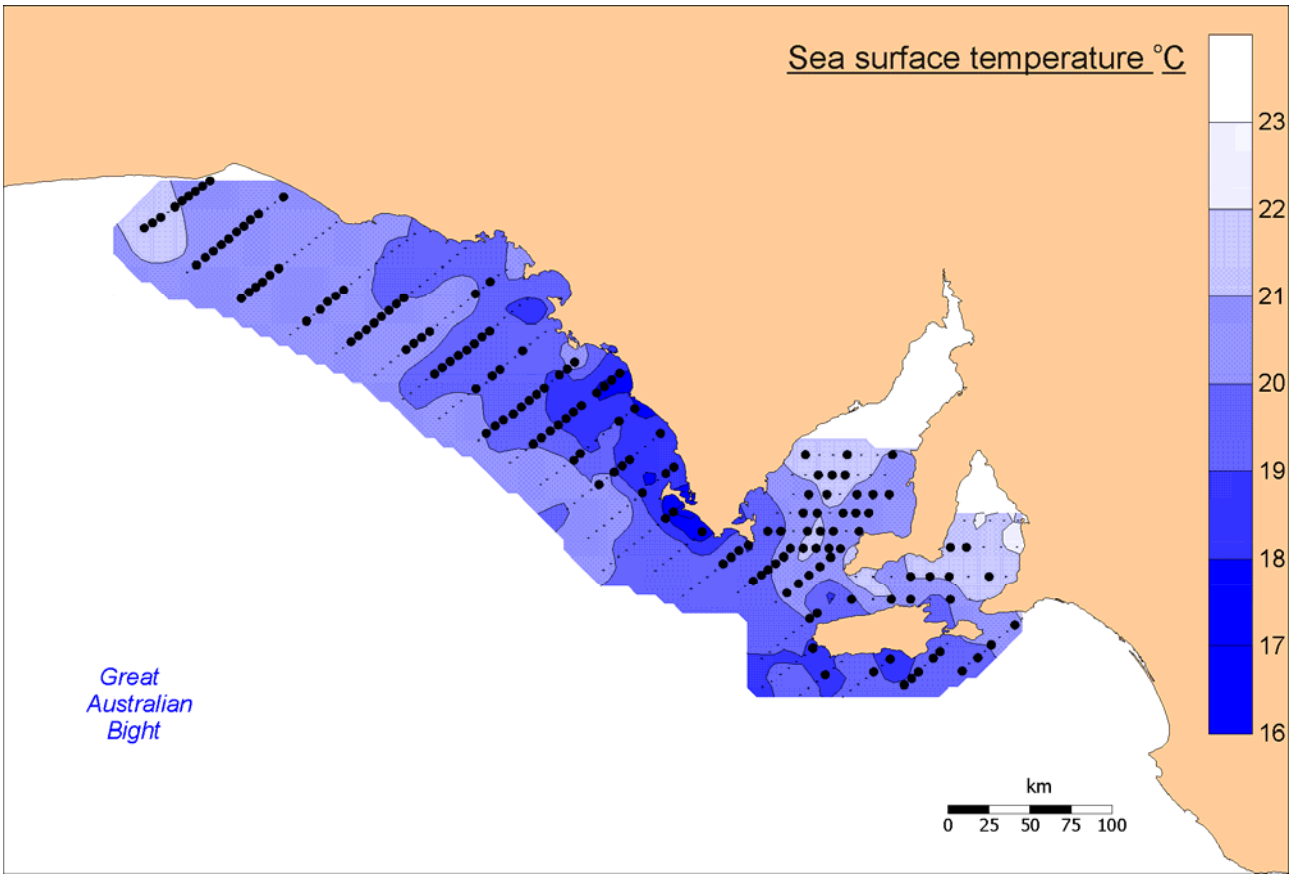

Figure 3. Sea-surface temperature profile across the 2009 survey area, showing stations where sardine eggs were collected (●).

*3.2.2 Fluorescence (chlorophyll-*a)

Chlorophyll-*a* concentration at each station ranged between 0.001 and 0.39 µg.L^{-1} (Fig. 4). The highest values were recorded in Avoid Bay off Coffin Bay Peninsula, near Cape Adieu, Anxious Bay and in the mouth of Spencer Gulf. The remainder of coastal and shelf waters mainly had chlorophyll-*a* concentrations ranging between 0.01 and 0.2 µg.L^{-1}.

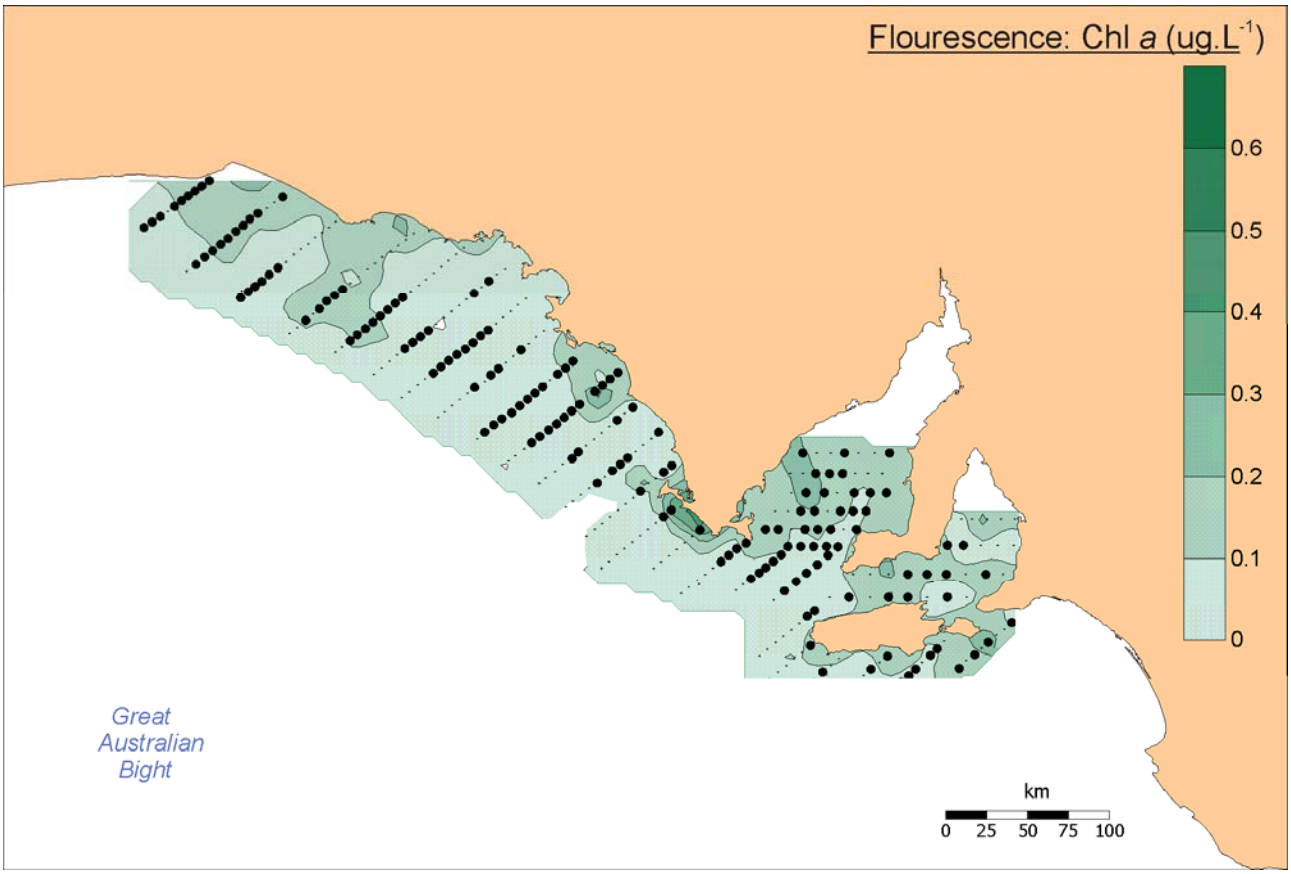

Figure 4. Surface concentration of chlorophyll-*a* across the 2009 survey area, showing stations where sardine eggs were collected (●).

3.2.3 Zooplankton abundance

Total small fraction densities of zooplankton ranged between 0.102 and 6.56 ml.m^{-3} (Fig. 5). Large fraction densities of zooplankton ranged between 0 and 8.29 ml.m^{-3} (Fig. 6). The patches of large zooplankton taxa observed on the outer shelf between Streaky Bay and Coffin Bay Peninsula were comprised mostly of salps. The highest densities of small zooplankton taxa were found in the mouth of Spencer Gulf, Investigator Strait, inshore west of Eyre Peninsula, south of Kangaroo Island and the Head of Bight.

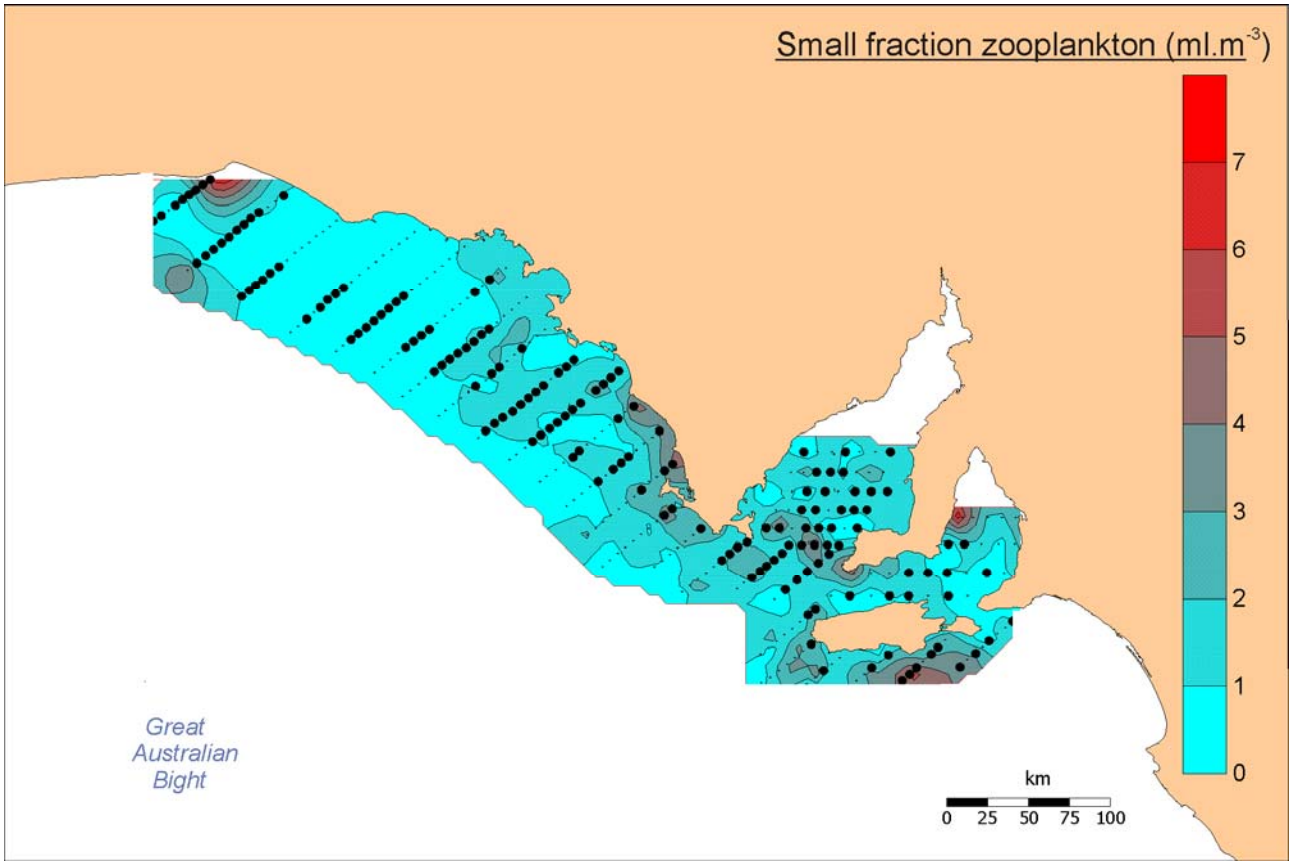

Figure 5. Distribution and abundance (ml.m^{-3}) of zooplankton (small fraction) across the 2009 survey area, showing stations where sardine eggs were collected (●).

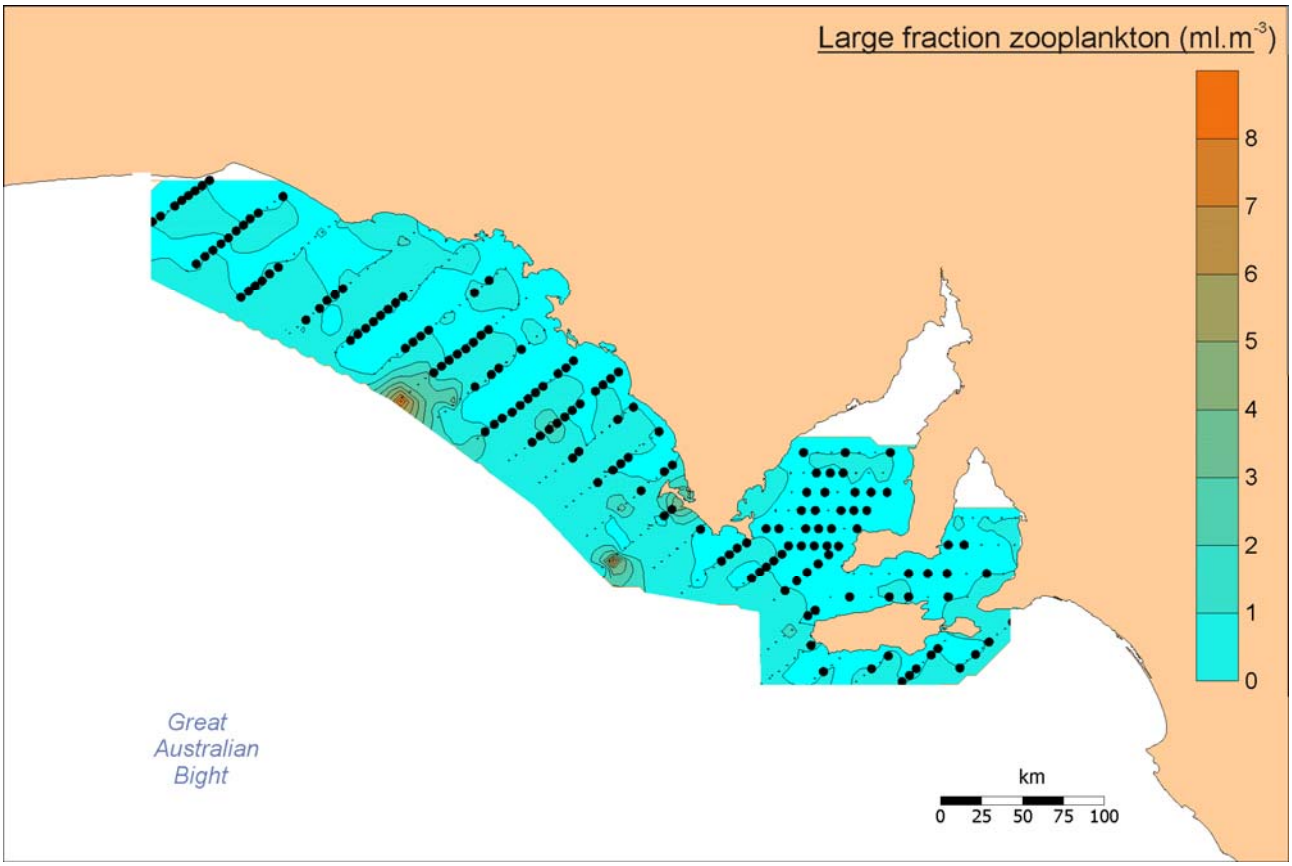

Figure 6. Distribution and abundance (ml.m^{-3}) of zooplankton (large fraction) across the 2009 survey area, showing stations where sardine eggs were collected (●).

3.2 Distribution and Abundance of Eggs and Larvae

3.2.1 Distribution and abundance of eggs

A total of 2,582 sardine eggs were collected at 159 of 340 (46.8%) stations on 34 transects between the Head of Bight and Victor Harbor (Fig. 7). The stations with the highest egg densities were located in southern Spencer Gulf, north of Pt Sir Isaac and south of Kangaroo Is. Egg densities up to 1,618 eggs.m^{-2} were recorded in these regions. In contrast to other years when strong upwelling events occurred, eggs were also found in higher densities in waters between Cape Carnot and Cape Finniss. Egg densities at positive stations west of Cape Finniss were lower than in southern Spencer Gulf and ranged between 5 and 392 eggs.m^{-2}.

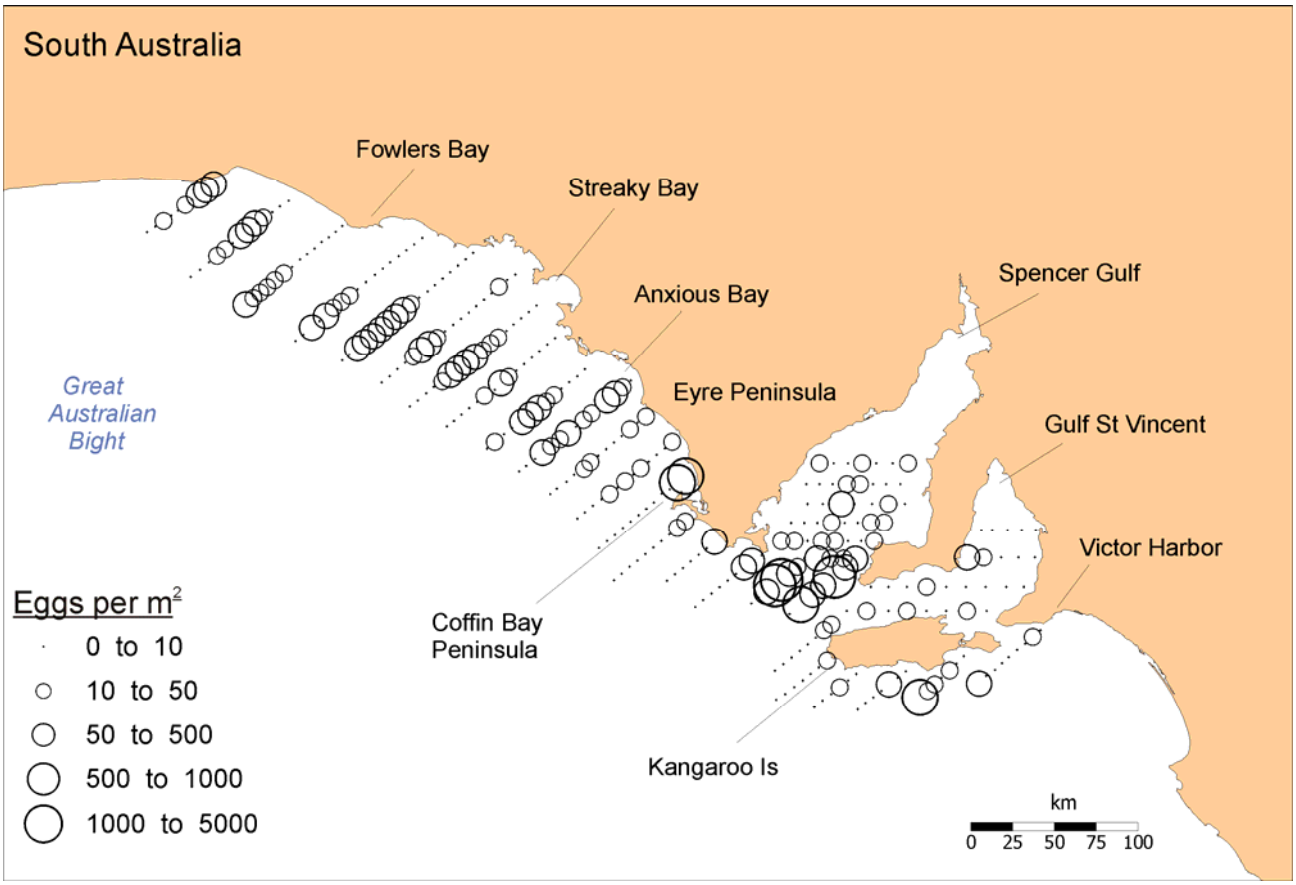

Figure 7. Spatial patterns of sardine egg distribution and abundance in 2009.

3.2.2 Larval abundance and distribution

A total of *n* = 3,708 sardine larvae were collected at 217 of 340 stations (63.8%) between the Head of Bight and Victor Harbor (Fig. 8). The spatial distribution of larvae was similar to that of sardine eggs. Densities were highest west of Venus Bay, adjacent to Coffin Bay Peninsula, in southern Spencer Gulf, and south of Kangaroo Island, and ranged between 5 and 1,410 larvae.m^{-2}.

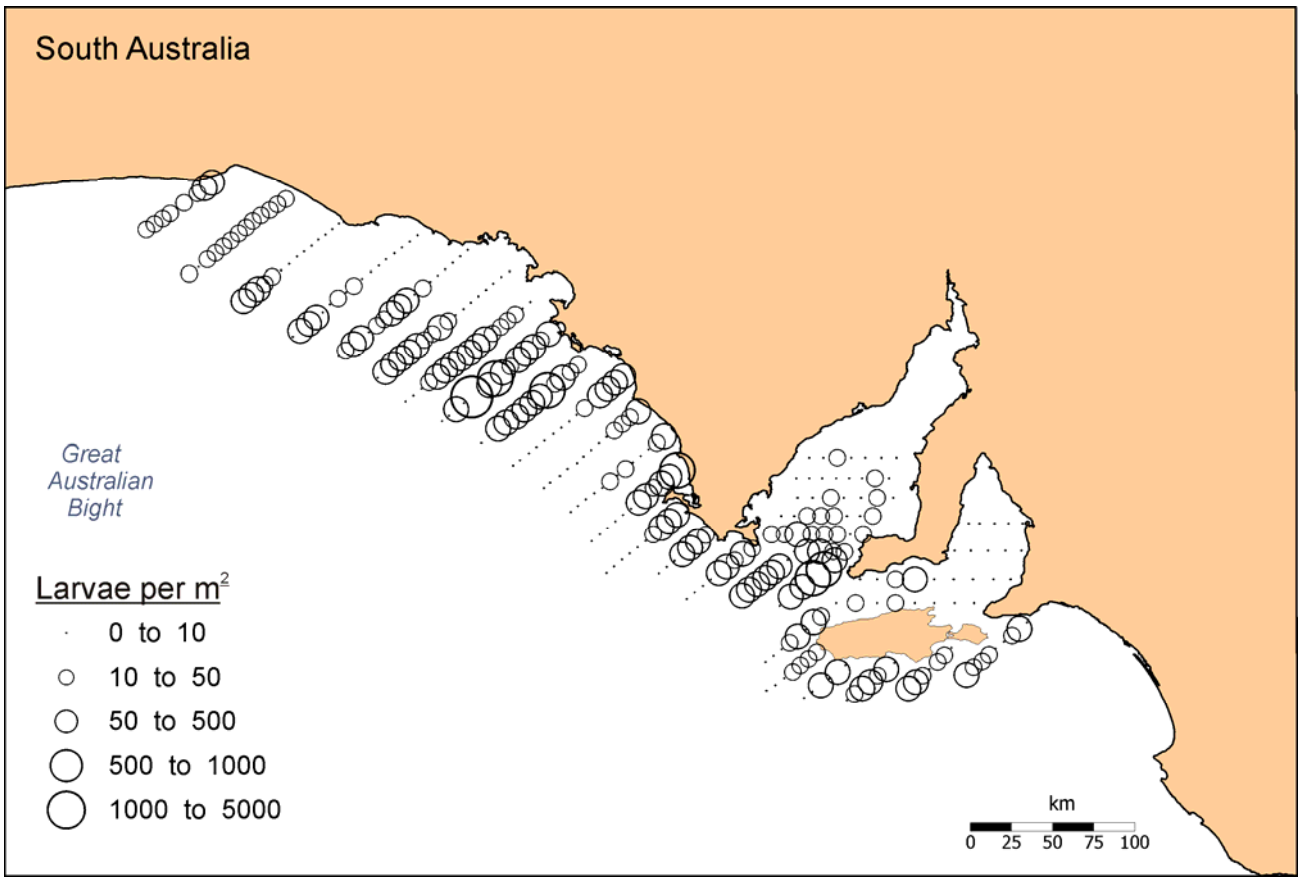

Figure 8. Spatial patterns of sardine larval distribution and abundance in 2009.

3.3 Spawning Area

The estimated spawning area for the entire survey area was 53,553 km^2, comprising 46.7% of the total area sampled (114,746 km^2) (Table 1).

Table 1. Mean daily egg production (P_0, log-linear model), spawning area (A) and spawning biomass (Parameters S, R from 1998 – 2007, all other adult parameters from 2009). Table shows the variance (σ^2) term used in estimate.

	Area sampled	Spawning area A (km ^{2})	Percentage of area sampled	$\sigma^2 P_b$	P_0 (eggs.d^{-1}.m^{-2})	Spawning biomass (t)
Total survey	114,745	53,553	46.7	1.27	63.2	171,531

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. 2009b) was 63.2 eggs.day^{-1}.m^{-2} (95% CI = 46.1 – 87.1, Fig. 9, Table 1). The other four models provided estimates of P_0 that ranged from 87.4 eggs.day^{-1}.m^{-2} (exponential model and Gaussian GLM) to 96.0 eggs.day^{-1}.m^{-2} (Quasi^2 GLM) (Table 5).

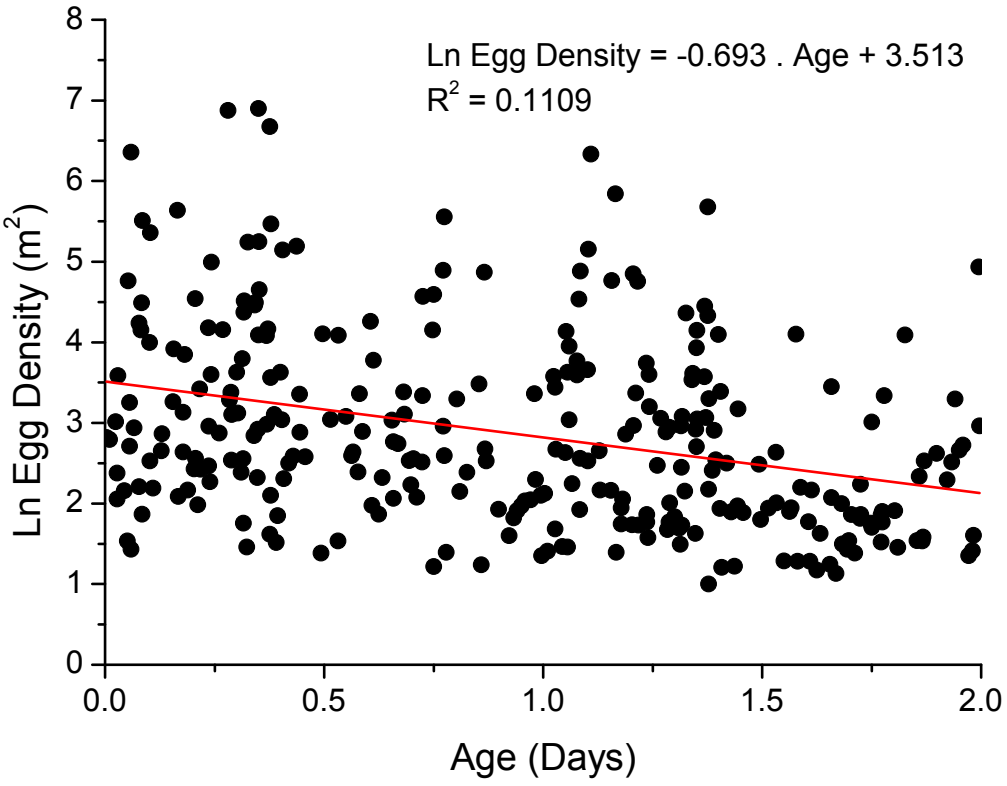

Figure 9. Linear regressions between ln-transformed sardine egg density (eggs.m^{-2}) and age (days) data in 2009.

3.5 Adult Reproductive Parameters

A total of 19 samples comprising 3767 mature sardines was collected at Scotts Cove, Wedge Is., North Neptune Is., Greenly Is. and Pearson Is. during the 2009 survey (Table 2). Estimates of the adult female reproductive parameters used in calculations of spawning biomass are provided in Tables 3, 4 and 5. Adult parameters calculated from samples collected between 1998 and 2007 are provided in Table 5. Bootstrapped parameter estimates that provided 95% confidence intervals are shown in Table 5. Male samples collected during the first cruise in 2009 were not used in the analysis due to a freezer break down onboard the R.V. Ngerin.

Table 2. Sampling details for adult sardine collected in Investigator Strait and the eastern Great Australian Bight during the 2009 DEPM surveys.

Date	Location	Survey	N samples	*n* fish
18/02/2009	Scotts Cove	1	3	397
20/02/2009	N. Neptune Is.	1	4	346
22/02/2009	Greenly Is.	1	3	599
26/03/2009	Pearson Is.	2	3	432
27/03/2009	Pearson Is.	2	1	234
27/03/2009	Greenly Is.	2	1	96
28/03/2009	Greenly Is.	2	1	42
28/03/2009	N. Neptune Is.	2	3	1621
		Total	19	3767

3.5.1 Mean female weight

The mean weight of mature females in samples ranged from 48.4 to 75.0 g (Table 3). The weighted mean weight of mature females in 2009 was 59.9 g (95% CI = 55.5 – 65.8, Table 3) and for 1998 – 2007 was 62.1 g (95% CI = 59.2 – 65.0, Table 5).

3.5.2 Sex ratio

The sex ratio calculated from the 2009 data was abnormally low (0.36, 95% CI = 0.285 – 0.436) (Table 5). The sex ratio from data collected between 1998 and 2007 was 0.527 (95% CI = 0.493 – 0.561).

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 2009. 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	18/02/2009	28	90	N/A	59.6	N/A
2	Scotts Cove	18/02/2009	53	92	N/A	59.1	N/A
3	Scotts Cove	18/02/2009	43	91	N/A	57.1	N/A
4	Neptune Island	20/02/2009	8	48	N/A	71.0	N/A
5	Neptune Island	20/02/2009	90	90	N/A	69.0	N/A
6	Neptune Island	20/02/2009	9	32	N/A	65.5	N/A
7	Neptune Island	20/02/2009	16	53	N/A	60.1	N/A
8	Greenly Island	22/02/2009	103	52	N/A	74.6	N/A
9	Greenly Island	22/02/2009	183	126	N/A	71.7	N/A
10	Greenly Island	22/02/2009	36	99	N/A	70.0	N/A
11	Pearson Island	26/03/2009	99	29	50.9	73.3	0.30
12	Pearson Island	26/03/2009	168	50	51.9	71.8	0.29
13	Pearson Island	26/03/2009	78	8	53.0	75.0	0.13
14	Pearson Island	27/03/2009	195	39	51.6	66.4	0.20
15	Greenly Island	27/03/2009	54	42	44.6	51.6	0.47
16	Greenly Island	28/03/2009	27	15	43.1	61.8	0.44
17	Neptune Island	28/03/2009	623	271	42.2	51.6	0.35
18	Neptune Island	28/03/2009	195	155	39.4	48.4	0.49
19	Neptune Island	28/03/2009	222	155	41.1	50.4	0.46
			2230*	**1537***		**59.9#**	

Table 4. Number of female sardine in samples and estimates of spawning fraction (*S)* for samples collected in 2009. Values in bottom row are sums* and weighted means#.

Sample	Location	Date	POF 0	POF 1+2	Total	Spawning Fraction (S)	POF 0s only
1	Scotts Cove	18/02/2009	18	11	90	0.11	0.20
2	Scotts Cove	18/02/2009	8	13	92	0.08	0.09
3	Scotts Cove	18/02/2009	3	13	91	0.06	0.03
4	Neptune Island	20/02/2009	2	2	48	0.03	0.04
5	Neptune Island	20/02/2009	8	13	90	0.08	0.09
6	Neptune Island	20/02/2009	4	6	32	0.10	0.13
7	Neptune Island	20/02/2009	11	6	53	0.11	0.21
8	Greenly Island	22/02/2009	26	8	52	0.22	0.50
9	Greenly Island	22/02/2009	40	13	126	0.14	0.32
10	Greenly Island	22/02/2009	22	15	99	0.12	0.22
11	Pearson Island	26/03/2009	22	0	29	0.25	0.76
12	Pearson Island	26/03/2009	24	1	50	0.17	0.48
13	Pearson Island	26/03/2009	7	1	8	0.33	0.88
14	Pearson Island	27/03/2009	25	14	39	0.33	0.64
15	Greenly Island	27/03/2009	13	4	42	0.13	0.31
16	Greenly Island	28/03/2009	4	3	15	0.16	0.27
17	Neptune Island	28/03/2009	210	1	271	0.26	0.77
18	Neptune Island	28/03/2009	73	5	155	0.17	0.47
19	Neptune Island	28/03/2009	53	16	155	0.15	0.34
Total			573*	145*	1537*	0.16#	0.37#

Table 5. Parameters used in the calculations of spawning biomass.

Adult Parameters	Estimate	Lower 95% CI	Upper 95% CI
Sex Ratio 2009	0.364	0.285	0.436
Sex Ratio 98-07	0.527	0.493	0.561
Spawning Fraction 2009	0.156	0.114	0.196
Spawning Fraction 98-07	0.123	0.109	0.138
Female Weight 2009	59.9	55.5	65.8
Female Weight 98-07	62.1	59.2	65.0
Fecundity 2009	18,187	16,313	20,457
Fecundity 98-07	18,542	17,598	19,528
P_0 Estimates			
Log Linear	63.2	56.5	87.1
Exponential	87.4	73.6	133.9
Gaussian GLM	87.4	73.6	133.9
Quasi GLM	91.4	76.5	140.8
Quasi^2 GLM	96.0	80.4	150.0

3.5.3 Batch fecundity

Batch fecundity ranged from 4,382 to 42,397 hydrated oocytes for the 108 hydrated female sardine examined in 2009. Based on the relationship (Batch Fecundity = 349.9 x Gonad Free Female Weight – 1240, R^2 = 0.47, Fig. 10) and the mean gonad free female weight (55.8 g) for all samples collected in 2009, mean batch fecundity was 18,187 hydrated oocytes per batch (95% CI = 16,312 – 20,457). This resembled the batch fecundity calculated for samples collected between 1998 and 2007 (18,542, 95 % CI = 17,598 – 19,528) (Table 5).

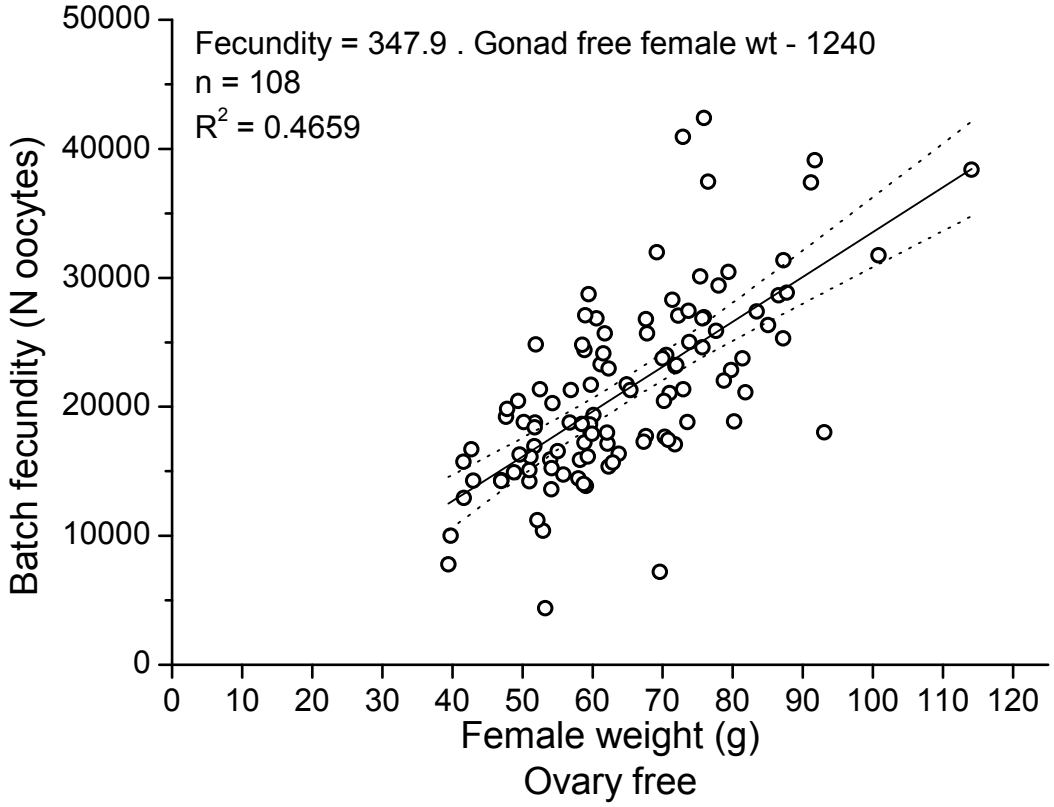

Figure 10. Relationship between gonad-free weight and batch fecundity in 2009 (dotted line = 95% CI).

3.5.4 Spawning fraction

Of the 1537 ovaries examined, 573 had hydrated oocytes and/or day-0 POFs and 145 had day-1 POFs or day-2 POFs (Table 4). The percentage of females in samples with hydrated oocytes and/or day-0 POFs ranged from 3 to 77%. Some samples (e.g. S17, Neptune Island, 28/3/09, Table 4) had very high numbers of females with hydrated oocytes and/or day-0 POFs. The weighted mean spawning fraction for all 2009 data was 0.156 (95% CI = 0.114 – 0.196) whereas using data from 1998 – 2007 the spawning fraction was 0.123 (95% CI = 0.109 – 0.138, Table 5).

3.6 Re-sampling: Bootstrapping Procedures

The distributions for each variable calculated using 'bootstrap replacement' procedures and the percentile method are shown in Table 5.

3.7 Spawning Biomass

The best estimate of spawning biomass, calculated using the log-linear version of the exponential egg mortality model, female weight and batch fecundity from 2009 adult samples and sex ratio and spawning fraction from 1998-2007 (all previous years) adult samples was 171,531 t (95% CI = 122,100 – 242,497, Table 5, Fig.11). Estimates of spawning biomass obtained using the best estimates of adult parameters (i.e. female weight and batch fecundity from 2009 samples and sex ratio and spawning fraction from 1998-2007 samples) and the five models for estimating egg production ranged from 171,531 t (log-linear) to 260,399 t (Quasi^2, Fig. 12). Estimates of spawning biomass obtained using the best estimate of egg production (log-linear model) and different estimates of adult parameters ranged between 171,531 t (female weight and batch fecundity from 2009 samples and sex ratio and spawning fraction from 1998-2007 samples) and 196,969 t (all adult paramaters from 2009 samples, Fig. 11).

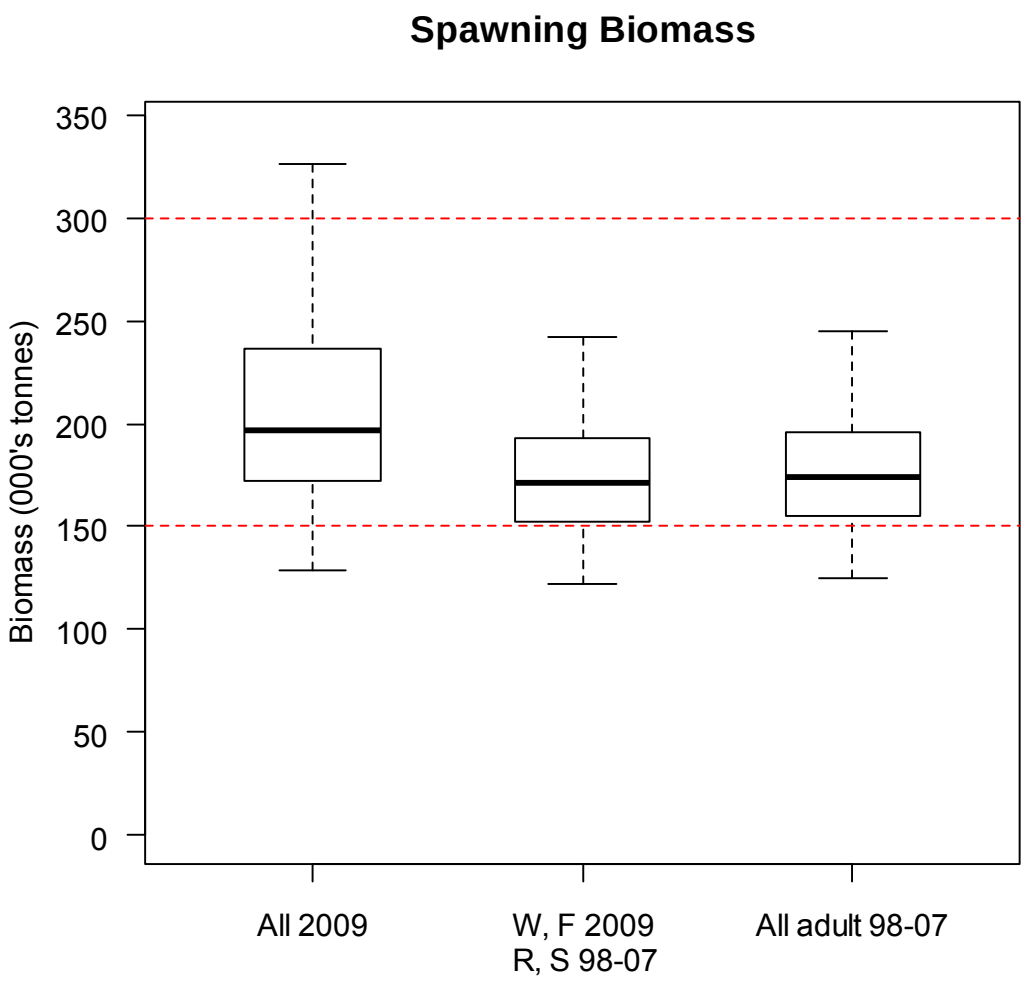

Figure 11. Estimates of spawning biomass obtained using estimate of egg production obtained with the log-linear model and 1), all adult parameters estimated from 2009 samples; 2), female weight and batch fecundity estimated from 2009 samples and sex ratio and spawning fraction estimated from 1998-2007 (all previous years) samples; and 3), all adult parameters estimated from 1998-2007 samples. The whiskers (outer values) represent the 95% confidence intervals and the box represents the 50% confidence interval. Dotted lines are the 150,000 – 300,000 t biomass range for TACC.

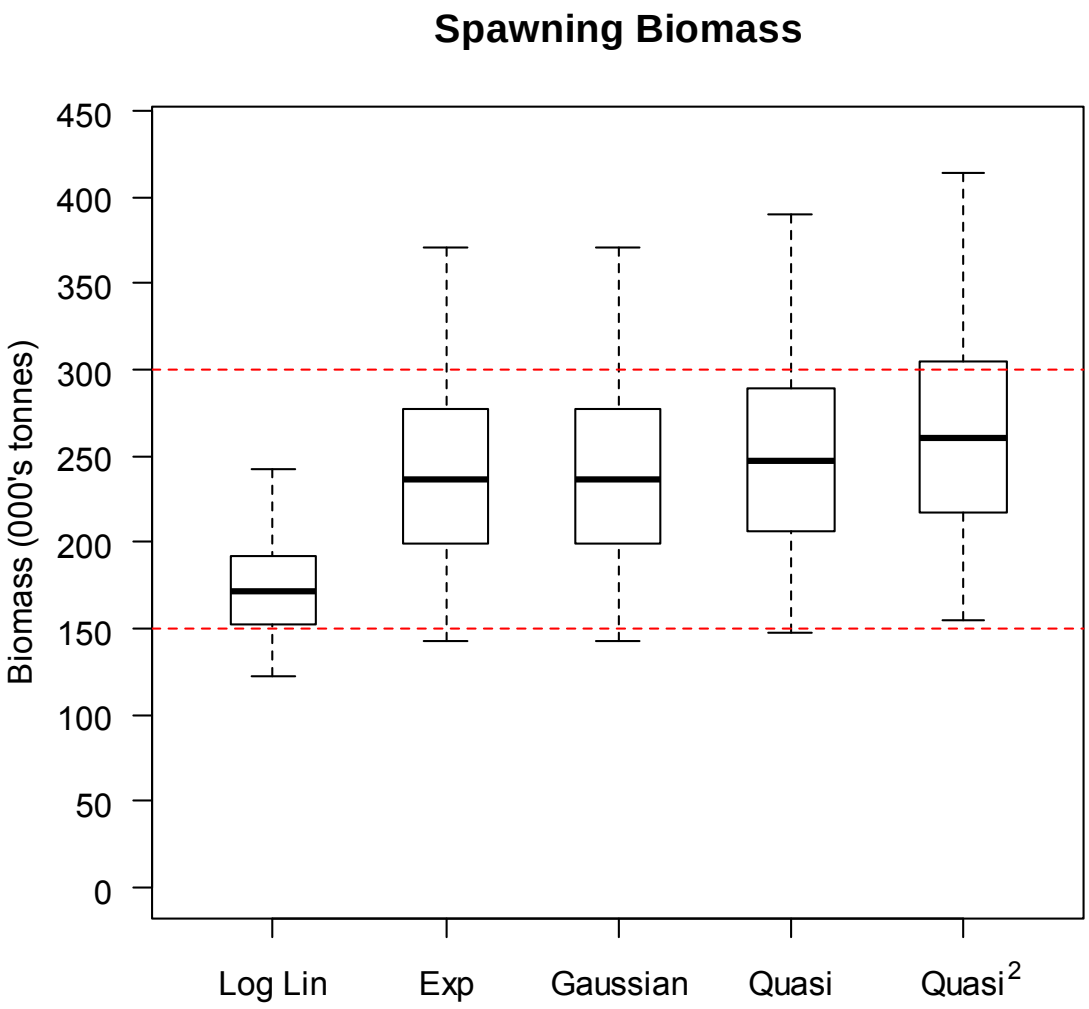

Figure 12. Estimates of spawning biomass using female weight and batch fecundity estimated from 2009 samples and sex ratio and spawning fraction estimated from 1998-2007 (all previous years) and the five models used to estimate $P_{0.}$ The whiskers (outer values) represent the 95% confidence interval and the box represents the 50% confidence interval. Dotted lines are the 150,000 – 300,000 t biomass range for TACC.

4. DISCUSSION

4.1 Biophysical variables and egg and larval distribution patterns

The lowest SSTs (<18.0^{o}C) and highest levels of chlorophyll-*a* (>0.2 µg.L^{-1}) recorded in 2009 were at sites located in coastal waters along the southern Eyre Peninsula. This pattern has also been observed in previous DEPM surveys, which are always conducted during the summer-autumn upwelling period (e.g. Ward et al. 2007). However, SSTs and concentrations of chlorophyll-*a* in 2009 were generally lower than those recorded in past surveys (i.e. 14 – 15^{o}C; up to 3.3 µg.L^{-1}), suggesting that upwelling was not as strong during the 2009 cruises as it has been in previous years. Other studies conducted by SARDI Aquatic Sciences also suggest that 2009 was not a strong upwelling year (Dr John Middleton, pers. comm.). Zooplankton densities recorded in 2009 were also lower than in most previous years.

During the 2009 surveys, large numbers of sardine eggs and larvae were collected over a large area. For example, the total number of eggs collected in 2009 was the fourth highest recorded since 2000 (2004, 2006 or 2007 were higher). Importantly, egg and larval densities in Spencer Gulf, where the SASF mainly operate, were high in comparison to previous years, which is a positive sign for the status of the stock. During the 2009 surveys, sardine eggs and larvae were also collected from waters between Cape Carnot and Cape Finniss (Figs. 7, 8). This contrasts with observations made during strong upwelling years, when SSTs are low in these waters and sardine eggs and larvae are often absent or rare. In 2009, sardine eggs and larvae were widely distributed in waters west of Cape Finniss, but densities were relatively low compared to those recorded in this area during previous years. This could reflect the dispersal of adults in response to the low densities of plankton observed west of Cape Finniss during 2009. However, the low densities recorded in these waters compared to those recorded in Spencer Gulf may also indicate that the level of spawning activity during the second 2009 cruise was lower than during the first.

4.2 Spawning area

The estimate of spawning area for 2009 (53,553 km^{2}, Table 1) is the highest recorded for DEPM surveys in South Australian waters. This is a positive indicator of the current status of the stock as spawning area is the DEPM parameter that is most strongly correlated with spawning biomass (Gaughan et al. 2004). The large spawning area suggests wide dispersal of adults, possibly in response to the low plankton densities across South Australian waters during the 2009 survey.

4.3 Egg production

The estimates of daily egg production obtained using five models ranged from 63.2 to 96.0 eggs. day^{-1}.m^{-2}. The estimate obtained using the linear version of the exponential egg mortality, which

was the method recommended for sardine in South Australian waters by Ward et al. (2009b), was 63.2 eggs. $day^{-1}.m^{-2}$ (95% CI = 46.1 – 87.1, Fig. 9, Table 1). Ward et al. (2009b) recommended that the linear version of the exponential egg mortality be used for estimating mean daily egg production of sardine because it consistently provides more precautionary estimates than the other models. Furthermore, estimates of mean daily egg production obtained using this method are not as strongly affected by one or two samples containing large numbers of eggs as estimates obtained using other models.

The estimate of mean daily egg production obtained using the log-linear model is lower than the estimate obtained in the previous study in 2007 of 116.6 $eggs.day^{-1}.m^{-2}$ (95% CI = 74.17-182.42). This decrease may be related to the wide dispersal of sardines across the shelf, and subsequently large spawning area, in 2009. However, it could also reflect the potentially low level of spawning activity in the western part of the survey area during the second cruise. If spawning activity was actually lower during the second cruise than during the first, it would suggest that there would be benefits in estimating spawning biomass separately for each cruise. This approach would require the development of new methods for sampling spawning adults that could be applied throughout the survey area (see section 4.4. below).

The large confidence intervals for the estimates of egg production obtained using all models support the widely held view that the DEPM is imprecise and that much of this imprecision is associated with the estimation of egg production. As the DEPM is only applied every two years under the current harvest strategy, it could be argued that there is a need to ensure that estimates of egg production and spawning biomass are as precise as possible. However, this need could be further increased if a new, less precautionary harvest strategy were to be established for the fishery. As indicated by Ward et al. (2009b), the best way to increase the precision of estimates of egg production would be to develop a numerical model that integrates data from CUFES and CalVET nets to estimate egg production.

4.4 Adult sampling

During the 2009 survey, 19 samples of adult sardine containing 1,537 females were collected from throughout South Australian waters, including Scotts Cove, North Neptune Island, Pearson Island and Greenly Island. No samples were collected west of Pearson Island, due to the absence of locations (i.e. protected bays on offshore islands) that are suitable for sampling adults using the existing methodology (i.e. gill-net and lights). Alternative adult sampling methods which can be deployed in open water need to be developed as a high priority.

Several of the adult samples collected during 2009 (e.g. samples 11, 14 and 17; Table 3 and 4) included high proportions of males and actively spawning females (POF *d*0) which suggests that the samples were from ephemeral, male-dominated spawning aggregations that form during the daily spawning period (Stratoudakis et al. 2006). As the sex ratio of the population is unlikely to differ significantly from 0.5, the low overall estimate of sex ratio obtained in 2009 provides strong evidence that spawning aggregations were over-represented in samples collected during 2009. The high estimate of spawning fraction for 2009 also supports this conclusion. For this reason, estimates of sex ratio (*R*) and spawning fraction (*S*) calculated from data obtained during all previous years (1998-2007) appear to provide the most suitable basis for estimating the spawning biomass for 2009. The dominance of spawning aggregations in areas where adults were sampled during 2009 further emphasises the need to develop adult sampling methods that can be employed in additional locations to those that can be sampled using the current methodology.

4.5 Spawning biomass estimates

The best estimate of spawning biomass for 2009 was 171,531 t (95% CI = 122,100 – 242,479). This estimate is based on the estimates of egg production obtained using the linear version of the exponential model, which was the method recommended for use on South Australian sardine by Ward et al. (2009b), and precautionary estimates of adult parameters, namely estimates of female weight and batch fecundity obtained from samples collected in 2009 and estimates of sex ratio and female weight obtained from samples collected from 1998-2007. The best estimate of spawning biomass for 2009 (171,531 t) lies within the target range for spawning biomass of 150,000 to 300,000 t. A TACC for 2010 of 30,000 t would equate to an exploitation rate of 17.5% of the estimate of spawning biomass for 2009.

4.6 Future research directions

The best option for improving the precision of estimates of spawning biomass obtained using the DEPM is to develop a numerical model to integrate data collected by a CUFES and CalVET nets to estimate mean daily egg production. The other opportunity to improve estimates of spawning biomass is to develop alternative adult sampling methods. This would ideally be done in collaboration with members of the SASF and involve investigation of the potential for obtaining adult samples from both trawling and purse-seining.

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