

Spawning biomass of Sardine, *Sardinops sagax*, in waters off South Australia in 2016



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

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SARDI Aquatics Sciences
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Report to PIRSA Fisheries and Aquaculture

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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 obtained using this method is the key biological performance indicator for the South Australian Sardine Fishery (SASF). This report provides an estimate of the spawning biomass of Sardine in waters off South Australia in February-March 2016.

EXECUTIVE SUMMARY

This report provides an estimate of the spawning biomass of Sardine, *Sardinops sagax*, in waters off South Australia in 2016.

Surveys were conducted during 4–16 February and 2–13 March 2016. The total survey area was 122,567 km².

Sea surface temperatures (SSTs) ranged from 14.1 to 22.5°C. Low SSTs and elevated concentrations of chlorophyll-*a* in coastal waters of the western Eyre Peninsula reflected seasonal upwelling during the surveys.

A total of 1,979 live Sardine eggs were collected from 139 sites. Most eggs were collected from shelf waters of the eastern and central Great Australian Bight.

The spawning area (*A*) in 2016 was 50,105 km².

Mean daily egg production (P_0) calculated using the linear version of the exponential egg mortality was 58.1 eggs.day⁻¹.m⁻² (95% CI = 37.7–88.4 eggs.day⁻¹.m⁻²). P_0 was estimated to be 53.4 (95% CI = 31.3–92.4), 61.7 (95% CI = 34.1–100.4) and 62.2 (95% CI = 34.3–101.4) eggs.day⁻¹.m⁻² using the non-linear least squares model, negative binomial GLM and quasi GLM, respectively.

Nine samples comprising 1,061 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 from these samples were: female weight, $W = 48.4$ g (95% CI = 41.8–59.8); batch fecundity, $F = 15,355$ hydrated oocytes (95% CI = 12,818–18,379); sex ratio, $R = 0.64$; spawning fraction, $S = 0.088$ (95% CI = 0.064–0.117).

As no females with hydrated oocytes were collected in 2016, F was estimated by applying the batch fecundity relationship for all years to the mean gonad free female weight for 2016.

The historical mean R (0.53) was used to estimate spawning biomass because samples used to obtain the estimate for 2016 (0.64) were considered to be biased towards females.

The estimate of spawning biomass calculated using the log linear model was 201,316 t (95% CI = 112,712–378,335 t). Estimates of spawning biomass calculated using other egg production models ranged from 185,579 to 214,387 t.

There is a need to investigate methods for sampling egg and adult Sardines that may refine estimates of egg production and spawning fraction.

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:

Equation 1

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

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 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; 2011a). 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 *et al.* 2015). FRDC Project 2014/026 has also highlighted the need to improve methods for collecting egg samples to estimate egg production.

1.3 Aim and Objectives

This report provides an estimate of the spawning biomass of Sardine in gulf and shelf waters of South Australia during February-March 2016. 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_0 , W , R , F , S);
3. To use the DEPM to estimate the spawning biomass in 2016;
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 during February and March 2016. Plankton samples were collected at 350 sites on 34 transects between Victor Harbor and the Head of Bight (Fig. 1). Of these 350 samples, 9 were additional to the pre-determined survey design. 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).

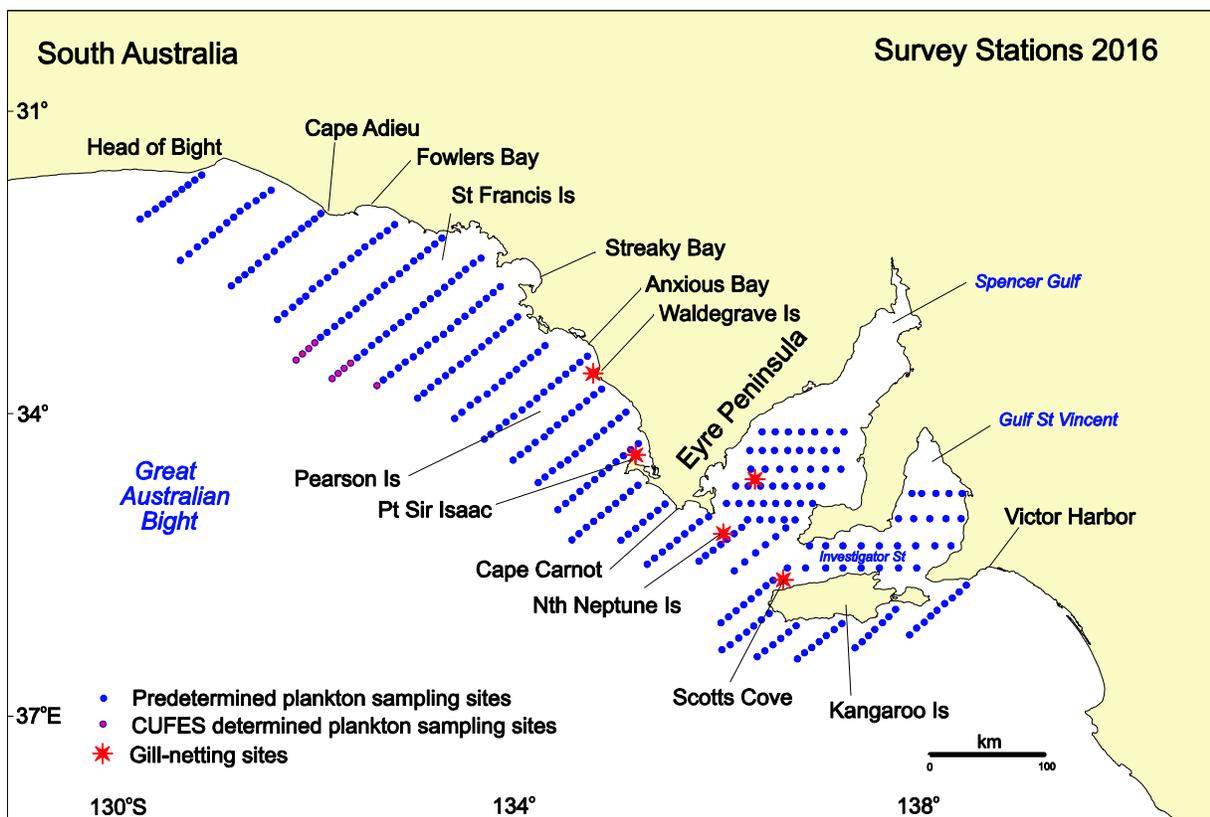


Figure 1. Map of South Australia showing sites where plankton and adult samples were collected during the 2016 DEPM surveys.

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 ($\mu\text{g.L}^{-1}$). 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 sampled (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 $\sim 1 \text{ m.s}^{-1}$. *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 5% 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 a day

class based on descriptions and temperature-development keys in White and Fletcher (1996). Approximate ages were determined from collection time and an assumed spawn time of 12 am.

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:

$$P_t = \frac{C \cdot D}{V} \quad \text{Equation 2}$$

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). 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 $<19.0^\circ\text{C}$, $19.0\text{--}20.0^\circ\text{C}$ and $>20.0^\circ\text{C}$, 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.

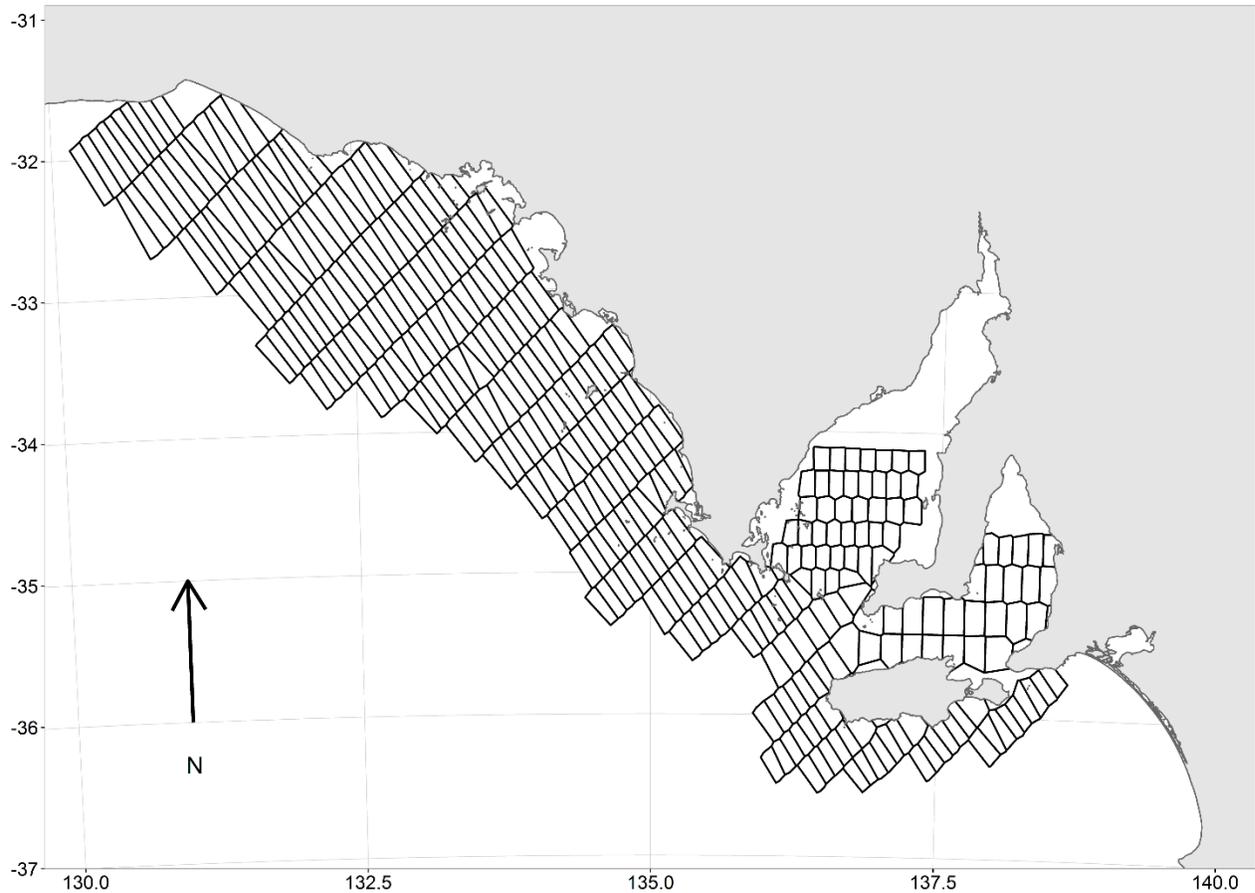


Figure 2. Voronoi nearest neighbour polygons generated in R, used to estimate the total spawning area in 2014.

2.2.6 Daily egg production (P_b) 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, zero-egg records were introduced at ages corresponding to these measurements. The linear version of the exponential egg mortality model is:

$$\ln P_b = \ln(P_i + 1) - Zt, \quad \text{Equation 3}$$

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):

$$P_0 = e^{(\ln P_b + \sigma^2/2)} - 1 \quad \text{Equation 4}$$

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, Figure 1).

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:

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

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:

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

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:

$$\overline{R}_i = \frac{F}{(F + M)} \quad \text{Equation 7}$$

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

2.3.5 Batch fecundity (F)

No fish with hydrated oocytes were collected in 2016. Batch fecundity was estimated from historical data. In previous years, 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 weighed ovarian sub-sections counted. 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 mean 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 (*d0*) (assumed to be spawning or have spawned on the night of capture), day-1 POFs (*d1*) (assumed to have spawned the previous night) and day-2 POFs (*d2*) (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.

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

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:

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

where, *d0*, *d1* and *d2* 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

2.4.1 Spawning biomass estimates

Spawning biomass was calculated according to Equation 1 using the estimate of P_0 obtained using the log-linear model, spawning area (A) and adult parameters for S and W estimated from the 2016 survey. Due to the absence of hydrated females collected in 2016, the batch fecundity relationship for all years was applied to the mean gonad free female weight for 2016 to estimate F . The historical mean R was used instead of the 2016 estimate because it was considered to be biased towards females.

Spawning biomass was also estimated using three other models for estimating egg production (*i.e.* the non-linear least squares model, negative binomial GLM and quasi GLM).

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 and S were calculated from the bootstrapped survey using the method described above. Batch fecundity (F) was calculated from the bootstrapped gonad-free female weight using the batch relationship obtained from all females with hydrated oocytes sampled since 1998. The adult parameter R was sampled from a normal distribution chosen such that the 2.5% and 97.5% quantiles aligned to the minimum and maximum observed values, respectively. For each bootstrap iteration the sampled values of W , R , F , and S were used in the calculation of 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 , and S using the percentile method. Parameter estimates were calculated independently in Excel 2013 and R 3.3.1 (for quality assurance) with confidence intervals estimated with R 3.3.1.

2.4.3 Sensitivity Analysis

Sensitivity analyses were conducted to assess the effects on estimates of spawning biomass of variations in the range of values obtained for each parameter in 2016 and between 1998 and 2014.

3. RESULTS

3.1 Distribution and Abundance of Eggs

3.1.1 Distribution and abundance of eggs

A total of 1,979 live Sardine eggs were collected at 139 of 350 (39.7%) sites on 34 transects between the Head of Bight and Victor Harbor between February and March 2016 (Fig. 3). The sites with the highest egg densities were located in the mouth of Spencer Gulf, north-west of Anxious Bay and in mid/outer shelf waters of the eastern and central GAB. The highest egg density recorded was 1,199 eggs.m⁻².

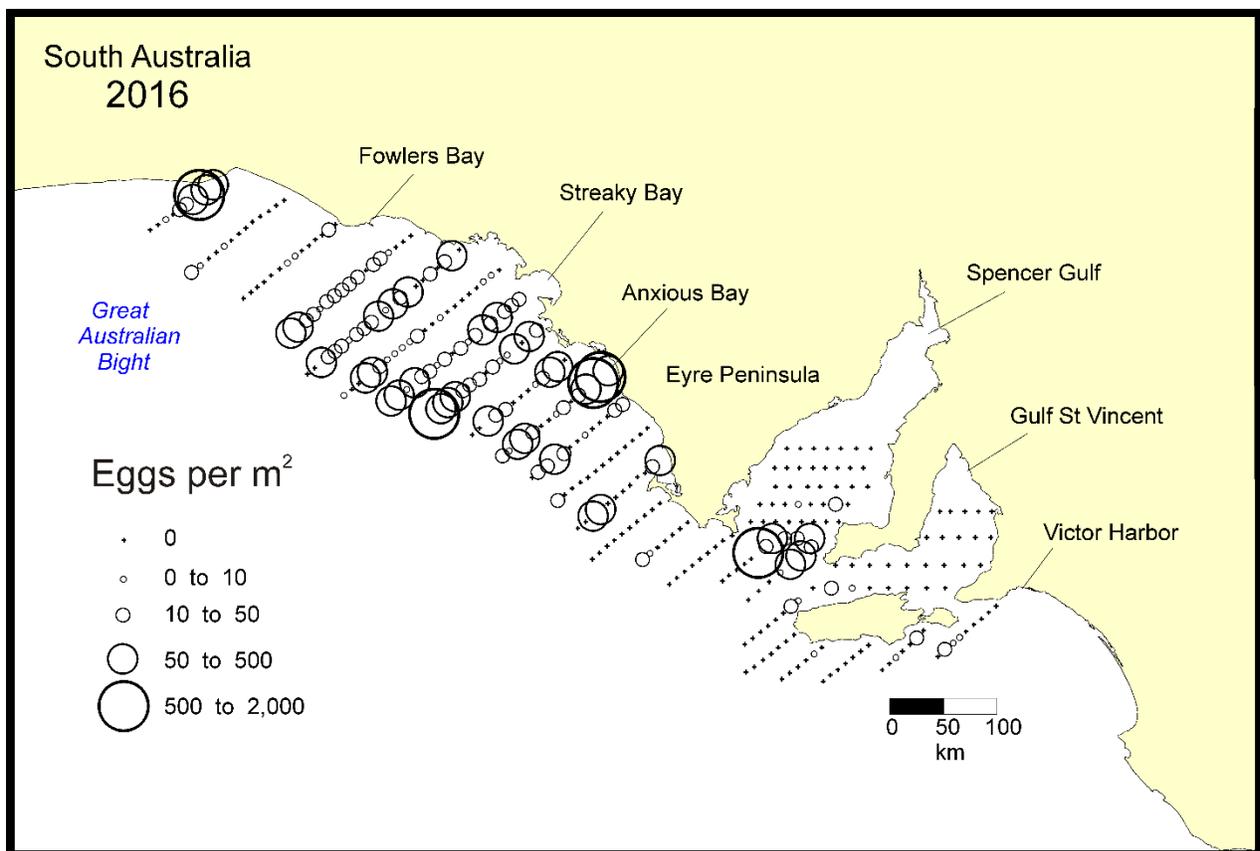


Figure 3. Spatial patterns of live Sardine egg distribution and abundance between February and March 2016.

3.2. Biophysical Variables

3.2.1 Sea surface temperature

Sea surface temperatures (SSTs) ranged from 14.1 to 22.5°C (Fig. 4) between February and March 2016. High SSTs (>19°C) were recorded in Spencer Gulf, Gulf St Vincent and throughout the central Great Australian Bight (GAB). A plume of cool water (<19°C) extended from western Kangaroo Island to Streaky Bay, with particularly cold water (<16°C) along the coast of the western Eyre Peninsula.

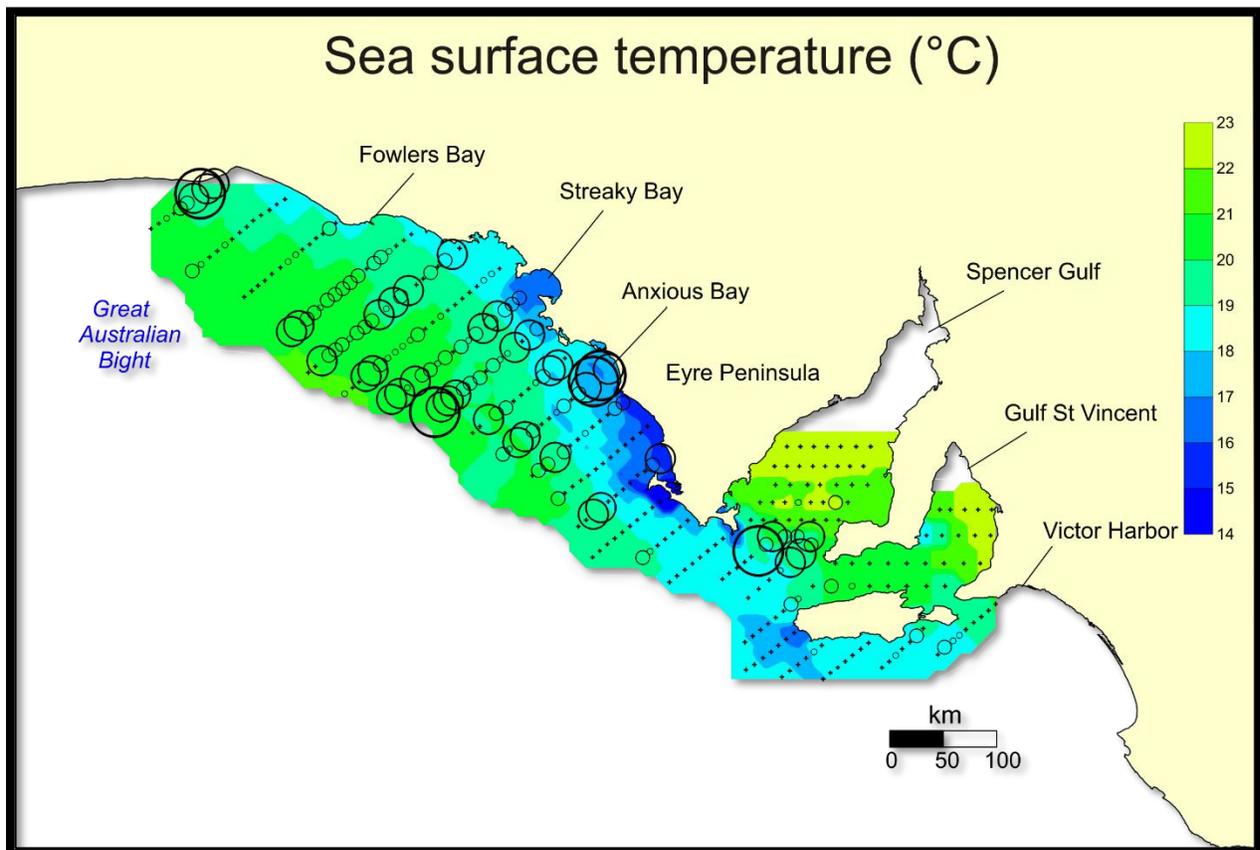


Figure 4. Sea surface temperature profile across the 2016 February – March survey, overlaid with Sardine egg distribution and abundance.

3.2.2 Fluorescence (chlorophyll-a)

Chlorophyll-a concentration at each site ranged between 0.211 and 16.195 $\mu\text{g.L}^{-1}$ (Fig. 5) between February and March 2016. The highest values were recorded off the western coast of Eyre Peninsula and west of Kangaroo Island. The remainder of coastal and shelf waters mainly had chlorophyll-a concentrations ranging between 0.2 and 2.0 $\mu\text{g.L}^{-1}$.

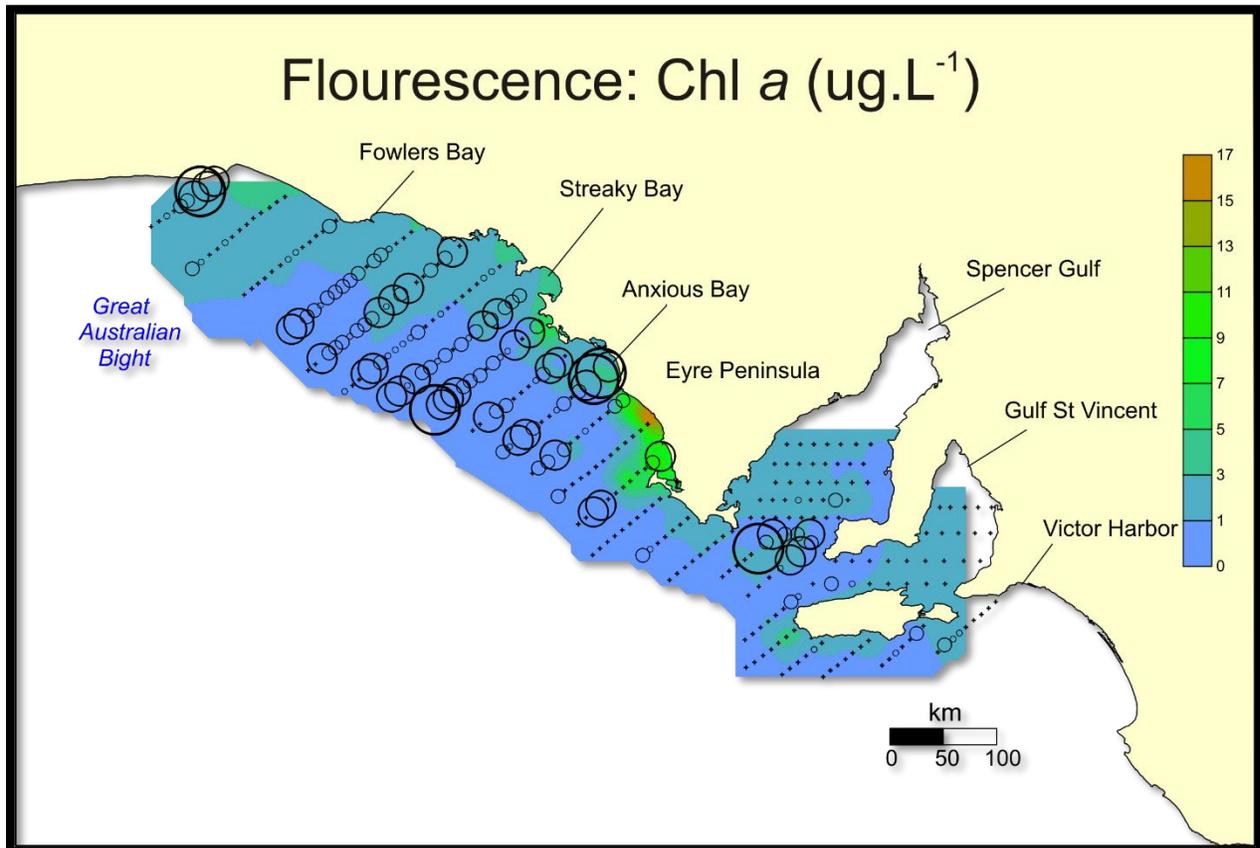


Figure 5. Surface concentration of chlorophyll-a inferred from fluorescence readings across the 2016, February - March survey area, overlaid with Sardine egg distribution and abundance.

3.2.3 Zooplankton abundance

Total densities of zooplankton ranged between 0 and 44.6 ml.m⁻³ (Fig. 6) between February and March 2016. The highest densities occurred in the mouth of Spencer Gulf, and along the south western coast of Eyre Peninsula and were comprised predominately of salps.

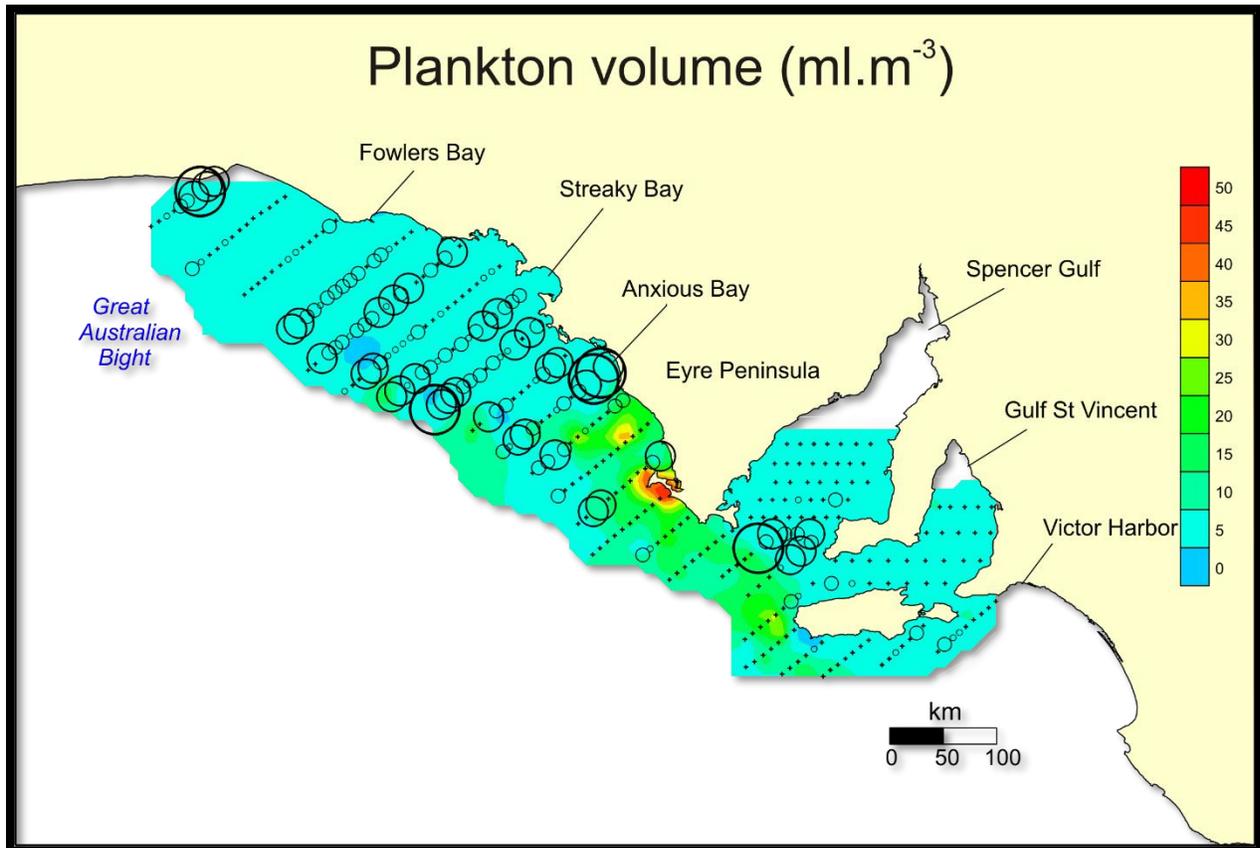


Figure 6. Distribution and abundance (ml.m⁻³) of zooplankton across the 2016, February - March survey area, overlaid with Sardine egg distribution and abundance.

3.3 Spawning Area

The estimated spawning area was 50,105 km², comprising 40.9% of the total area sampled (122,567 km², Table 1). The presence of eggs in CUFES samples was used as a basis for extending some transects (Fig. 1). An additional 9 sites were sampled in 2016, representing an additional 5,601 km²; 5 of these samples contained live Sardine eggs and contributed 1,753 km² to the spawning area.

Table 1. Total area surveyed and spawning area (*A*) estimated in 2016.

Total area sampled (km ²)	Spawning area, <i>A</i> (km ²)	Spawning area percentage
122,567	50,105	40.9

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 58.1 eggs.day⁻¹.m⁻² (95% CI = 37.7–88.4, Fig. 7,8, Table 2). The use of alternate egg production models produced estimates of 53.4 (95% CI = 31.3–92.4) for the non-linear least square model and 61.6 (95% CI = 34.1–100.4) and 62.2 (95% CI = 34.3–101.4) for the negative binomial and the quasi GLMs, respectively (Fig. 7, 8, Table 2).

Table 2. Mean daily egg production (P_0) and instantaneous daily mortality (Z) estimated with four alternate models.

Model fit	P_0 (eggs·day ⁻¹ ·m ⁻²) (95% CI)	Z
Linear version of exponential model, $\ln(\rho+1) \sim \text{age}$, corrected	58.1 (37.7–88.4)	0.58
Exponential model, $\rho \sim \exp(\text{age})$, NLS	53.4 (31.3–92.4)	0.57
GLM, $\rho \sim \text{age}$, Negative Binomial family	61.6 (34.1–100.4)	0.80
GLM, $\rho \sim \text{age}$, Quasi family, log link, $\text{var} \sim \mu^2$	62.2 (34.3–101.4)	0.81

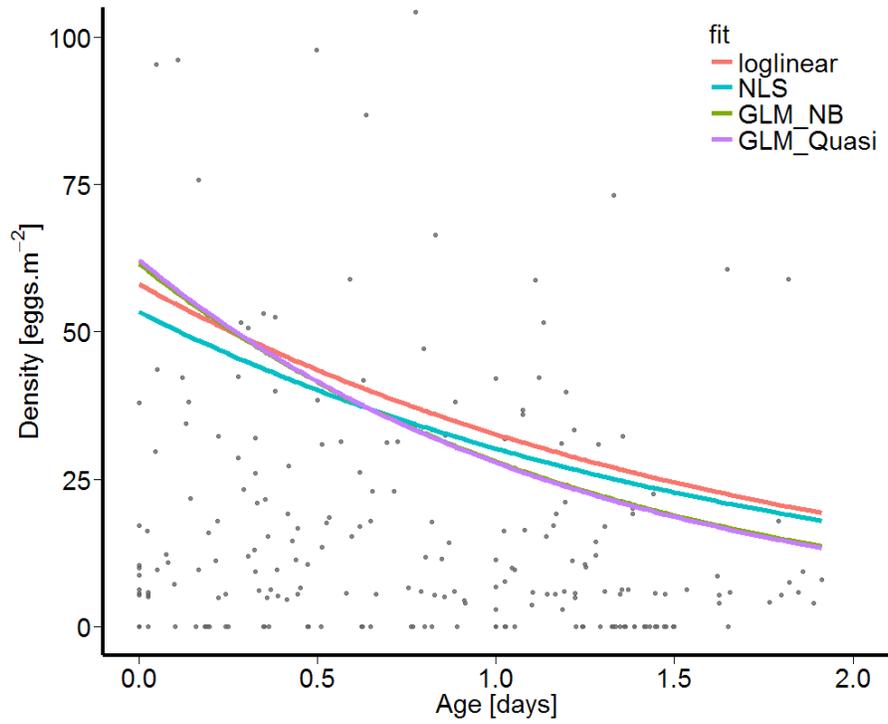


Figure 7. Regressions between Sardine egg density (eggs.m⁻²) and age (days) data in 2016.

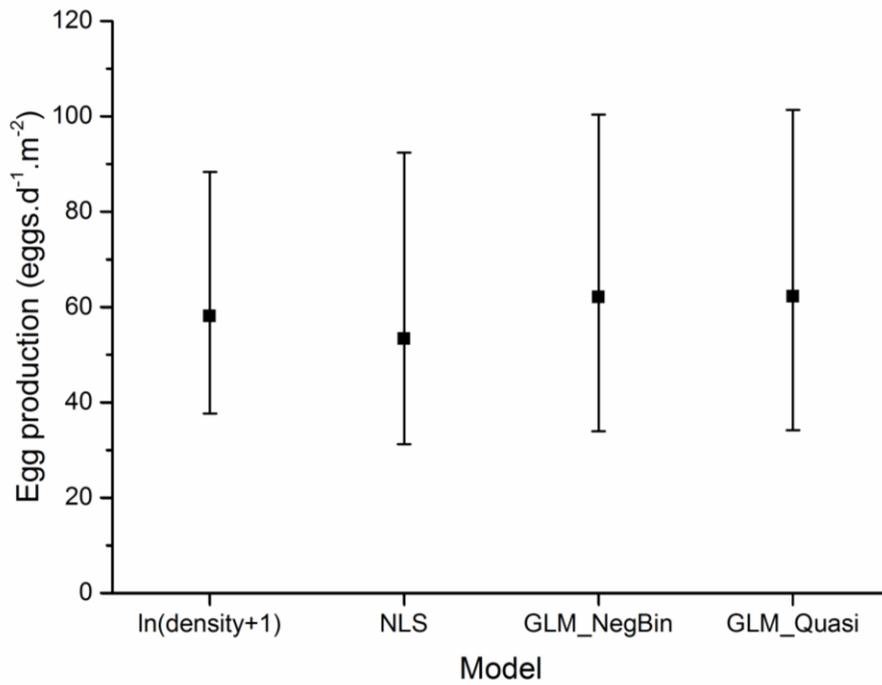


Figure 8. Egg production estimates (eggs.d⁻¹.m⁻²) from the four alternate models (Error bars are 95% CI).

3.5 Adult Reproductive Parameters

A total of nine samples comprising 1,061 mature Sardines were collected at Scotts Cove, North Neptune Island and Waldegrave Island (Table 3). 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 2014 and the bootstrapped 95% confidence intervals are shown in Table 6.

Table 3. Sampling details for adult Sardine collected during the 2016 DEPM surveys.

Date	Location	Survey	N samples	<i>n</i> fish
07/02/2016	Scotts Cove	1	3	434
09/02/2016	Neptune Island	1	2	285
12/03/2016	Waldegrave Island	2	4	342
		Total	9	1,061

3.5.1 Mean female weight

The mean weight of mature females in samples collected in 2016 ranged from 35.0 to 71.7 g (Table 4). The weighted mean weight of mature females in 2016 was 49.9 g (95% CI = 41.8–59.8, Table 4, 6). The mean weight of mature females collected between 1998 and 2014 was 57.5 g (45.2–78.7).

3.5.2 Sex ratio

The sex ratio calculated from the 2016 survey was 0.64 (Table 4). The mean sex ratio between 1998 and 2014 was 0.53 and ranged between 0.36 and 0.68 (Table 6).

Table 4. 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 2016. Values in bottom row are sums (*) and weighted means (#).

Sample	Location	Date	Male	Female	Mean Male Weight (g)	Mean Female Weight (g, W)	Sex Ratio by weight (R)
1	Scotts Cove	07/03/2016	53	90	37.7	42.5	0.66
2	Scotts Cove	07/03/2016	38	89	38.0	42.5	0.72
3	Scotts Cove	07/03/2016	75	89	38.2	43.7	0.58
4	N. Neptune Is.	09/02/2016	56	90	61.6	75.0	0.66
5	N. Neptune Is.	09/02/2016	63	76	66.0	69.0	0.56
6	Waldegrave Is.	11/03/2016	48	55	32.6	36.0	0.56
7	Waldegrave Is.	11/03/2016	27	46	34.3	40.2	0.67
8	Waldegrave Is.	11/03/2016	15	31	35.9	43.6	0.71
9	Waldegrave Is.	11/03/2016	30	90	37.8	45.6	0.78
			405*	656*	44.6#	49.9#	0.64#

3.5.3 Batch fecundity

No females with hydrated oocytes were collected in 2016. Based on the relationship (Batch Fecundity = $329.6 \times$ Gonad Free Female Weight – 593.7, $R^2 = 0.53$) for all females with hydrated oocytes collected between 1998 and 2014 (Fig. 9) and the mean gonad free female weight for all samples collected in 2016 (48.4 g), mean batch fecundity was 15,355 oocytes per batch (95% CI = 12,818–18,379; Table 6). The mean batch fecundity for samples collected between 1998 and 2014 was 17,241 oocytes per batch (10,904–24,790, Table 6).

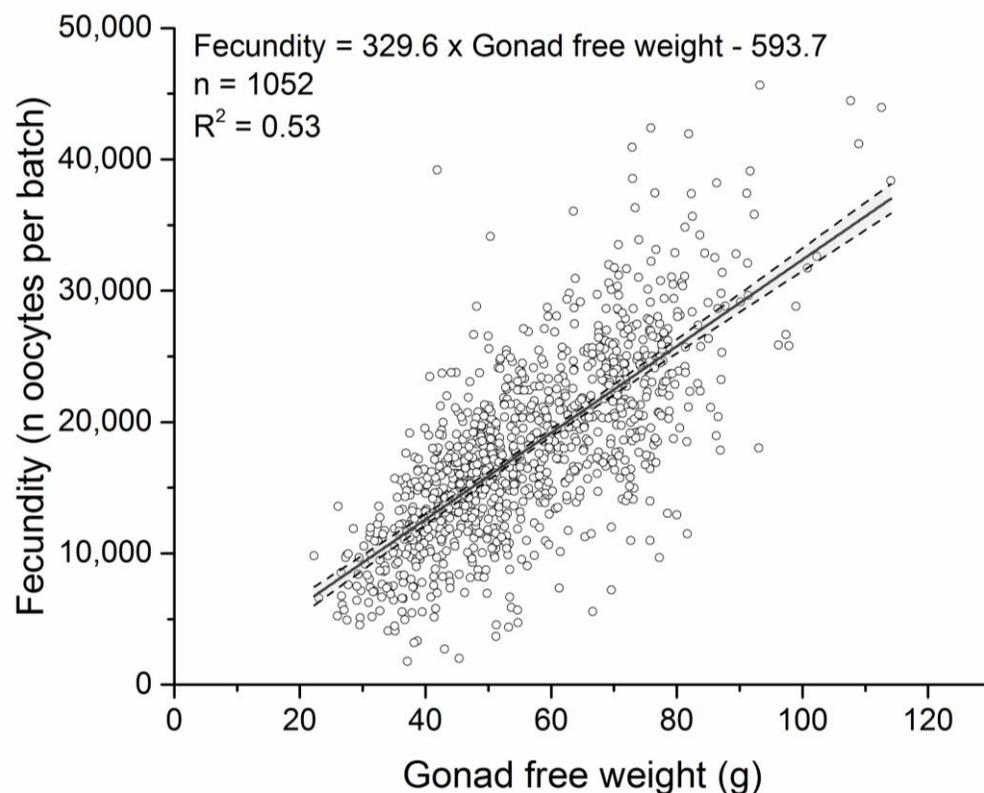


Figure 9. Relationship between gonad-free weight and batch fecundity for all hydrated Sardine collected between 1998 and 2014 (dotted line = 95% CI).

3.5.4 Spawning fraction

Of the 656 ovaries examined, 25 had day-0 POFs, 28 had day-1 POFs and 121 day-2 POFs (Table 4). The spawning fraction of females in each sample ranged from 0.041 to 0.152. The

weighted mean spawning fraction for all 2014 data was 0.088 (95% CI = 0.064–0.117). For 1998–2014, the mean spawning fraction was 0.116 and ranged between 0.040 and 0.179 (Table 6).

Table 5. Number of female Sardine in samples and estimates of spawning fraction (S) for samples collected in 2016. 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	07/03/2016	2	2	15	90	0.070
2	Scotts Cove	07/03/2016	2	7	21	89	0.122
3	Scotts Cove	07/03/2016	4	5	9	89	0.076
4	N. Neptune Is.	09/02/2016	0	0	11	90	0.041
5	N. Neptune Is.	09/02/2016	0	0	11	76	0.048
6	Waldegrave Is.	11/03/2016	4	6	15	55	0.152
7	Waldegrave Is.	11/03/2016	2	2	16	46	0.145
8	Waldegrave Is.	11/03/2016	2	1	6	31	0.097
9	Waldegrave Is.	11/03/2016	9	5	17	90	0.115
			25*	28*	121*	656*	0.088#

Table 6. Parameters used in the calculations of spawning biomass. Values for 2016 and the mean, minimum and maximum for 1998 to 2014 are presented (for spawning area, 2005 to 2014 only are considered as the surveys were previously not of consistent area). Note * indicates historically estimated values which were adjusted to historical means.

Parameter	2016 (95% CI)	Mean 1998-2014 (min-max)
Egg Production (P_0 , eggs.day ⁻¹ .m ⁻²)	58.1 (37.7–88.4)	72.7 (38.1–120.9)
Sex Ratio (R)	0.64	0.53 (0.36*–0.68*)
Fecundity (F, eggs.female ⁻¹)	15,355 (12,818–18,379)	17,241 (10,904–24,790)
Spawning Fraction (S)	0.088 (0.064–0.117)	0.116 (0.040–0.179)
Female Weight (W, g)	49.9 (41.8–59.8)	57.5 (45.2–78.7)
Spawning Area (A, km ²)	50,105	49,100 (36,549–71,859)

3.6 Sensitivity Analysis

The sensitivity analyses show where parameter estimates from the 2016 surveys are in comparison to the range of values recorded between 1998 and 2014 and their influence on estimates of spawning biomass (Fig. 10).

The estimates of egg production (P_0) and spawning fraction in 2016 were both lower than the historical mean (Fig. 10). Female weight and batch fecundity were also marginally lower than the historical mean, however these two parameters are correlated and their combined effect on spawning biomass is low. The estimate of spawning area obtained in 2016 was similar to those in recent years.

The estimate of sex ratio (R , 0.64) in 2016 was high in comparison to previous surveys and the all years mean (0.53) was considered more appropriate for estimating spawning biomass.

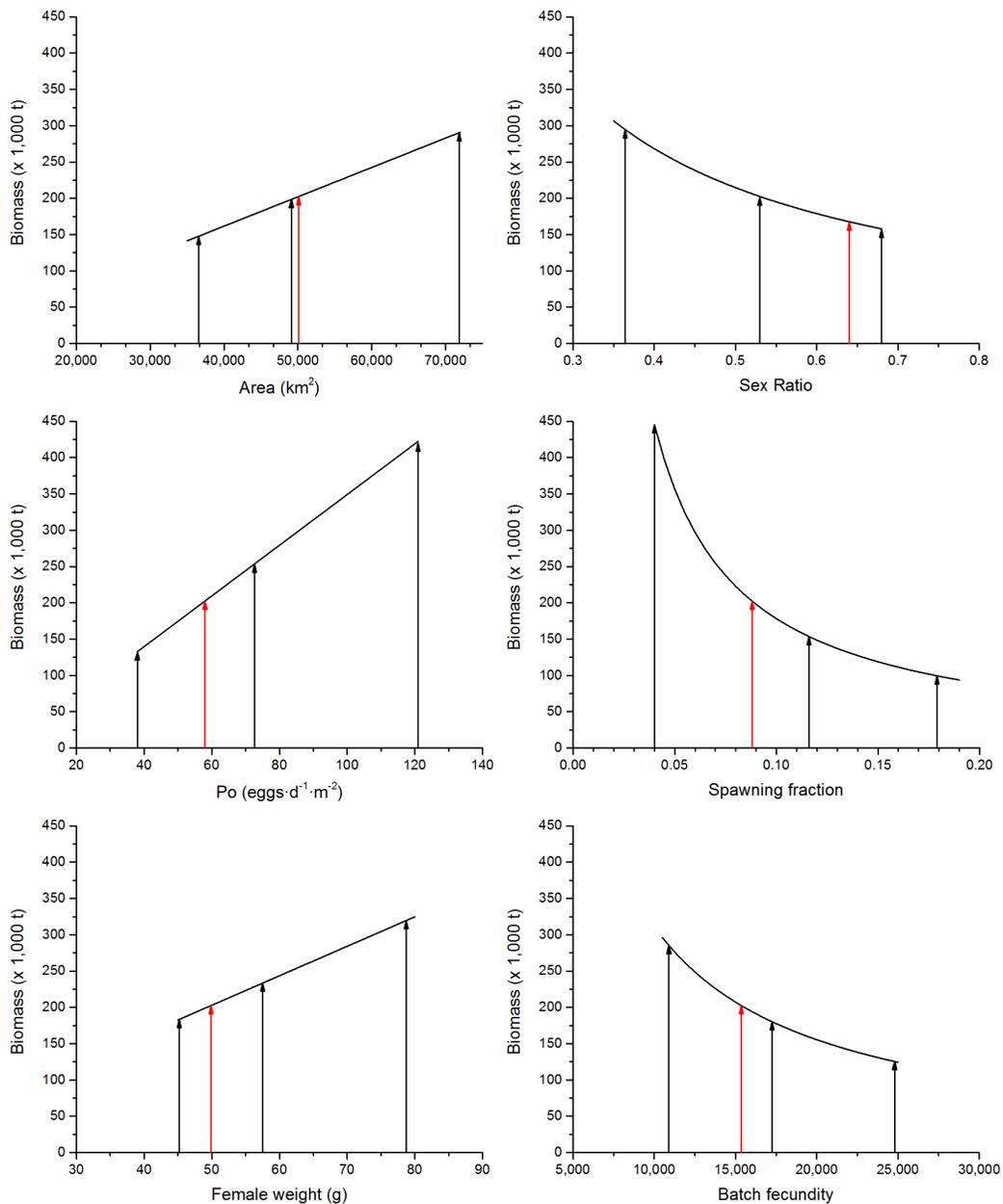


Figure 10. Sensitivity plots showing where 2016 parameter estimates (red arrow) are in comparison to the range of values recorded between 1998 and 2014 (2005 and 2014 for spawning area) and their influence on estimates of spawning biomass. Black arrows are the minimum, mean and maximum values for 1998-2014. Note that the influence of each 2016 estimate on spawning biomass was tested using all other 2014 parameters, except that mean sex ratio was used when testing other parameters rather than the value of sex ratio obtained for 2016.

3.7 Spawning Biomass

The estimate of spawning biomass calculated using the log linear model and parameter estimates obtained in 2016 (except sex ratio, R , where the historical average was used) was 201,316 t (95% CI = 112,712–378,335, Fig. 11). The spawning biomass calculated using the alternate egg production models were; 184,938, 213,532 and 215,494 t for the non-linear least squares model, negative binomial GLM and quasi GLM, respectively (Fig. 11).

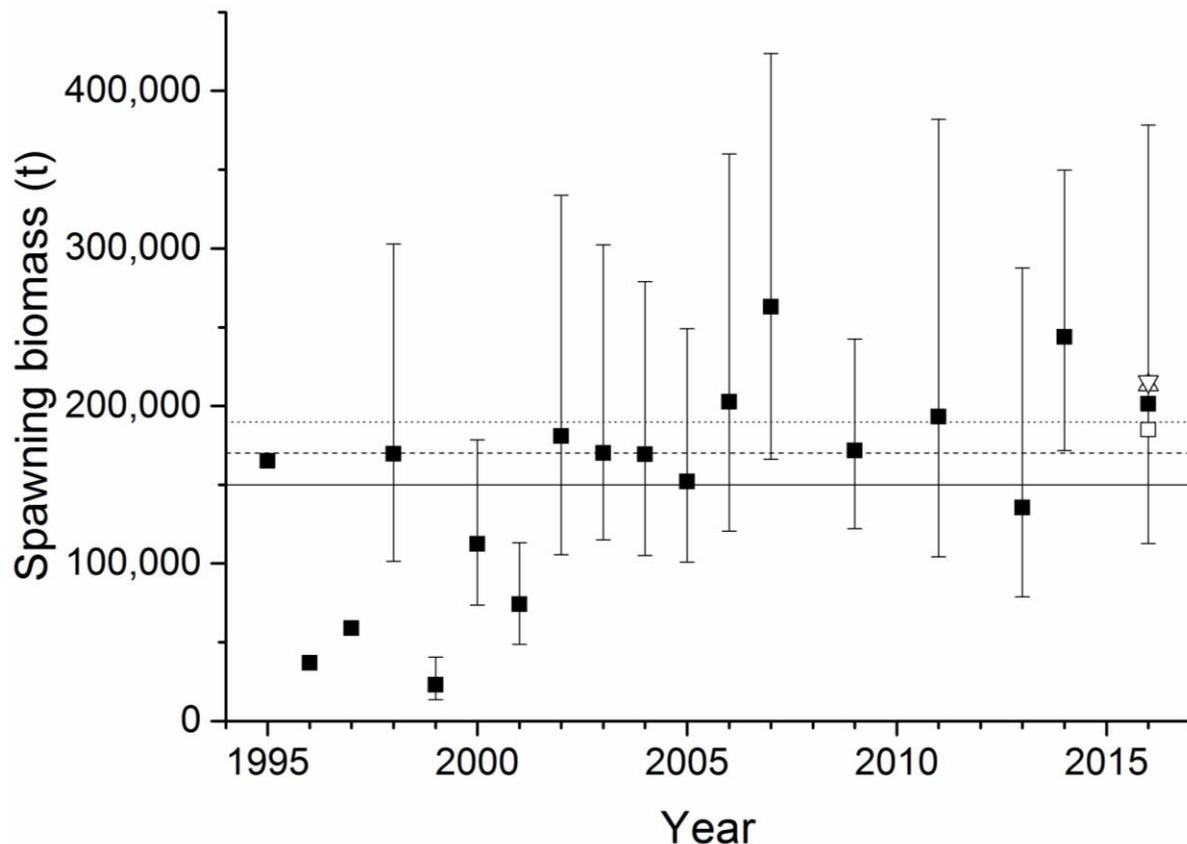


Figure 11. Spawning biomass estimates for Sardine in South Australian waters from 1995 to 2016 using the log-linear egg production model (solid squares). Error bars are 95% confidence intervals. Where alternative egg mortality models are also investigated, these estimates are shown (non-linear least squares (\square), negative binomial GLM (\triangle) and quasi GLM (∇)). The horizontal lines indicate the 150,000 (solid), 170,000 (dashed) and 190,000 t (dotted) reference points in the harvest strategy (PIRSA 2014).

4. DISCUSSION

4.1 Biophysical Variables and Egg Distribution

The low SSTs ($<18^{\circ}\text{C}$) and elevated concentrations of chlorophyll-*a* ($>0.3\ \mu\text{g}\cdot\text{L}^{-1}$) recorded in inshore waters off southern Eyre Peninsula during the surveys showed that strong upwelling occurred in the eastern GAB during February and March 2016 (e.g. McClatchie *et al.* 2006). The high plankton densities recorded in coastal waters of the southern Eyre Peninsula and south of Spencer Gulf reflect nutrient enrichment by upwelling processes (Ward *et al.* 2006). These patterns are consistent with observations during other strong upwelling years, such as 2014 (Ward *et al.* 2014).

Plankton samples collected in 2016 contained 1,979 live eggs, which is comparable to most previous years but lower than 2014, when 7,995 live eggs were collected (Ward *et al.* 2014). As was the case in 2013 and 2014 (Ward *et al.* 2014), the abundance of Sardine eggs was low at sites located in Gulf St Vincent, eastern Investigator Strait and Spencer Gulf north of Wedge Island. The presence of high egg densities at a few sites in southern Spencer Gulf and over a large area in shelf waters of the GAB was also consistent with results obtained in the last two surveys (i.e. 2013 and 2014).

A total of nine additional sites were sampled on the seaward-end of three 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 2013, 2014 and 2016 suggests that the adaptive sampling approach based on CUFES samples used in those years should be continued in future surveys.

4.2 Spawning Area

The estimate of spawning area for 2016 (50,105 km²; Table 1, 5) is close to the average spawning area recorded in DEPM surveys conducted off South Australia since the current survey design was adopted, but lower than the large spawning area of 71,859 km² recorded in 2014 (Ward *et al.* 2014). The occurrence of a large proportion of the total spawning area in shelf waters of the GAB continues the pattern observed in 2013 and 2014 (Ward *et al.* 2014), when few eggs were recorded east of Cape Carnot compared to the 1990s and early 2000s (Ward *et al.* 2012).

4.3 Egg Production

The estimate of egg production (P_0) obtained in 2016 using the linear version of the exponential egg mortality model, which was the method recommended for SA Sardine by Ward *et al.* (2011a), was 58.1 eggs.day⁻¹.m⁻² (95% CI = 37.7–88.4), which is lower than the mean value since 1998 of 72.7 eggs. day⁻¹.m⁻². Estimates of egg production (and their confidence intervals) obtained using other models (i.e. non-linear least squares model, negative binomial GLM and quasi GLM) were similar to the estimates from the linear model (i.e. 53.4, 61.6 and 62.2 eggs. day⁻¹.m⁻²), respectively.

4.4 Adult Sampling

During the 2016 survey, nine samples of adult Sardines containing 656 females were collected from three sites where gillnetting has been consistently successful, i.e. Scotts Cove, North Neptune Island and Waldegrave Island (e.g. Ward *et al.* 2014). However, no adult samples were obtained west of Waldegrave Island or from shelf waters of the GAB where the majority of eggs occurred, again emphasizing the need to develop an alternative sampling method (see Ward *et al.* 2014).

Adult samples collected during 2016 were comprised of a large proportion of females ($R = 0.64$) and overall had a relatively low spawning fraction (0.088, 95% CI = 0.064–0.117) compared to the mean for 1998 to 2014 of 0.116. Samples collected in 2016 also contained no females with hydrated oocytes. As females were over-represented in samples, the mean sex ratio for samples collected between 1998 and 2014 (0.53), was used to calculate spawning biomass. Batch fecundity was estimated by applying the relationship with mean weight established from all females with hydrated oocytes collected between 1998 and 2014.

4.5 Spawning Biomass

Spawning biomass is highly correlated with spawning area (Gaughan *et al.* 2004). The relatively large spawning area observed in 2016 (i.e. approximately equal to the long-term mean) supports the finding that the spawning biomass was also relatively large (i.e. close to the long-term mean). However, as was the case in 2014 (Ward *et al.* 2014), uncertainty associated with estimates of adult parameters highlights the need to develop an alternative method for sampling adults. The results for 2016 also highlight the uncertainty surrounding estimates of egg production and suggest that an alternative sampling method may also need to be developed for estimating this parameter.

The spawning biomass estimate obtained using the method recommended by Ward *et al.* (2011a), i.e. $58.1 \text{ eggs.day}^{-1}.\text{m}^{-2}$ (95% CI = 37.7–88.4), produces an estimate of spawning biomass of 201,316 t, which is above the upper reference point in the management plan of 190,000 t. This estimate is considered to provide a suitable basis for setting the Total Allowable Catch (TAC) for 2017. Other methods for estimating egg production produce similar estimates of spawning biomass, with only the estimate obtained using the non-linear least squares model (i.e. 184,938 t) being below 190,000 t.

4.6 Future Research Needs

The results for 2016 again emphasise the need to establish a reliable method for sampling adult Sardines that has been identified in previous reports (e.g. Ward *et al.* 2014). Uncertainty regarding the estimate of egg production, combined with results of a concurrent project that is investigating options for improving the DEPM (SARDI, unpublished data), also highlight the need to develop an improved method for collecting samples to further refine estimates of egg production.

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