

## Spawning biomass of Sardine, *Sardinops sagax*, in waters off South Australia in 2019



Ward, T.M., Ivey, A.R., Grammer, G.L., and Smart, J.

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PO Box 120 Henley Beach SA 5022

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Report to PIRSA Fisheries and Aquaculture

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**South Australian Research and Development Institute**

SARDI Aquatic Sciences  
2 Hamra Avenue  
West Beach SA 5024

Telephone: (08) 8207 5400  
Facsimile: (08) 8207 5415  
<http://www.pir.sa.gov.au/research>

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Author(s): Ward, T.M., Ivey, A.R., Grammer, G.L. and Smart, J.  
Reviewer(s): Noell, C., Rogers, P.J. (SARDI) and Shanks, S. (PIRSA)  
Approved by: A/Prof Linnane, A.  
Acting Science Leader - Fisheries

Signed:



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## EXECUTIVE SUMMARY

This report provides an estimate of the spawning biomass of Sardine, *Sardinops sagax*, in waters off South Australia during 2019. The estimate of spawning biomass obtained using the Daily Egg Production Method (DEPM) is the key performance indicator for determining the status of the southern Australian stock of Sardine.

An ichthyoplankton survey was conducted during February-April 2019. Adult samples were not collected in 2019 due to logistical constraints; covering the entire spawning area was given higher priority. Estimates of adult parameters were obtained from surveys conducted off South Australia from 1998 to 2018. Sensitivity analyses were conducted to evaluate the effects of variability in individual parameters on the uncertainty of the estimate of spawning biomass for 2019.

The total survey area was 119,369 km<sup>2</sup>. Live Sardine eggs were collected at 148 of 339 (43.7%) sites. The spawning area ( $A$ ) was 53,600 km<sup>2</sup>. Mean daily egg production ( $P_o$ , 95% CI) estimated from data collected in 2019 using the linear version of the exponential mortality model was 68.1 (42.0–107.7) eggs.day<sup>-1</sup>.m<sup>-2</sup>. The estimate of  $P_o$  obtained using all data collected from 1998 to 2019 was 81.4 (72.8–91.0) eggs.day<sup>-1</sup>.m<sup>-2</sup>. Four other models produced higher estimates of  $P_o$ .

Estimates of adult parameters (95% CI) calculated from all data obtained between 1998 and 2018 were: spawning fraction ( $S$ ): 0.11 (0.106–0.117); sex ratio ( $R$ ): 0.55 (0.52–0.58); mean female weight ( $W$ ): 58.0 (22.2–93.8) g; and batch fecundity ( $F$ ): 17,776 (6,408–29,144) oocytes. The ratio of  $F/W$  (i.e. 306.4 (258.5–354.3) eggs.g<sup>-1</sup>) was used instead of the individual parameters of  $F$  and  $W$  to calculate spawning biomass because this approach increased precision.

Sensitivity analyses demonstrated the benefits of using estimates of  $P_o$  and adult parameters obtained from historical data to estimate spawning biomass. Detailed justification of this refined approach to applying the DEPM is provided in the Sardine stock assessment report for 2019.

The estimate of spawning biomass (95% CI) of Sardine for 2019 was 233,684 (181,214–286,153) t which is above the upper target reference point of 190,000 t in the Management Plan. On this basis, the southern Australian stock of Sardine is classified as **Sustainable**. This classification is consistent with the findings of the spawning biomass report for 2018, the stock assessment report for 2019 and the most recent report on the Status of Australian Fish Stocks.

**Keywords:** Sardine, Spawning Biomass, South Australia.

## 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 approximately 20 species of small to medium-sized pelagic fishes (Stratoudakis et al. 2006, Dimmlich et al. 2009, Neira et al. 2009, Ward et al. 2009, 2016). The method is widely used in coastal fisheries because it is often the most practical option available for assessment of pelagic species (Ward et al. 1998).

The DEPM relies on the premise that spawning biomass 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; Parker 1980; 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 spawning fraction (proportion of mature females spawning each day/night,  $S$ ) and mean batch fecundity (number of oocytes in a batch,  $F$ ) by mean female weight ( $W$ ). Spawning biomass ( $SB$ ) is calculated according to the equation:

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

The DEPM can be applied to fishes that spawn multiple batches of pelagic eggs over an extended spawning season (Parker 1980, Lasker 1985). Data used to estimate total daily egg production are obtained from fishery-independent ichthyoplankton surveys. Adult samples used to estimate mean daily fecundity are obtained from research surveys or samples obtained from commercial vessels using a variety of methods (Stratoudakis et al. 2006; Ward et al. 2011). The main 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 logistical and statistical challenges have been encountered and estimates of spawning biomass are known to be imprecise (e.g. Stratoudakis et al. 2006; Bernal et al. 2012, Dickey-Collas et al. 2012; Ward et al. 2018). There are considerable uncertainties associated with the estimation of several parameters, especially  $P_0$  (Fletcher et al. 1996, McGarvey and Kinloch 2001, Gaughan et al. 2004). Ward et al. (2011, 2018) showed that

the log-linear model of Piquelle and Stauffer (1985) provides more precise estimates of  $P_0$  than other models and recommended its use for Sardine (*Sardinops sagax*) off South Australia. However, recent studies have shown that inter-annual variations in estimates of  $P_0$  for Sardine off South Australia are low in comparison to statistical uncertainty (e.g. Ward et al. 2018, SARDI unpublished a, b). These findings support previous studies (e.g. Mangel and Smith 1990; Gaughan et al. 2004) that have shown that the spawning biomass of Sardine is not correlated with  $P_0$  and that variations in total daily egg production are driven primarily by spawning area ( $A$ ). The potential benefits of using estimates of  $P_0$  obtained from multiple years and the estimate of  $A$  obtained from the annual ichthyoplankton survey to estimate the total daily egg production of Sardine off South Australia warrant investigation (see also SARDI unpublished b).

Inter-annual variability in mean daily fecundity may also be relatively low compared to the statistical uncertainty and sampling error associated with estimation of adult parameters (see SARDI unpublished b). For example, the sex ratio ( $R$ ) of a population is likely to be stable among years (i.e. at just over 0.5); however, adult samples obtained in some years are dominated by either males or females and provide estimates of  $R$  that are unlikely to be representative of the spawning population (e.g. 0.35–0.70). In addition, samples that are dominated by males/females tend to provide estimates of  $S$  that are quite different (i.e. 0.05 and 0.17) from the mean spawning fraction for Sardine worldwide (i.e. ~0.10–0.12, Ganais et al. 2009). The potential benefits (e.g. increased precision) of using estimates of adult parameters obtained from data obtained from all surveys conducted between 1998 and 2018 warrant investigation (see SARDI unpublished b).

## 1.2. Rationale, objective and approach

The DEPM has been used to estimate the spawning biomass of Sardine in South Australian waters since 1995 (Ward et al. 1998, 2011, 2017, 2018). The estimate of spawning biomass obtained using the DEPM is the key performance indicator for determining the status of the southern Australian stock of Sardine. The objective of this report is to estimate the spawning biomass of Sardine in waters off South Australia in 2019. Estimates of mean daily egg production and spawning area were obtained from an ichthyoplankton survey conducted during February to April 2019. Mean egg production was also estimated using all of the data obtained between 1998 and 2019. No adult samples were taken in 2019; weather was poor and covering the spawning was afforded a higher priority. Estimates of adult parameters were calculated from data obtained from surveys conducted in waters off South Australia between 1998 and 2018. Sensitivity analyses were undertaken to evaluate the effects of variability in estimates of individual parameters on the uncertainty associated with the estimate of spawning biomass for 2019.

## 2. METHODS

### 2.1. Study area and biophysical variables

#### 2.1.1. Study area

An ichthyoplankton survey was conducted from the *RV Ngerin* in shelf and gulf waters of South Australia during February to April 2019 (Fig. 1). Eastern sites were sampled during 28 February to 11 March 2019. Western sites were sampled during 28 March to 7 April 2019. Plankton samples were collected at a total of 339 sites on 34 transects between Victor Harbor and the Head of Bight (Fig. 1). Of these 339 samples, 2 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).

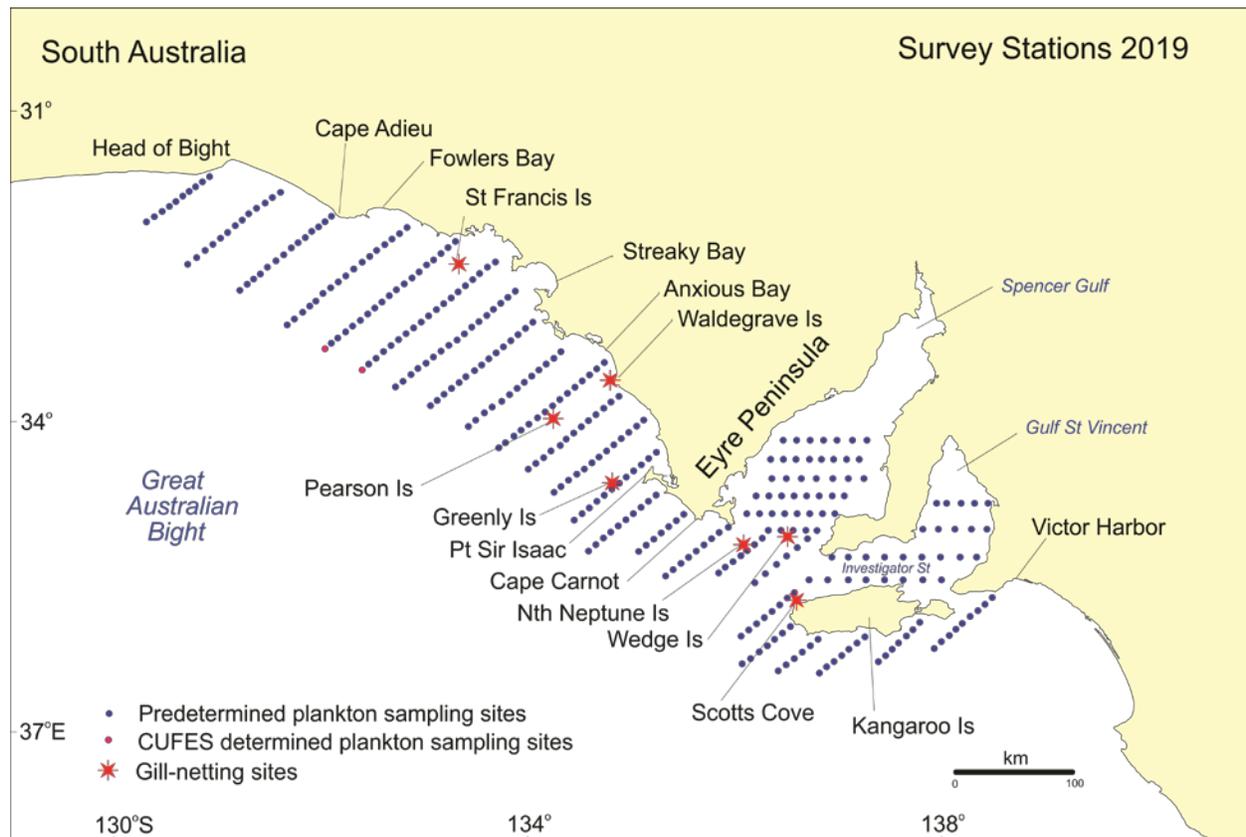


Figure 1. Map of South Australia showing sites where plankton samples were collected during 2019 and where adult samples were collected with gill-nets between 1998 and 2018.

### 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 the surface were extracted from each profile. At sites where water temperature was not measured, the average temperature of the adjacent stations was applied. 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 sea surface temperature (SST) and chlorophyll-*a* concentration were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

## 2.2. Mean 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, length of 1.8 m, 330  $\mu\text{m}$  mesh and plastic removable 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. The nets were 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 and flow-meter units was used to determine which was correct and that value was used for both nets. Upon retrieval of the nets, the samples from each of the two cod-ends were washed using seawater into a single one litre container. Samples were fixed using 75 ml of a 40% formaldehyde solution.

### 2.2.2. *Laboratory analysis*

Sardine eggs and larvae were identified in each plankton sample using published descriptions (Neira et al. 1998, White and Fletcher 1998). Eggs in each sample were staged based on descriptions in White and Fletcher (1998). Total counts of eggs of each stage in each sample were recorded. Eggs in the first and last stages were excluded from the statistical analyses as they were under- and over-represented in samples, respectively (see SARDI unpublished a, b, Stratoudakis et al. 2006, Bernal et al. 2012, Dicky-Collas et al. 2012).

### 2.2.3. *Egg ageing and treatment of zero count egg samples*

The development time of Sardine eggs is dependent on water temperature (Picquelle and Stauffer 1985, Pauly and Pullin 1988). Egg samples were allocated to three temperature bins that covered the range of temperatures typically sampled during Sardine DEPM surveys off South Australia (14–18°C, 18–22°C, and 22–26°C). These temperature bins were similar to those used in the published temperature egg development rates of Le Clus and Malan (1995). These rates were used to assign the mean age to each egg (Ward et al. 2018).

After the eggs were assigned an age, eggs in each sample were aggregated into daily cohorts by stage. This was done because more than one night's spawning could be represented in a sample. Total egg count and average age for each daily cohort was calculated by assigning each egg stage to a day of spawning (e.g. day 0, day 1, day 2), summing the number of eggs, and averaging their ages across the stages within the daily cohort. Average cohort ages were weighted by the number of eggs observed in each stage.

Samples were also identified where a zero count should (and should not) be allocated to one or more daily egg cohorts (Ward et al. 2018). Samples with no eggs were excluded from the analyses and not considered part of the spawning area. Samples with eggs could contain several possible combinations of daily cohorts depending on water temperature, spawning time and sampling time. Since spawning occurs each night (peak around 2:00 am), zero counts were allocated for daily cohorts where the cohort was expected to be present but not found in the sample.

### 2.2.4. *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 ( $\text{m}^3$ ), 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).

### 2.2.5. *Spawning area (A)*

The Voronoi natural neighbour (VNN) method (Watson 1981) was applied using the statistical package 'R' (Baddeley and Turner 2005; R Core Team 2019) 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 ( $\text{km}^2$ ) was then determined.  $A$  was defined as the total area of grids where live Sardine eggs were found.

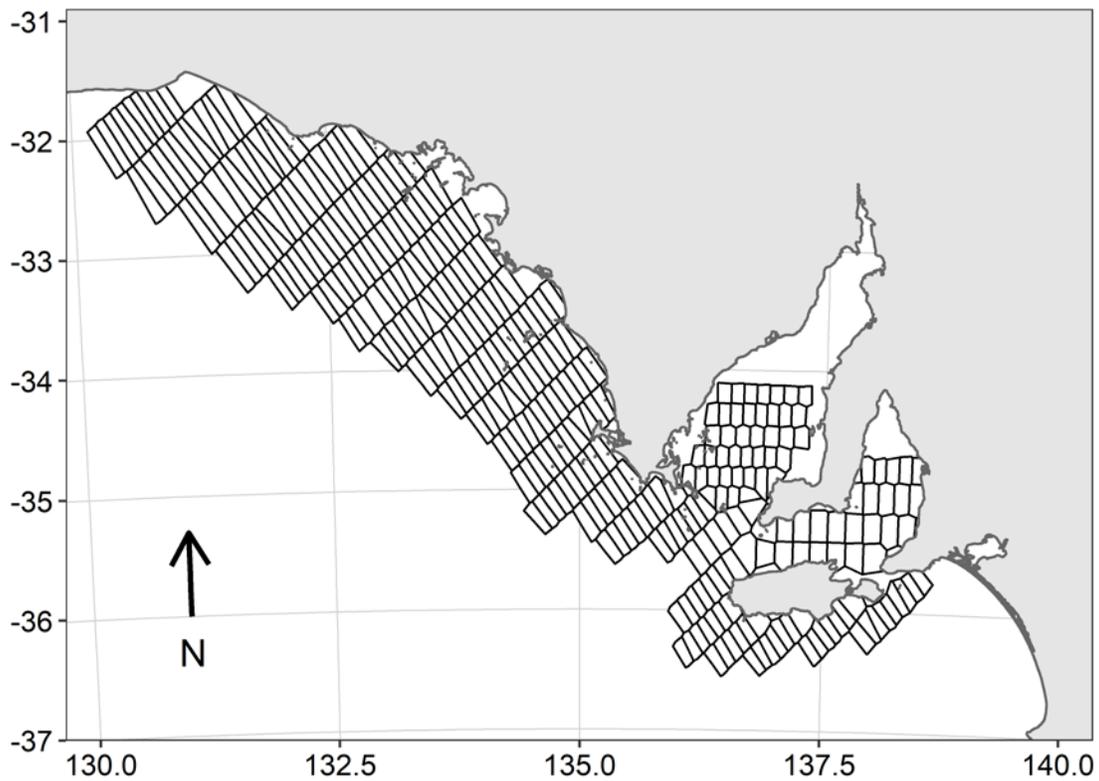


Figure 2. Voronoi nearest neighbour polygons used to estimate the total spawning area in 2019.

### 2.2.6. Mean daily egg production ( $P_0$ ) and egg mortality ( $Z$ )

The underlying model used to calculate  $P_0$  was the exponential egg mortality model (Equation 3) with a bias correction factor (Equation 4, the 'log-linear model'). 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,  $\sigma^2$  is the variance of the estimate of biased mean daily egg production ( $P_b$ ).

Two general linear models (GLMs) and a general linear mixed model (GLMM) negative binomial and quasi error structures, respectively, were also used to estimate  $P_0$  (Equation 5):

$$E[P_0] = g^{-1}(-zt + \varepsilon) \quad \text{Equation 5}$$

where  $E[P_0]$  is the expected value of  $P_0$ ,  $g^{-1}$  is the inverse-link function,  $zt$  is the instantaneous rate of daily egg mortality at age  $t$ , and  $\varepsilon$  is the error term. Negative binomial and quasi error structures are considered suitable for zero-inflated and over-dispersed data, such as egg density by age (e.g. Ward et al. 2011, 2018). Instantaneous egg mortality rate ( $z$ ) was estimated as a free parameter in each of the models. The value of  $P_0$  from the log-linear model was used to estimate spawning biomass for Sardine (see Ward *et al.* 2018).

$P_0$  was calculated using data collected solely in 2019, as well as with data from all years (combined) between 1998 and 2019. The all-years estimate of  $P_0$  is considered more robust than the individual year estimate of  $P_0$ , because sampling error within a year is greater than inter-annual variability of egg density and egg production (Ward et al. 2018, SARDI unpublished a, b).

### 2.3. Adult reproductive parameters

Adult samples were not collected in 2019 due to logistical constraints (mainly poor weather); ensuring ichthyoplankton samples were collected across the entire spawning area was given higher priority. Adult parameters used to estimate spawning biomass were derived from all adult samples of Sardine collected from waters off South Australia between 1998 and 2018.

#### 2.3.1. Sampling methods

A dual frequency echo sounder (*Simrad* 60 and 180 KHz) was used to search for schools of Sardines, in areas where they were known to aggregate (Fig. 1). The *RV Ngerin* 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 (150 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 male and immature fish were frozen. Mature females

were fixed in 10% buffered formaldehyde seawater solution. Calculations adult parameters are based on samples collected between 1998 and 2018 from Scotts Cove, Wedge, North Neptune Waldegrave, Greenly, Pearson, Flinders and St Francis Islands (GAB, Fig. 1).

### 2.3.2. Female weight ( $W$ )

Mature females from each sample were removed from the formalin solution and weighed ( $\pm 0.01$  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 ( $\pm 0.01$  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 ( $F$ )

Batch fecundity was estimated from ovaries containing hydrated oocytes using the methods of Hunter and Macewicz (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 all mature females.

Eggs per gram of female weight ( $F/W$ ) was calculated by using the linear relationship of batch fecundity determined from all years data (1998-2018) to estimate  $F$  and then dividing by the mean weight of all mature females collected ( $W$ ).

### 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

Spawning biomass was calculated according to Equation 1 using the all-years estimate of  $P_0$  obtained from the log-linear model, spawning area ( $A$ ) estimated in 2019 and estimates of  $S$ ,  $R$  and  $F/W$  obtained from adult samples collected between 1998 and 2018. Spawning biomass was also calculated separately using the estimates of  $P_0$  obtained from data collected in 2019.

The reliability of model fits, 95% confidence intervals (CIs) and coefficients of variation (CVs) for  $P_0$  were estimated using bootstrap resampling methods with 10,000 iterations. Coefficients of variation and CIs for  $R$ ,  $S$ ,  $F$ ,  $W$  and  $F/W$ , were calculated from the all-years adult data. A ratio estimator was used calculate the coefficients of variation (CVs) for  $S$ ,  $R$ , and  $F/W$  (see Rice 1995). The variance around the spawning biomass estimate was calculated by the summing the squared CVs for each parameter and multiplying by the square of the estimate of spawning biomass. Uncertainty estimates presented for all parameters are 95% CIs. Data analyses were done in the R programming environment (R Core Team, 2019).

## 2.5. Sensitivity analysis

Sensitivity analyses were conducted to assess the effects of variations in the range of values obtained for each parameter in each years between 1998 and 2019 on estimate of spawning biomass for 2019.

### 3. RESULTS

#### 3.1. Distribution and abundance of eggs

A total of 3,020 live Sardine eggs were collected at 148 of 339 (43.7%) sites on 34 transects between the Head of Bight and Victor Harbor between February and April 2019 (Fig. 3). Sites with the high egg densities were located in the mouth of Spencer Gulf, around the south-western part of Kangaroo Island and on the shelf in the eastern Great Australian Bight (GAB). The highest egg density (i.e. 1,402 eggs.m<sup>-2</sup>) was recorded in the eastern

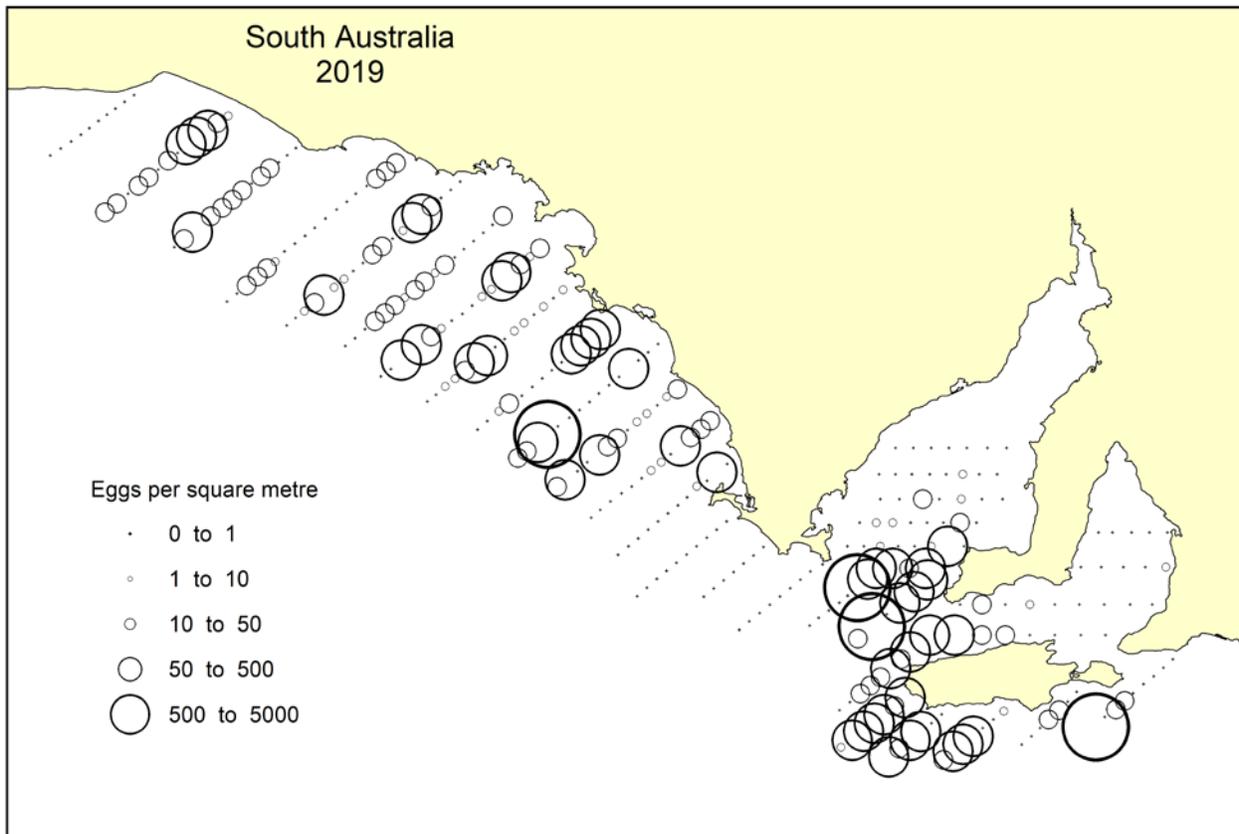


Figure 3. Densities of live Sardine eggs at sites sampled during February to April 2019.

## 3.2. Biophysical variables

### 3.2.1. Sea surface temperature

Sea surface temperatures (SSTs) ranged from 16.5 to 24.0°C (Fig. 4) between February and April 2019. High SSTs (>20°C) were recorded in Spencer Gulf, Gulf St Vincent, Investigator Strait and throughout the central Great Australian Bight (GAB). Cooler, upwelled water (<19°C) was present off the lower west coast of Eyre Peninsula.

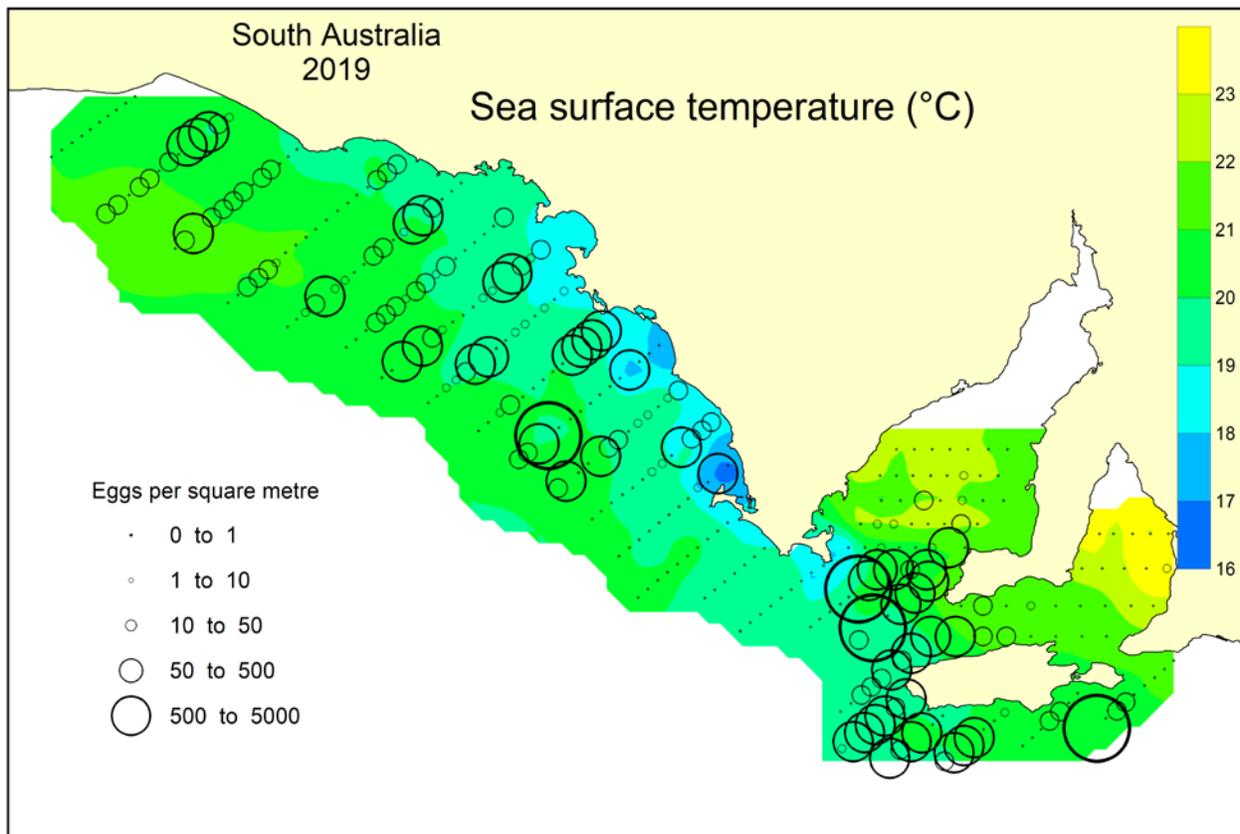


Figure 4. Sea surface temperatures and densities of live Sardines eggs at sites sampled during February to April 2019, overlaid with Sardine egg densities.

### 3.2.2. Fluorescence

Surface chlorophyll-*a* concentration at each site ranged between 0.006 and 1.863  $\mu\text{g.L}^{-1}$  (Fig. 5) between February and April 2019. The highest values were recorded in the central GAB, north-west of Coffin Bay and in Spencer Gulf. The remainder of coastal and shelf waters mainly had chlorophyll-*a* concentrations  $<0.2 \mu\text{g.L}^{-1}$ .

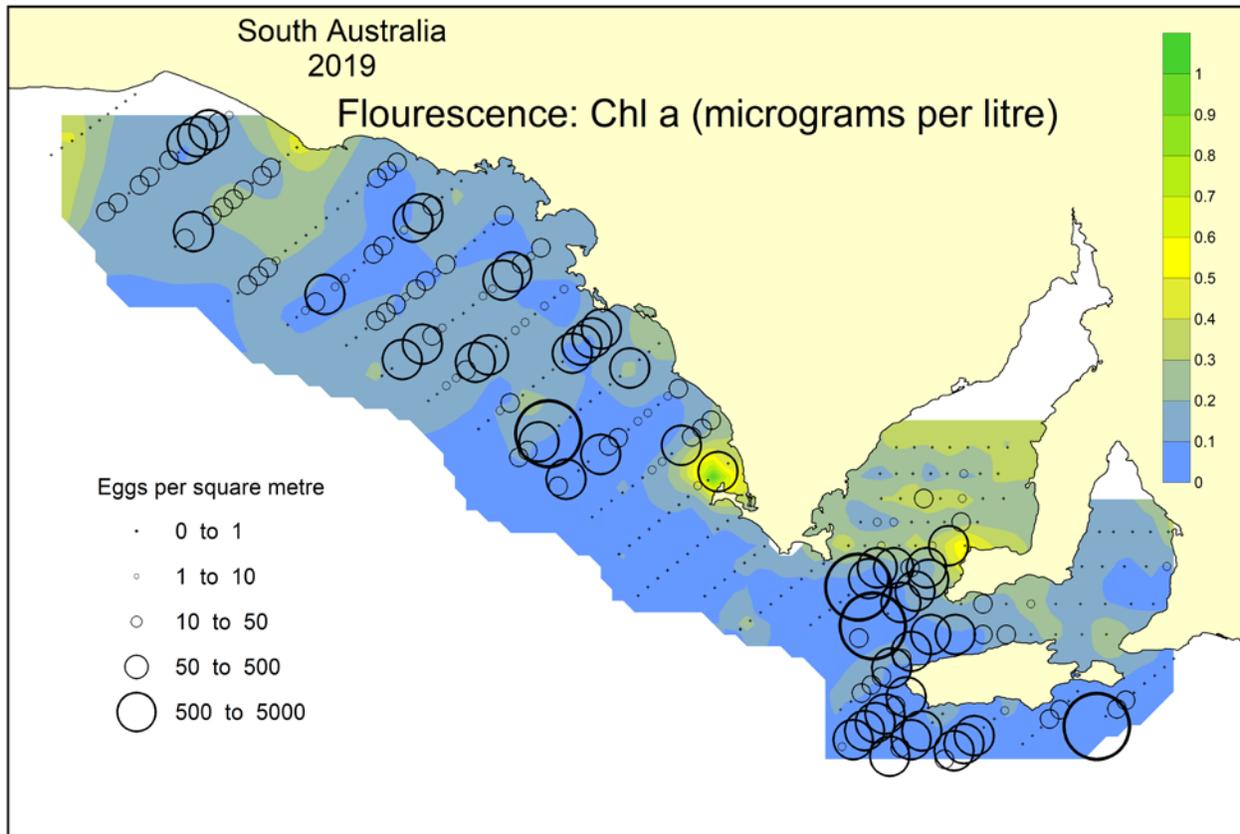


Figure 5. Surface concentration of chlorophyll-*a* and densities of live Sardines eggs at sites sampled during February to April 2019, overlaid with Sardine egg densities.

### 3.3. Spawning area

The estimated spawning area was 53,600 km<sup>2</sup> and comprised 44.9% of the total area sampled (119,369 km<sup>2</sup>, Table 1).

Table 1. Total area surveyed and spawning area (*A*) estimated in 2019.

Total area sampled (km <sup>2</sup> )	Spawning area, <i>A</i> (km <sup>2</sup> )	Spawning area percentage
119,369	53,600	44.9

### 3.4. Mean daily egg production (*P*<sub>0</sub>)

The estimate of *P*<sub>0</sub> (95% CI) obtained by fitting the linear version (Eq. 3) of the exponential egg mortality model to data obtained in 2019 was 68.1 (42.0–107.7) eggs.day<sup>-1</sup>.m<sup>-2</sup> (Fig. 6, 7, Table 2). The other models produced estimates of *P*<sub>0</sub> between 72.7 and 97.6 eggs.day<sup>-1</sup>.m<sup>-2</sup> when fitted to the data for 2019 (Fig. 6, 7, Table 2).

Table 2. Mean daily egg production (*P*<sub>0</sub>) and instantaneous daily mortality (*Z*) estimated using the log-linear model and four alternative models using data collected 2019.

Model fit	<i>P</i> <sub>0</sub> (eggs.day <sup>-1</sup> .m <sup>-2</sup> ) (95% CI)	<i>Z</i>
Linear version of exponential model, corrected	68.1 (42.0–107.7)	0.60
GLM, Negative Binomial	95.9 (30.4–266.9)	0.76
Exponential model, Non-linear least squares	74.3 (44.3–127.3)	0.39
GLM, Quasi	97.6 (30.2–285.0)	0.78
GLMM, Negative Binomial, log link	72.7 (46.0–113.0)	0.41

The estimate of *P*<sub>0</sub> obtained by fitting the linear model (Eq. 3) to all data from 1998 to 2019 was 81.4 (72.8–91.0) eggs.day<sup>-1</sup>.m<sup>-2</sup> (Fig. 6, 7, Table 3). The alternative models produced estimates of *P*<sub>0</sub> between 97.0 and 171.0 eggs.day<sup>-1</sup>.m<sup>-2</sup> (Fig. 6, 7, Table 3) when fitted these data.

Table 3. Mean daily egg production ( $P_0$ ) and instantaneous daily mortality ( $Z$ ) estimated using the log-linear model and four alternate models, based on all data collected from 1998 to 2019.

Model fit	$P_0$ (eggs·day <sup>-1</sup> ·m <sup>-2</sup> ) (95% CI)	$Z$
Linear version of exponential model, corrected	81.4 (72.8–91.0)	0.51
GLM, Negative Binomial	169.9 (117.2–248.9)	1.10
Exponential model, NLS	146.7 (95.4–315.0)	0.88
GLM, Quasi, log link	171.0 (118.3–249.1)	1.10
GLMM, Negative Binomial, log link	97.0 (80.5–119.6)	0.35

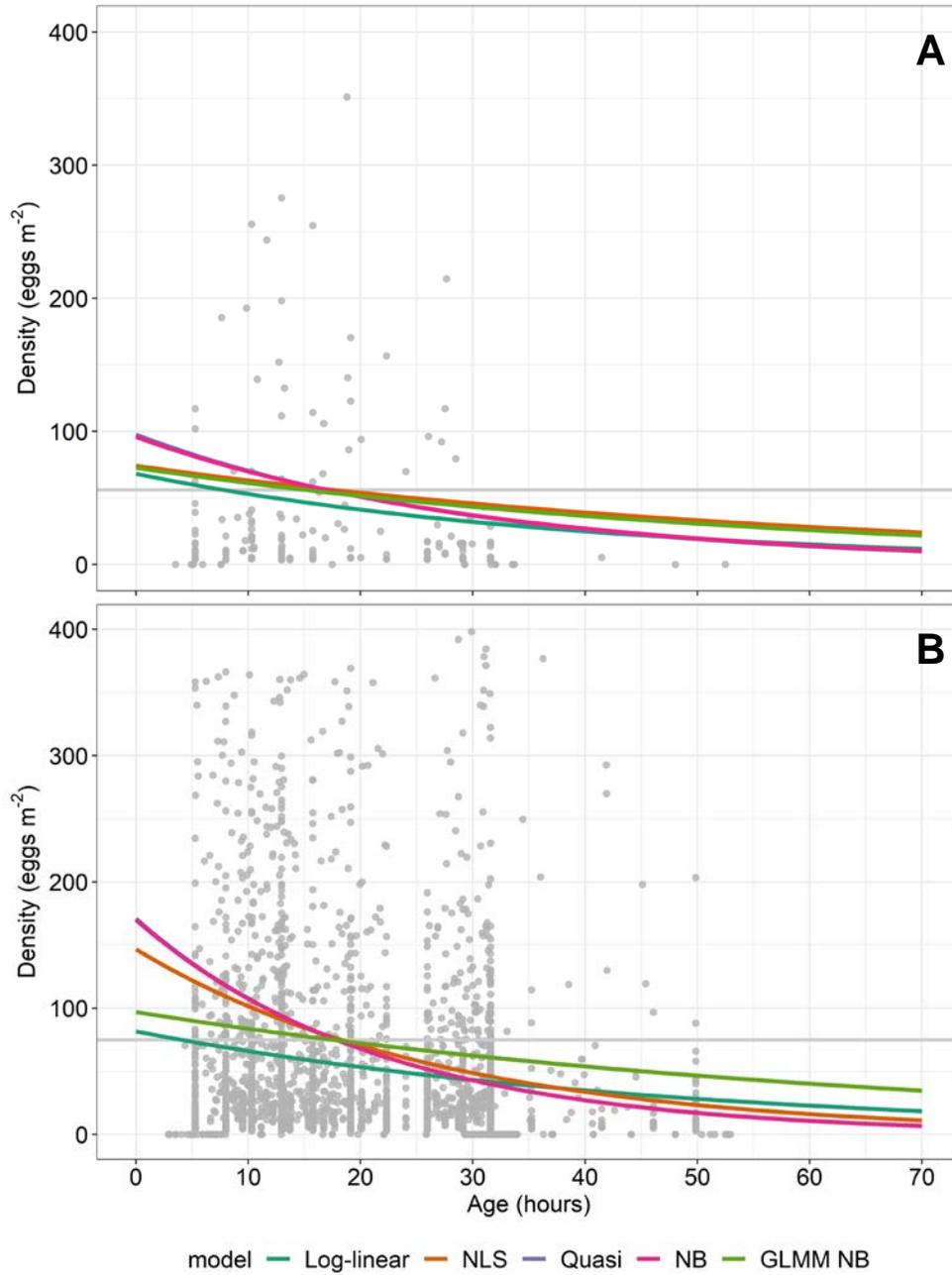


Figure 6. Models fitted to egg densities (eggs.m<sup>-2</sup>) and egg age (hours) of Sardine cohorts in 2019 (A) and all years combined (1998 to 2019; B). Grey horizontal line (bottom plot): mean egg density for all-years.

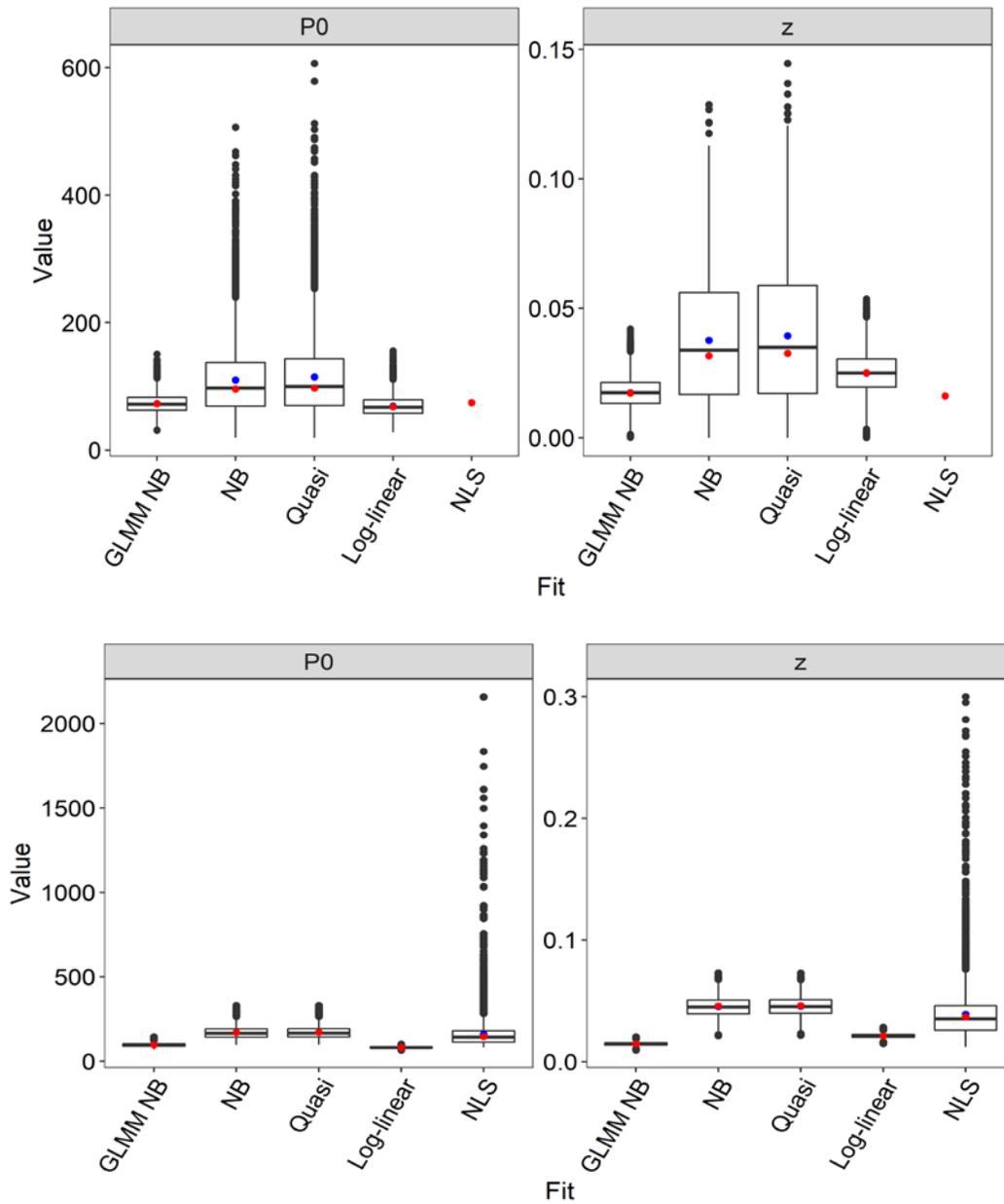


Figure 7. Mean daily egg production ( $P_0$ , egg·day<sup>-1</sup>·m<sup>-2</sup>) and instantaneous daily mortality ( $z$ , day<sup>-1</sup>) for Sardine from the five egg production models for data collected in 2019 (top) and all years from 1998 to 2019 (bottom). Horizontal black line is the median and box is the quartiles. Red dot: model point estimate; blue dot: bootstrapped mean; solid line: 99% Confidence Interval, black dots: outliers.

### 3.5. Adult parameters

Adult parameters used to estimate spawning biomass in 2019 were derived from samples collected off South Australia between 1998 and 2018 (see Appendix 1).

#### 3.5.1. Mean female weight

The mean weight of mature females ( $W$ , 95% CI) estimated from 16,986 fish (255 samples) collected between 1998 and 2018 was 58.0 (22.2–93.8) g (Table 4). Estimates of  $W$  for individual years ranged between 45.0 g in 1998 and 78.7 g in 2004 (Appendix 1).

#### 3.5.2. Sex ratio

The mean sex ratio by weight ( $R$ , 95% CI) calculated from all fish collected between 1998 and 2018 was 0.55 (0.52–0.58) (Table 4). The total numbers of females and males collected were 14,286 (51.1% of fish) and 13,645 (48.9%), respectively (Appendix 1). Estimates of  $R$  for individual years ranged from 0.36 in 2009 to 0.70 in 2018 (Appendix 1).

#### 3.5.3. Batch fecundity

Between 1998 and 2018, 1099 females with hydrated oocytes were collected (Fig. 8). The fecundity-weight relationship estimated from these samples was: Batch Fecundity =  $335 \times \text{Gonad Free Female Weight} - 797$  ( $R^2 = 0.53$ ). Mean gonad free female weight between 1998 and 2018 was 55.5 g and ranged between 43.2 and 75.0 g. Overall mean batch fecundity ( $F$ , 95% CI) was 17,776 (6,408–29,144) oocytes (Table 4).

The overall estimate of  $F/W$  was 306.4 (258.5–354.3) eggs.g<sup>-1</sup> (Table 4). Estimates of  $F/W$  for individual years ranged from 297.2 eggs.g<sup>-1</sup> in 2000 to 311.6 eggs.g<sup>-1</sup> in 2017 (Appendix 1).

#### 3.5.4 Spawning fraction

The spawning fraction ( $S$ , 95% CI) calculated from all data collected between 1998 and 2018 was 0.11 (0.100–0.123) (Table 4). A total of 15,448 ovaries were examined; 2,578 had day-0 POFs or hydrated oocytes, 1,540 had day-1 POFs and 1,046 day-2 POFs. Estimates of  $S$  for individual years ranged from 0.44 in 2011 to 0.70 in 2018 (Appendix 1).

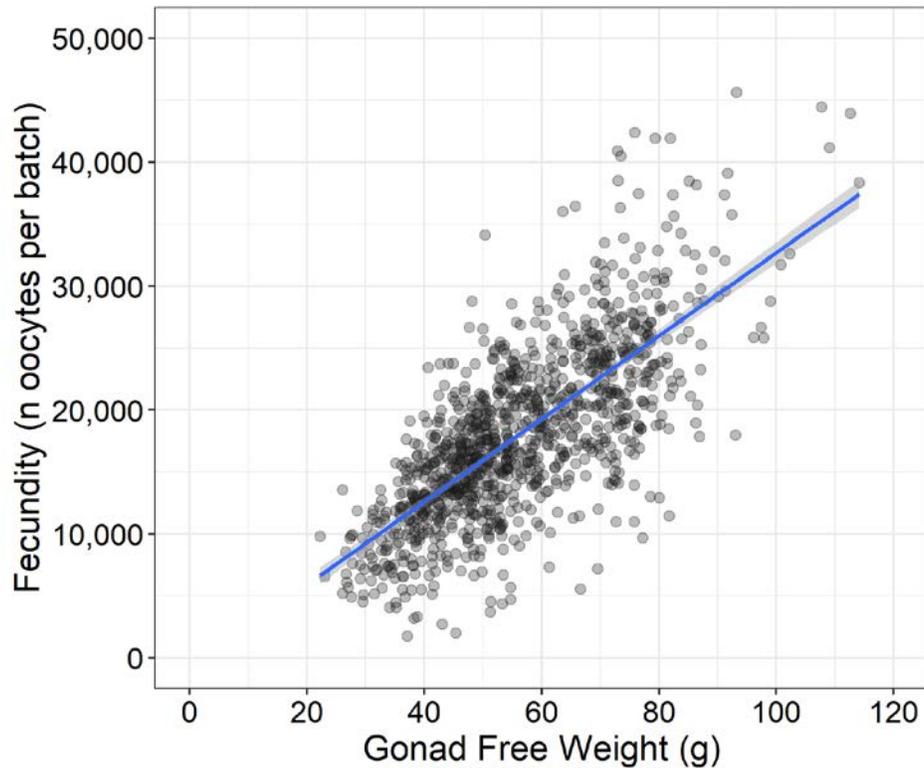


Figure 8. Relationship between gonad-free weight and batch fecundity ( $F$ ) for all hydrated Sardine collected from 1998 to 2018 (blue shading = 95% CI).  $F = 335 \cdot \text{Gonad Free Weight} - 797$ , ( $R^2 = 0.53$ ).

Table 4. Parameter estimates used in the calculations of spawning biomass

Parameter	All Years	95% CI	CV	Range (among years) 1998 - 2018
Egg Production ( $P_0$ , eggs.day <sup>-1</sup> .m <sup>-2</sup> )	81.6	72.8–91.0	0.06	39.0–145.3
Sex Ratio (R)	0.55	0.52–0.58	0.03	0.36–0.70
Fecundity (F, eggs.female <sup>-1</sup> )	17,776	6,408–29,144	0.26	13,722–24,259
Spawning Fraction (S)	0.111	0.100–0.123	0.05	0.041–0.179
Female Weight (W, g)	58.0	22.2–93.8	0.31	45.0–78.7
F/W (eggs.g <sup>-1</sup> )	306.4	258.5–354.3	0.31	297.2–311.6
Spawning Area (A, km <sup>2</sup> )	-	-	-	15,637–73,981

### 3.6. Spawning biomass

The estimate of spawning biomass (95% CI) calculated using the estimate of  $A$  obtained from the survey conducted in 2019, and the all-years estimates of  $P_0$  (log-linear model),  $S$ ,  $R$ , and  $F/W$  was 233,684 (181,214–286,153) t (Figure 10). The estimate calculated using the value of  $P_0$  obtained from 2019 survey was 195,034 t.

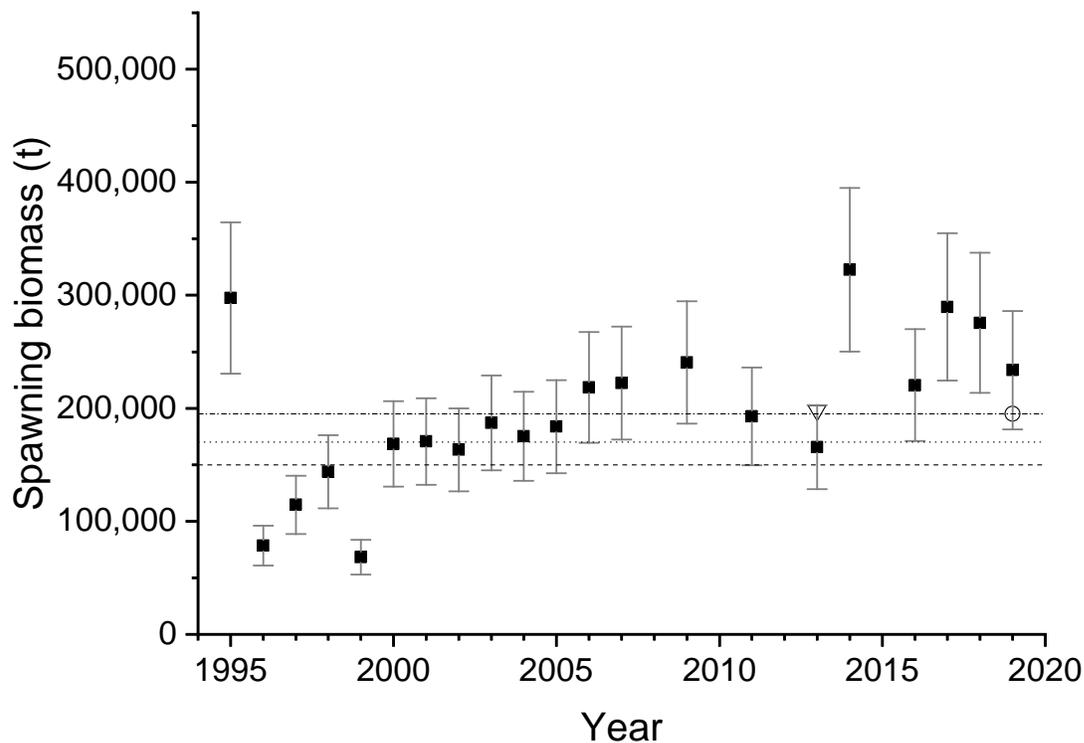


Figure 9. Estimates of spawning biomass (95% CI) for Sardine in South Australian waters from 1995 to 2019 using the log-linear egg production model and all-years data for all parameters, except for spawning area ( $A$ ). The open circle for 2019 is the estimate of spawning biomass obtained using estimate of  $P_0$  from 2019. The open triangle for 2013 (when the survey did not cover the entire spawning area) is the estimate of spawning biomass using the mean  $A$  from 2002 to 2011 (45,406 km<sup>2</sup>). The horizontal lines indicate the 150,000 t (dash), 170,000 t (dotted) and 190,000 t (dash/dot) reference points in the harvest strategy (PIRSA 2014).

### 3.7. Sensitivity analysis

The sensitivity analysis shows the effects of inter-annual variability in parameters (i.e.  $P_0$ ,  $R$ ,  $S$ ,  $F$ ,  $W$  and  $F/W$ ) on the estimate of spawning biomass for 2019 (Table 4, Fig. 10, Appendix 1).

Inter-annual variations in  $W$  and  $F$  between 1998 and 2018 were large and had a strong influence on the estimate of spawning biomass (i.e.  $W = 183,011$  to  $320,067$  t;  $F = 187,139$  to  $391,360$  t). However, the ratio of  $F/W$  was similar among years and inter-annual variation in this combined parameter had a much smaller effect on spawning biomass (i.e.  $232,455$  to  $241,785$  t, Fig. 10).

The estimates of  $R$  obtained in individual years were variable (Table 4; Appendix 1). It is likely that extreme values (e.g. 0.36 and 0.70) are more reflective of the limitations of the adult sampling program than the relative abundance of sexes in the population. The implausibly large fluctuations in  $R$  between consecutive surveys supports this conclusion. The variations in  $R$  had a large influence on the estimate of spawning biomass for 2019 (i.e.  $185,335$  to  $355,438$ ). This finding demonstrates the benefits of using all-years data to estimate this parameter.

The estimates of spawning fraction ( $S$ ) obtained in individual years were also highly variable (i.e. ranging from 0.041 to 0.179). Other studies have shown that annual values of  $S$  are correlated with  $R$  (e.g. SARDI unpublished b). Inter-annual variations in  $S$  are more likely to reflect the limitations of the adult sampling program than differences in the spawning rates occurring in the population. Inter-annual variability in  $S$  had the strong influence of all of the parameters on the estimate of spawning biomass (i.e.  $144,150$  to  $648,674$  t). The sensitivity analysis demonstrated the benefit of using all available data to estimate  $S$ .

The high level of inter-annual variability in the estimates of  $P_0$  appears to reflect the high level of statistical uncertainty associated with annual estimates of this parameter (Fig. 10, Appendix 1). This range of variability had a strong influence on the estimate of spawning biomass (i.e.  $112,737$  to  $420,019$  t, Fig. 10). The estimate of spawning biomass obtained using the  $P_0$  estimated from 2019 survey was lower than the estimate obtained using all-years data. Both estimates were within the range of values of  $P_0$  obtained in individual years between 1998 and 2018 (Table 4).

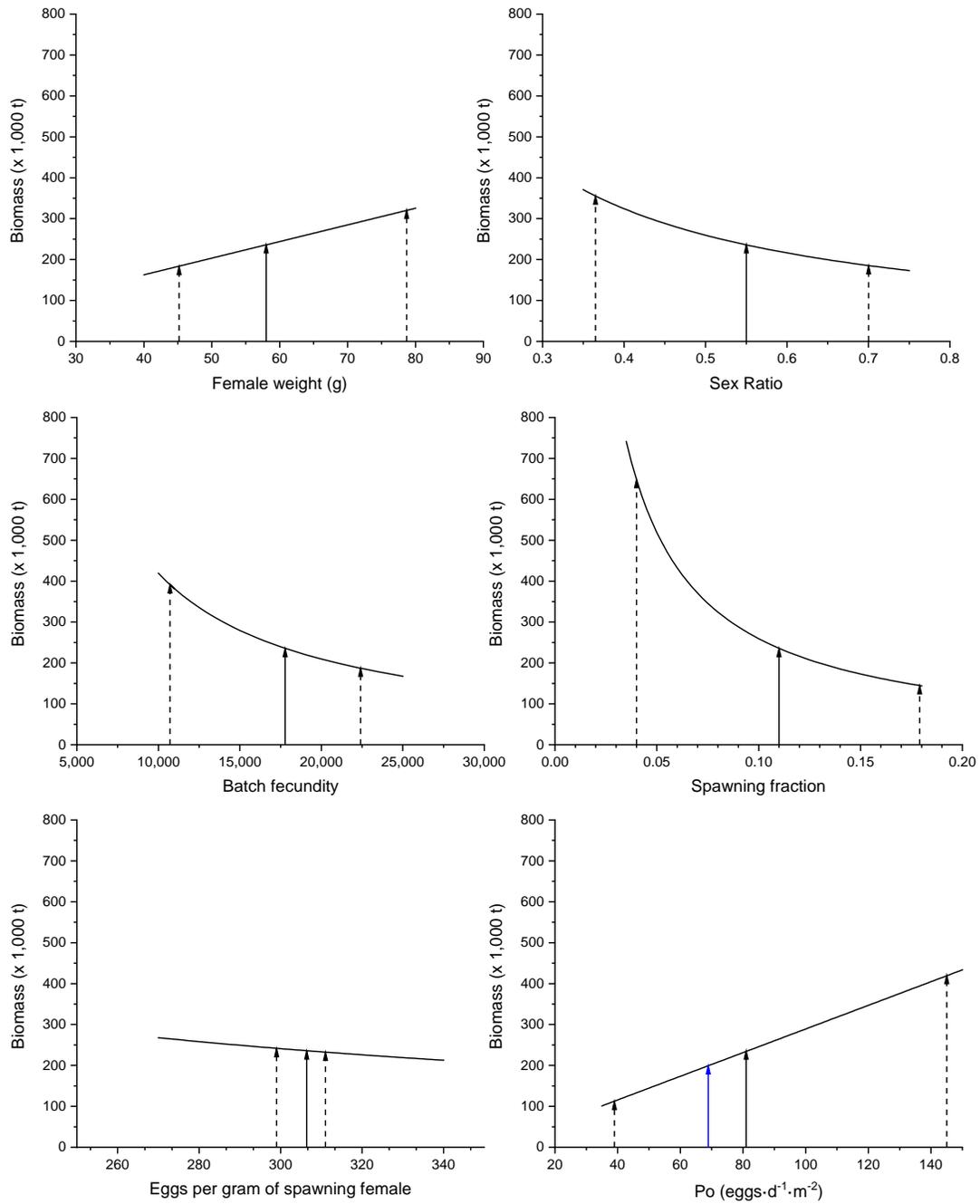


Figure 10. Sensitivity plots showing effects of variability in adult parameters and egg production on estimates of spawning biomass. Solid black arrows: parameter estimates for all years combined; Dashed arrows: range of values recorded between 1998 and 2018; Blue arrow:  $P_0$  estimate using only data collected in 2019.

## 4. DISCUSSION

### 4.1. Egg distribution

The distribution of Sardine eggs off South Australia in 2019 was similar to that observed in 2018, but different to the patterns observed in most years prior to 2018 (see SARDI unpublished b). In both 2018 and 2019, Sardine eggs were more widespread and abundant in southern Spencer Gulf and south of Kangaroo Island than in most previous years. Future surveys may need to be extended further to the east to ensure that the future spawning area of Sardine off South Australia is covered. The adaptive approach to sampling that has been applied at the end of shelf transects since 2013 should also be maintained in future surveys to ensure that plankton samples are collected from the entire spawning area.

### 4.2. Spawning area and mean daily egg production

The estimate of spawning area in 2019 was 53,600 km<sup>2</sup>, which is the fifth highest on record. Spawning area has been consistently above 50,000 km<sup>2</sup> since peaking at 73,981 km<sup>2</sup> in 2014. Spawning area is strongly correlated with Sardine abundance (Mangel and Smith 1990, Gaughan et al. 2004). The large spawning area observed in this study provides strong evidence that Sardines were widespread and abundant off South Australia in 2019.

Recent studies (e.g. Ward et al. 2018, SARDI unpublished a, b) have shown that for Sardine off South Australia inter-annual variability in estimates of  $P_0$  is low compared to statistical uncertainty (imprecision). In the present study, we addressed this issue by estimating  $P_0$  from data obtained from all years between 1998 and 2019. The estimate of  $P_0$  obtained using this approach was more precise (SD = 4.6) than the estimate obtained using data from 2019 only (SD = 16.3). This approach will prevent large inter-annual fluctuations in estimates of spawning biomass driven by variations in the annual estimate of  $P_0$  that are caused by statistical uncertainty. In future applications of the DEPM to Sardine off South Australia,  $P_0$  should be estimated using data obtained in all years since 1998.

### 4.3. Adult parameters

Evidence compiled in this report and elsewhere (e.g. SARDI unpublished b) suggest that the large variations among years observed in the estimates of the adult parameters of Sardine off South Australia are more likely to reflect the limitations of the adult sampling program, rather than actual differences among years in the reproductive patterns of the population. Re-analysis of adult samples collected off South Australia since 1998 suggest that both individual parameters and

mean daily fecundity are relatively stable among years, especially when inter-annual variability is evaluated within the context of potential sources of statistical uncertainty (i.e. precision and bias).

Inter-annual variability in the estimates of sex ratio ( $R$ ) exemplifies the problems associated with annual estimation of individual adult parameters. One of the sexes often dominates adult samples taken in a given year, with values of  $R$  for individual years ranging between 0.36 in 2009 and 0.07 in 2018. Large variations in  $R$  occurred between consecutive surveys. Annual estimates of  $R$  near the upper and lower ends of the observed range are unlikely to reflect the sex ratio in the broader population. The mean value of 0.55 obtained by combining all available data is likely to be a better approximation of the sex ratio of the population in any one year than the estimate obtained from that year's data. A value of sex ratio by weight of greater than 0.5 (i.e. 0.55) is appropriate because on average adult females sampled during the spawning season are slightly heavier at any given size than males. The marginally higher proportion of females (51.1%) than males (49.8%) obtained in samples collected between 1998 and 2018 also helps to explain why  $R$  was greater than 0.5. Uncertainty in the estimate of spawning biomass is reduced by using the mean value of  $R$  from the entire dataset rather than the estimate obtained in any single year.

Other studies have shown that  $S$  is correlated with  $R$  (e.g. Ward et al. 2016; SARDI unpublished b). Samples obtained in years when estimates of  $R$  were low (e.g. 0.35) typically produced estimates of  $S$  that were high (e.g. 0.18). This correlation exists because a large proportion of the females present in samples dominated by males were actively spawning, and vice-versa (Ganias et al. 2009). This dominance of males and females in samples has previously been interpreted as an artifact of differential sampling of spawning and non-spawning schools, respectively (Ganias et al. 2009). The mean value of  $S$  (0.11) obtained using all available data from South Australia is similar to the global mean spawning fraction for Sardine of 0.12 (Ganias et al. 2009). Like  $R$ , the all-years values of  $S$  is likely to be a better approximation of spawning fraction in any one year than the estimate obtained only using data collected in that year.

The data collected since 1998 used in the sensitivity analysis shows that estimates of  $F$  and  $W$  are highly variable among years. This variability may be explained, at least in part, by the sampling limitations discussed for  $R$  and  $S$ . However, the adult population includes fish of a wide range of sizes and the number of eggs produced by individual fish of similar sizes is also variable, so the variance of both parameters is high. Despite these sampling limitations and high levels of variability in  $F$  and  $W$  among years, the estimates of  $F/W$  obtained in individual years are remarkably similar (i.e. range 300–311 eggs.g<sup>-1</sup>). This low variability among years in  $F/W$  means

that these combined values have minimal influence on estimates of spawning biomass. For this reason, there is limited benefit in estimating  $F$  and  $W$  annually.  $F/W$  rather than  $F$  and  $W$  estimated separately should be used to calculate spawning biomass as this approach improves precision.

For reasons outlined above, in the foreseeable future, adult parameters used to calculate the spawning biomass of Sardine off South Australia should be estimated from data obtained in adult surveys conducted between 1998 and 2018.

#### **4.4. Spawning biomass**

The estimate of spawning biomass for 2019 of 233,684 (181,214–286,153) t is above the upper target reference point in the harvest strategy for the SASF of 190,000 t (PIRSA 2014). On this basis, the southern Australian stock of Sardine is classified as **Sustainable**. This classification is consistent with recent assessments provided in the spawning biomass report for 2018, the stock assessment report for 2019 and the most recent report on the Status of Australian Fish Stocks (<http://www.fish.gov.au/Reports>).

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**APPENDIX 1.** Annual and all-years parameters used to calculate estimates of Spawning Biomass. Total *A*: total area sampled (km<sup>2</sup>), *A*: spawning area (km<sup>2</sup>); *P<sub>0</sub>*: mean daily egg production (egg·m<sup>-2</sup>·day<sup>-1</sup>); *S*: spawning fraction; *R*: sex ratio; *W*: mean female weight (g); *F*: batch fecundity (oocytes·batch<sup>-1</sup>); *F/W*: Fecundity / Female Weight. Errors around the estimates are standard deviation (SD). *N*: number of samples; *n*: number of individuals. *F/W* was calculated using the all-years *F* relationship with *W* from that year.

Time	Total A	A	<i>P<sub>0</sub></i>	<i>P<sub>0</sub></i> SD	N· <i>P<sub>0</sub></i>	<i>S</i>	<i>S</i> SD	N· <i>S</i>	n· <i>S</i>	<i>R</i>	<i>R</i> SD	N· <i>R</i>	n· <i>R</i>	<i>W</i>	<i>W</i> SD	N· <i>W</i>	n· <i>W</i>	<i>F</i>	<i>F</i> SD	N· <i>F</i>	n· <i>F</i>	<i>F/W</i>	<i>F/W</i> SD
<b>All Years</b>	-	-	81.6	4.6	5672	0.111	0.006	231	15448	0.55	0.01	210	27931	58.0	18.3	255	16995	17776	5800	255	16995	306.4	24.4
1998	48379	32980	99.0	30.8	164	0.139	0.015	12	530	-	-	-	-	45.0	12.1	12	554	13722	3813	12	554	304.7	37.5
1999	65956	15637	50.0	14.9	213	0.169	0.021	15	763	-	-	-	-	50.6	14.6	16	785	15597	4395	16	785	308.5	51.9
2000	102198	38658	52.9	12.7	290	0.158	0.012	15	1012	0.52	0.05	15	2179	48.2	13.0	16	1071	14319	4373	16	1071	297.2	40.6
2001	132382	39131	59.7	15.6	316	0.179	0.014	10	743	0.56	0.04	10	1397	50.7	9.1	11	1002	15571	3210	11	1002	306.9	24.3
2002	131574	37462	97.4	29.1	319	0.077	0.014	22	1631	0.60	0.04	22	2932	61.8	19.5	22	1841	18958	6179	22	1841	307.0	15.3
2003	133058	42905	113.5	27.4	320	0.103	0.008	7	435	0.48	0.03	7	986	52.4	8.5	7	435	16210	2730	7	435	309.5	16.3
2004	105621	40219	145.3	41.3	284	0.166	0.016	10	412	0.52	0.04	10	879	78.7	16.2	10	413	24259	5013	10	413	308.2	15.1
2005	122831	42142	59.5	14.3	334	0.100	0.019	32	2223	0.51	0.04	32	4827	73.7	16.2	33	2234	22663	5199	33	2234	307.2	21.9
2006	119038	50121	102.4	26.5	341	0.095	0.018	20	1332	0.59	0.05	20	2445	63.1	21.9	21	1337	19116	6786	21	1337	303.0	16.6
2007	119036	50972	104.9	27.1	341	0.130	0.019	20	1084	0.54	0.07	20	2244	71.0	16.9	21	1086	21762	5446	21	1086	306.5	19.8
2009	119031	55179	66.3	14.1	340	0.156	0.022	19	1537	0.36	0.04	9	2425	59.9	13.3	19	1537	17935	4457	19	1537	299.6	24.6
2011	119449	44245	51.5	15.4	340	0.044	0.006	14	1169	0.65	0.05	13	1798	46.6	12.5	15	1181	14367	4100	15	1181	308.4	10.3
2013	119297	37953	39.0	8.7	340	0.072	0.016	9	703	0.69	0.03	9	1089	51.2	12.4	9	723	15707	4079	9	723	307.0	23.1
2014	125249	73981	92.7	20.0	355	-	-	-	-	0.57	0.02	16	1574	47.2	14.3	16	886	14666	4761	16	886	310.6	15.9
2016	122598	50551	47.7	9.9	350	0.088	0.012	9	656	0.65	0.03	9	1088	49.7	12.3	9	681	15337	5429	9	681	308.7	5.1
2017	119661	66453	136.3	25.8	343	0.120	0.019	8	504	0.52	0.05	9	1042	59.6	7.2	9	511	18557	4276	9	511	311.6	19.6
2018	120043	63215	112.4	24.6	343	0.054	0.009	9	714	0.70	0.03	9	1026	46.5	17.3	9	718	14261	2436	9	718	306.6	17.2
2019	119369	53600	68.1	16.3	339	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-