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# Spawning biomass of Sardine, *Sardinops sagax*, in waters off South Australia in 2020

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

**SARDI Publication No. F2007/000566-11**
**SARDI Research Report Series No. 1074**

**SARDI Aquatics Sciences**
**PO Box 120 Henley Beach SA 5022**

**September 2020**

**Report to PIRSA Fisheries and Aquaculture**

SOUTH AUSTRALIA

Government of South Australia
Department of Primary Industries and Regions

SARDI
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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 2020. 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-March 2020. The survey area was expanded in 2020 to include 40 new sites south-east of Kangaroo Island. The total survey area was 129,700 km$^2$. Live Sardine eggs were collected at 241 of 379 (63.6%) sites. The total spawning area ($A$) in 2020 of 82,627 km$^2$. Excluding the additional sites sampled in 2020 reduced the spawning area to 75,678 km$^2$, which is the largest on record. Mean daily egg production ($P_0$, 95% CI) estimated using the linear version of the exponential mortality model and all data collected from 1998 to 2020 was 82.6 (74.2–91.7) eggs.day$^{-1}$.m$^{-2}$.

Estimates of adult parameters (95% CI) calculated from all data obtained between 1998 and 2018 were: sex ratio ($R$): 0.55 (0.52–0.58); spawning fraction ($S$): 0.11 (0.10–0.12); and relative fecundity ($F'$): 305.0 (303.8–306.3) eggs.g$^{-1}$.

Sensitivity analyses demonstrated the benefits of using estimates of $P_0$, $R$, $S$ and $F'$ obtained from historical data to estimate spawning biomass.

The estimate of spawning biomass (95% CI) of Sardine for 2020 was 378,923 (318,777–439,068) t, which is the highest on record (1995-2020) and 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 2019, the stock assessment report for 2019 and the most recent report in 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, Parker 1980, Lasker 1985), 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 premise of the DEPM is 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 ($A$). In the original formulation of the DEPM (Parker 1980), mean daily fecundity was estimated from three parameters: sex ratio ($R$), spawning fraction ($S$) and relative fecundity ($F'$).

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

In more recent applications of the DEPM, female weight ($W$) and batch fecundity ($F$) have been estimated separately (Stauffer and Piquelle 1980; Picquelle and Stauffer 1985; Lasker 1985). Ward et al (2019) identified the need to compare the relative precision of the two formulations of the DEPM. Those studies also questioned whether or not all parameters need to be estimated annually, or if some parameters can be estimated with increased precision from datasets collected over multiple years. For example, we 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. 2019, 2020). These recent 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 ($A$).

## 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, 2019, 2020). 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 (PIRSA 2014). The objective of this report is to estimate the spawning biomass of Sardine in waters off South Australia in 2020. Estimates of mean daily egg production and spawning area were obtained from an ichthyoplankton survey conducted in 2020. Adult parameters were calculated from data obtained 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 2020.

## METHODS

### 1.3. Study area and biophysical variables

#### 1.3.1. *Study area*

An ichthyoplankton survey was conducted from the *RV Ngerin* in shelf and gulf waters of South Australia during February and March 2020 (Fig. 1). Plankton samples were collected at a total of 379 sites on 38 transects between Kingston and the Head of Bight (Fig. 1). The 379 sites sampled in 2020 included 40 additional sites south east of the traditional survey area (Fig. 1).

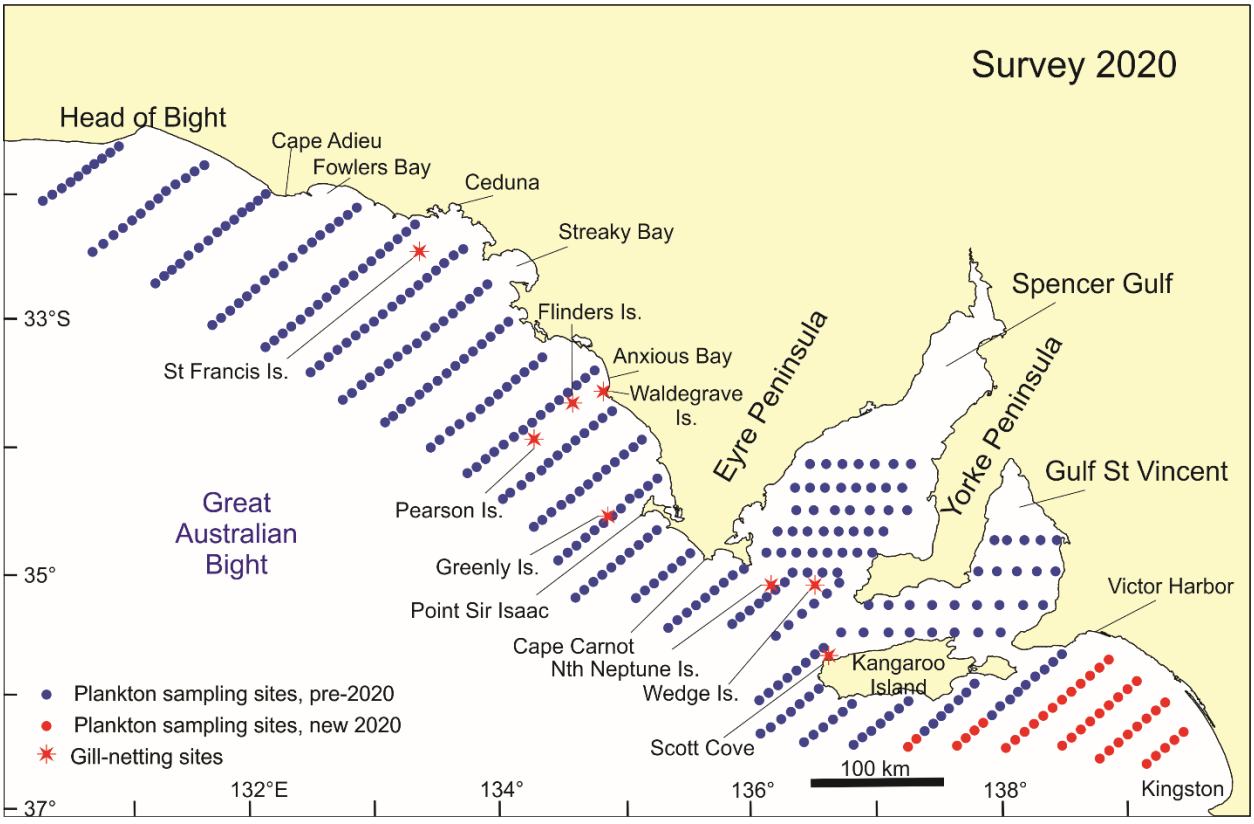


Figure 1. Map of South Australia showing sites where plankton samples were collected during 2020 and where adult samples were collected with gill-nets between 1998 and 2018.

### 1.3.2. *Water temperature and primary production*

At each sampling 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 CTD profile. At sites where water temperature was not recorded (due to technical difficulties), 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 ($\mu g.L^{-1}$). Spatial plots of sea surface temperature (SST) and chlorophyll-*a* concentration were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

## 1.4. Mean daily egg production and spawning area

### 1.4.1. *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 µm mesh and plastic removable cod-ends. During each tow the CalVET nets were deployed to a depth of 70 m or to 10 m of the seabed at depths <80 m. The nets were retrieved vertically at a speed of ~1 $m.s^{-1}$. *General Oceanics* 2030 flow-meters and factory calibration coefficients were used to estimate the distance travelled by the net during each tow. Where there was a discrepancy of more than 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.

### 1.4.2. *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 developmental 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 Ward et al. 2019, 2020).

### 1.4.3. *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.

### 1.4.4. *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:

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

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

### 1.4.5. *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 ($km^2$) was then determined. *A* was defined as the total area of grids where live Sardine eggs were found.

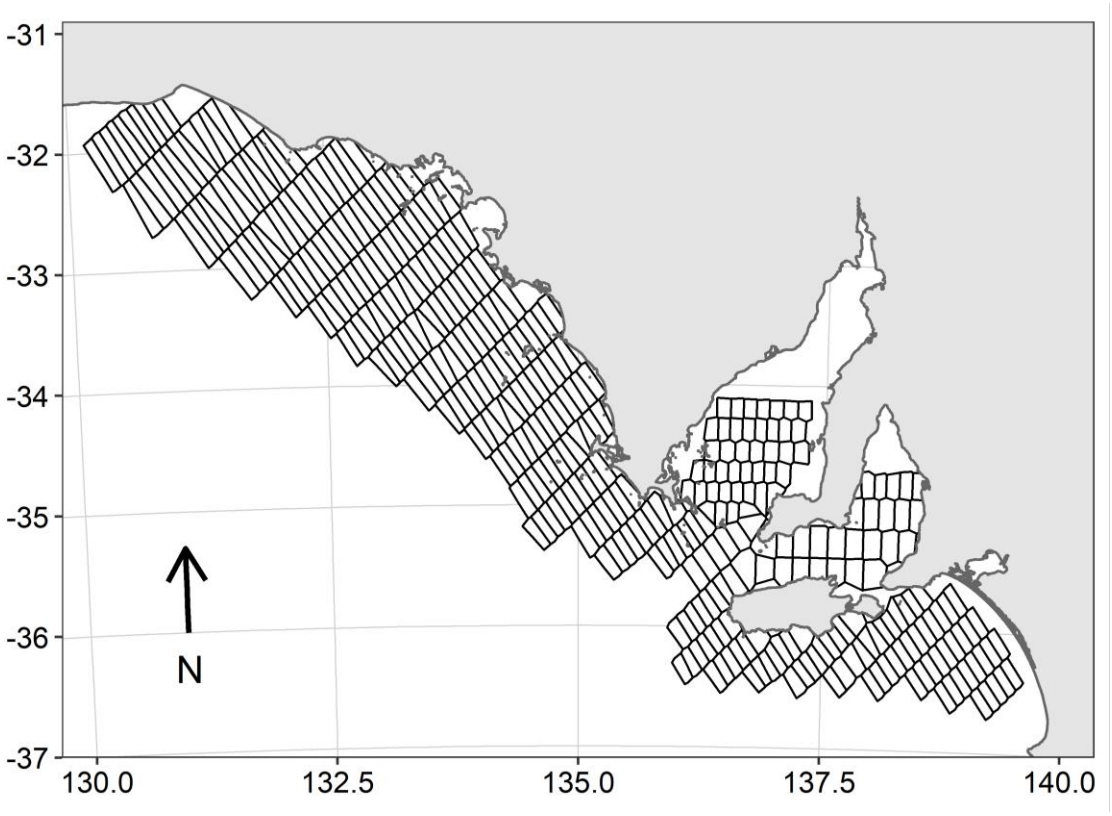


Figure 2. Voronoi nearest neighbour polygons used to estimate the total spawning area in 2020.

### 1.4.6. *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:

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

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):

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

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

A general linear model (GLM) with a negative binomial error structure (NB1) where the variance increases linearly with the mean (σ = μ*(1+ μ +φ)) was also used to estimate $P_0$ (Equation 5):

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

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. 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 2020, as well as with data from all years (combined) between 1998 and 2020. 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).

## 1.5. Adult reproductive parameters

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.

### 1.5.1. *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).

### 1.5.2. *Female weight (W)*

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

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

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

### 1.5.3. *Male weight*

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

### 1.5.4. *Sex ratio (R)*

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

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

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

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

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

### 1.5.5. *Spawning fraction (S)*

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

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

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

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

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

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

### 1.5.6. *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.

Relative Fecundity (*F'*) 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*).

## 1.6. 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 2020 and estimates of *S*, *R*

and *F'* 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 2020.

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',* were calculated from the all-years adult data. A ratio estimator was used calculate the coefficients of variation (CVs) for *S*, *R*, and *F'* (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 2020 on estimate of spawning biomass for 2020.

# 2. RESULTS

## 2.1. Distribution and abundance of eggs

A total of 6,630 live Sardine eggs were collected at 241 of 379 (63.6%) sites on 38 transects between the Kingston and Head of Bight between February and March 2020 (Fig. 3). Sites with the high egg densities were located in the mouth of Spencer Gulf, south of Kangaroo Island, off Anxious Bay and on the shelf in the central Great Australian Bight (GAB).

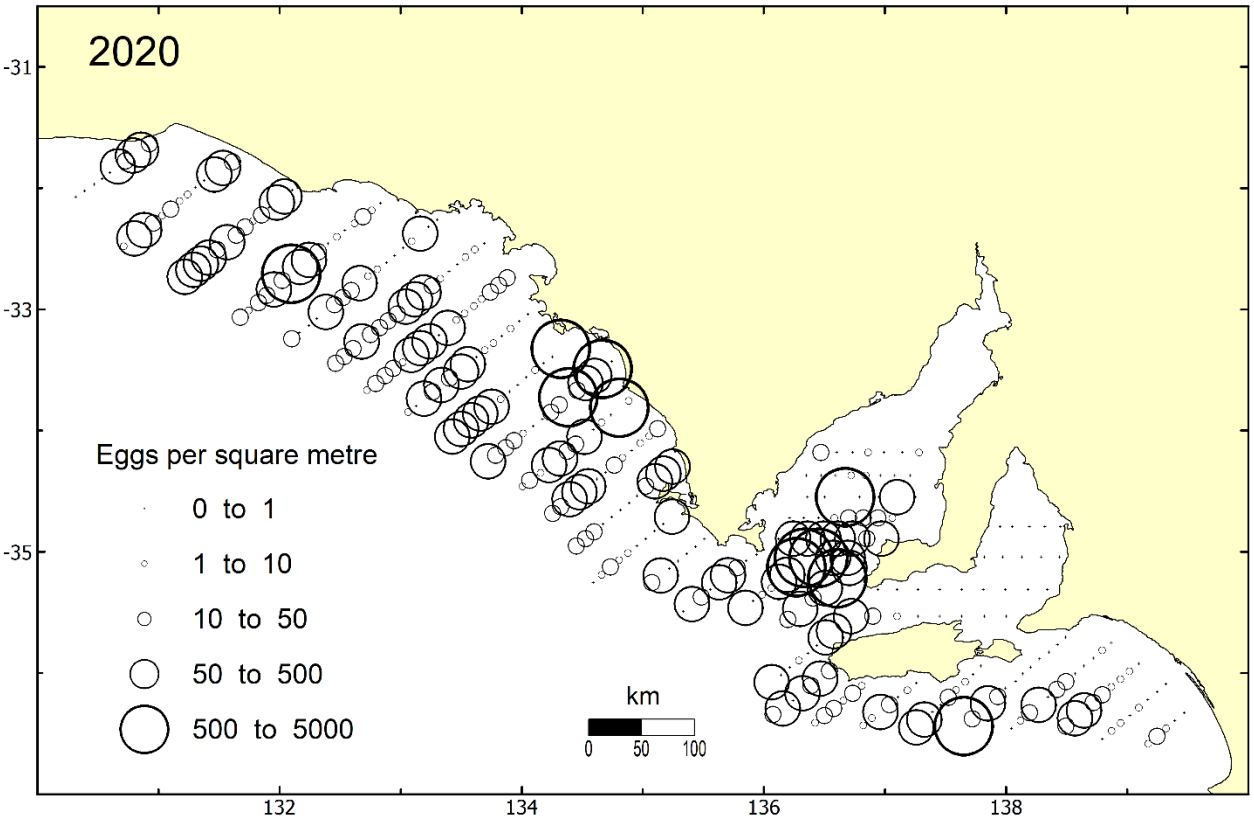


Figure 3. Densities of live Sardine eggs at sites sampled during February and March 2020.

## 2.2. Biophysical variables

### 2.2.1. *Sea surface temperature*

Sea surface temperatures (SSTs) ranged from 16.4 to 22.8˚C (Fig. 4) between February and March 2020. High SSTs (>20˚C) were recorded in Spencer Gulf, Gulf St Vincent and throughout the central Great Australian Bight (GAB). Cooler, upwelled water (<19˚C) was widespread throughout the eastern Great Australian Bight.

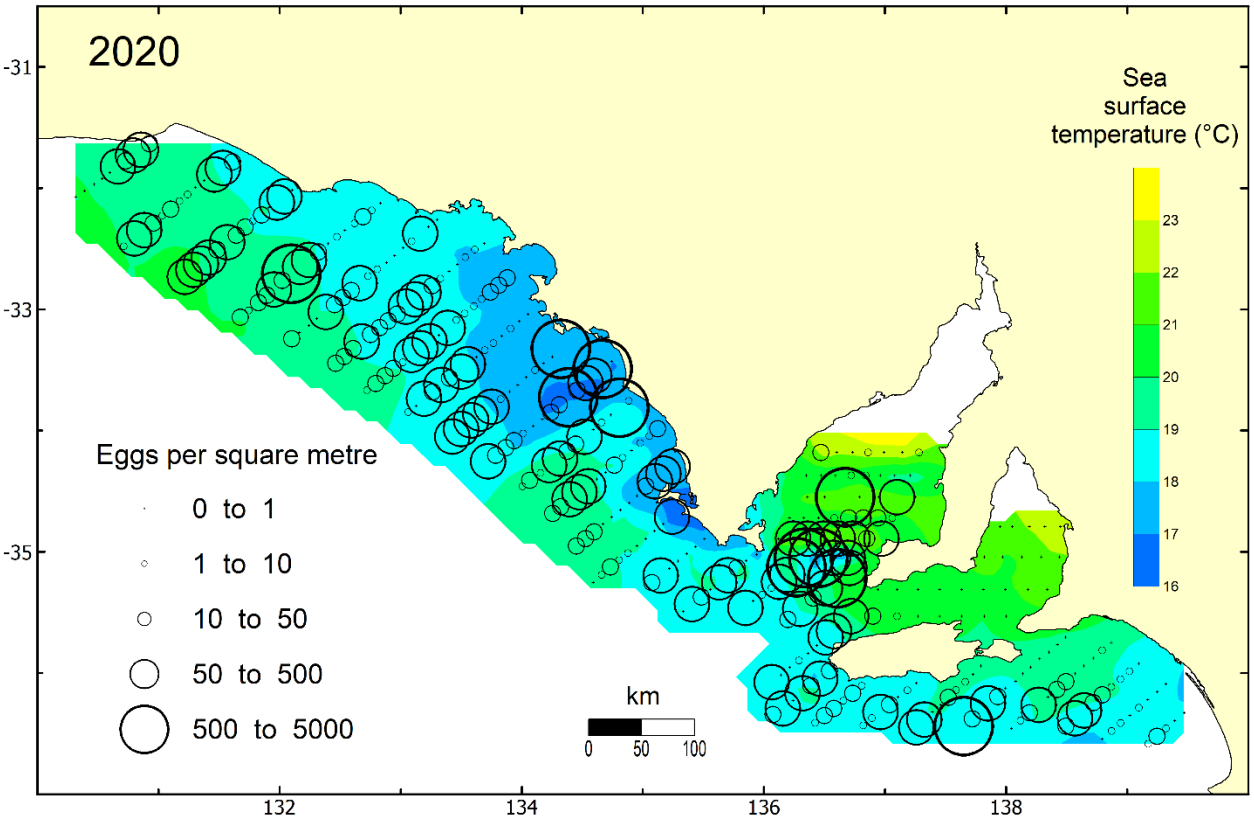


Figure 4. Sea surface temperatures and overlaid with densities of live Sardines eggs at sites sampled during February and March 2020.

### 2.2.2. *Fluorescence*

Surface chlorophyll-*a* concentration at each site ranged between 0.03 and 2.34 µg.L$^{-1}$ (Fig. 5) between February and March 2020. The highest values were recorded off Anxious Bay in the eastern GAB. The remainder of coastal and shelf waters mainly had chlorophyll-*a* concentrations <0.2 µg.L$^{-1}$.

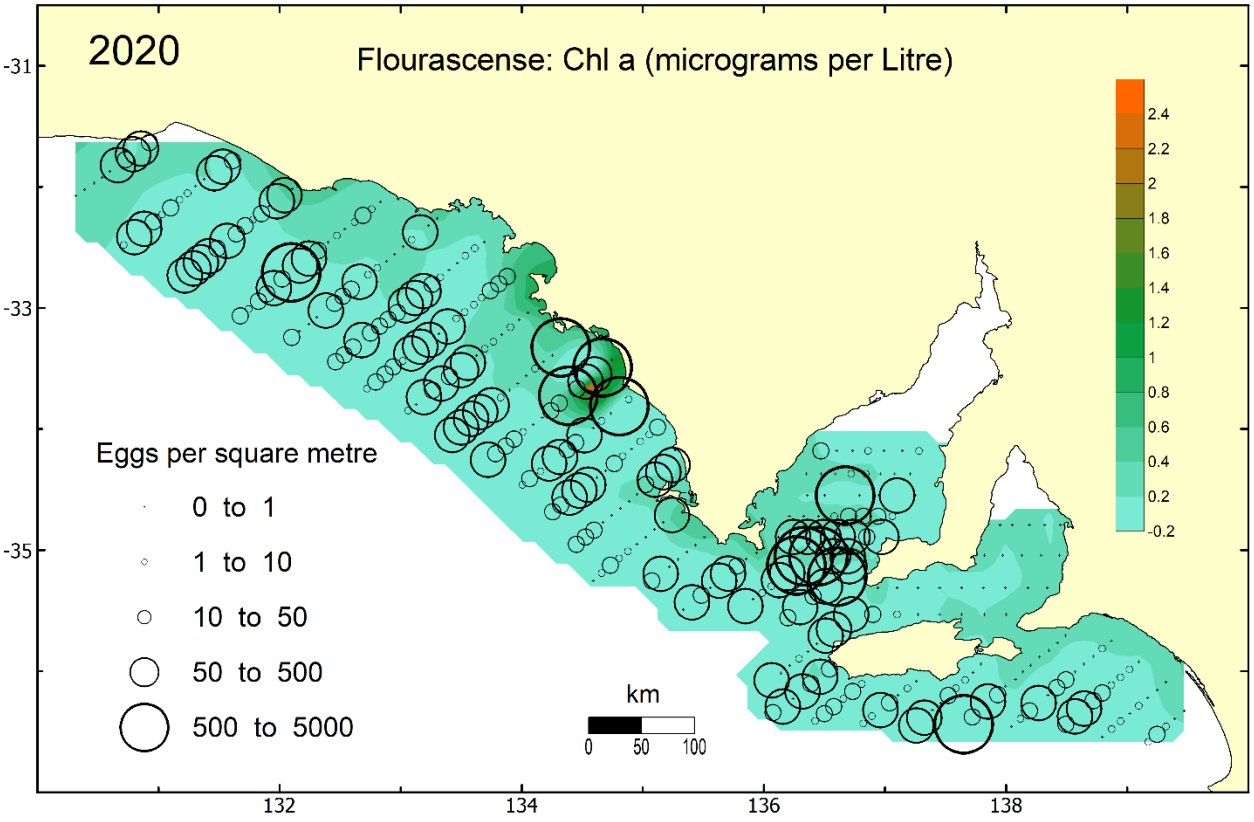


Figure 5. Surface concentration of chlorophyll-*a* overlaid with densities of live Sardines eggs at sites sampled during February and March 2020.

## 2.3. Spawning area

The estimated spawning area was 82,627 km$^2$ and comprised 63.7% of the total area sampled (129,700 km$^2$, Table 1, Fig. 6). If the additional transects sampled in 2020 were not included, the spawning area would have been 75,678 km$^2$ (Fig. 1, 6, Table 1).

Table 1. Total area surveyed and spawning area (*A)* estimated in 2020.

| | Area sampled (km$^2$) | Spawning area, *A* (km$^2$) | Spawning area percentage |
|---|---|---|---|
| Pre-2020 survey | 119,002 | 75,678 | 63.6 |
| With new stations | 129,700 | 82,627 | 63.7 |

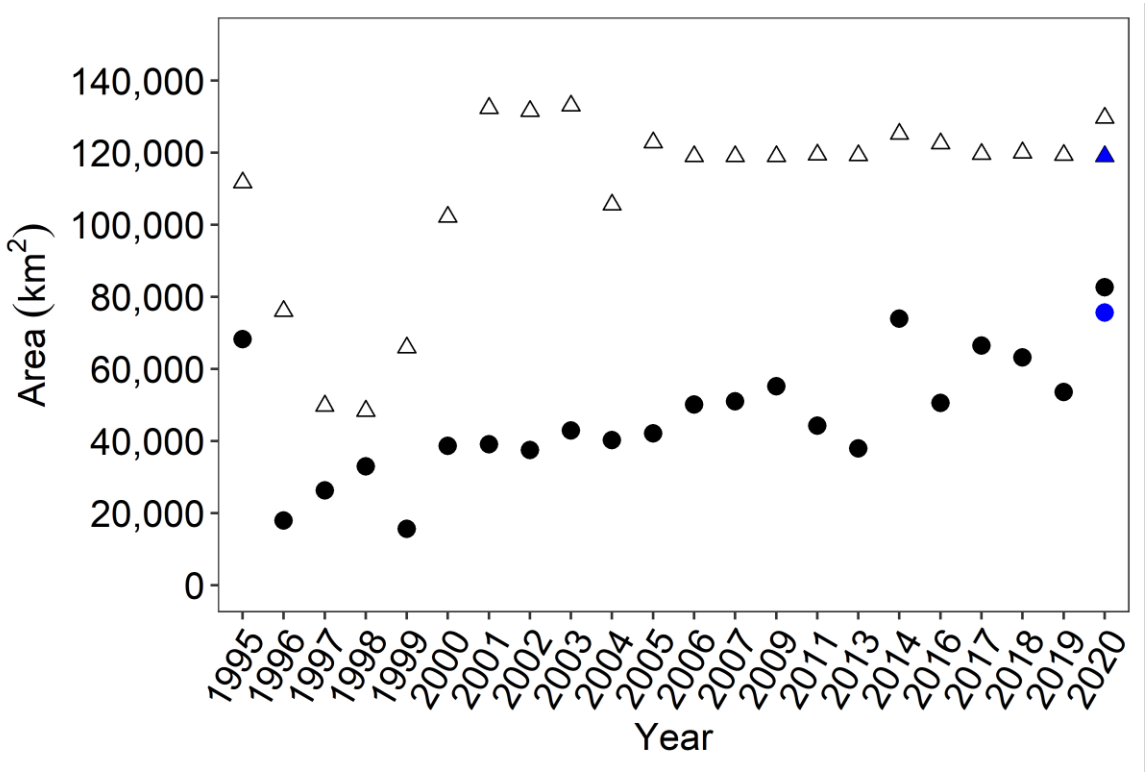


Figure 6. Sampling area (open triangles) and spawning area (closed circles) over the history of DEPM surveys in South Australia (blue points for 2020 are excluding additional sites).

## 2.4. Mean daily egg production ($P_0$)

The estimate of $P_0$ obtained by fitting the log-linear model (Eq. 3) to all data from 1998 to 2020 was 82.6 (74.2–91.7) eggs.day$^{-1}$.m$^{-2}$ (Fig. 7, Table 2). The alternative model produced an estimate of 97.6 eggs.day$^{-1}$.m$^{-2}$ (Fig. 7, Table 2) when fitted to these data.

Table 2. Mean daily egg production (P0) and instantaneous daily mortality (Z) estimated using the log-linear and GLM model, based on all data collected from 1998 to 2020.

| Model fit | $P_0$ (eggs·day$^{-1}$·m$^{-2}$) (95% CI) | $Z$ |
|---|---|---|
| Log-linear model | 82.6 (74.2–91.7) | 0.52 (0.43–0.60) |
| GLM, Negative Binomial | 97.6 (81.7–117.9) | 0.35 (0.29–0.42) |

The estimate of $P_0$ (95% CI) obtained by fitting the log-linear (Eq. 3) egg mortality model to data obtained in 2020 was 94.0 (64.7–136.7) eggs.day$^{-1}$.m$^{-2}$ (Fig. 7, Table 3). The GLM NB1 produced an estimate of $P_0$ of 103.0 eggs.day$^{-1}$.m$^{-2}$ when fitted to the data for 2019 (Fig. 7, Table 3).

Table 3. Mean daily egg production ($P_0$) and instantaneous daily mortality ($Z$) estimated using the log-linear and GLM model, using data collected in 2020.

| Model fit | $P_0$ (eggs·day$^{-1}$·m$^{-2}$) (95% CI) | $Z$ |
|---|---|---|
| Log-linear model | 94.0 (64.7–136.7) | 0.60 (0.28–0.89) |
| GLM, Negative Binomial | 103.0 (64.7–163.6) | 0.38 (0.17–0.60) |

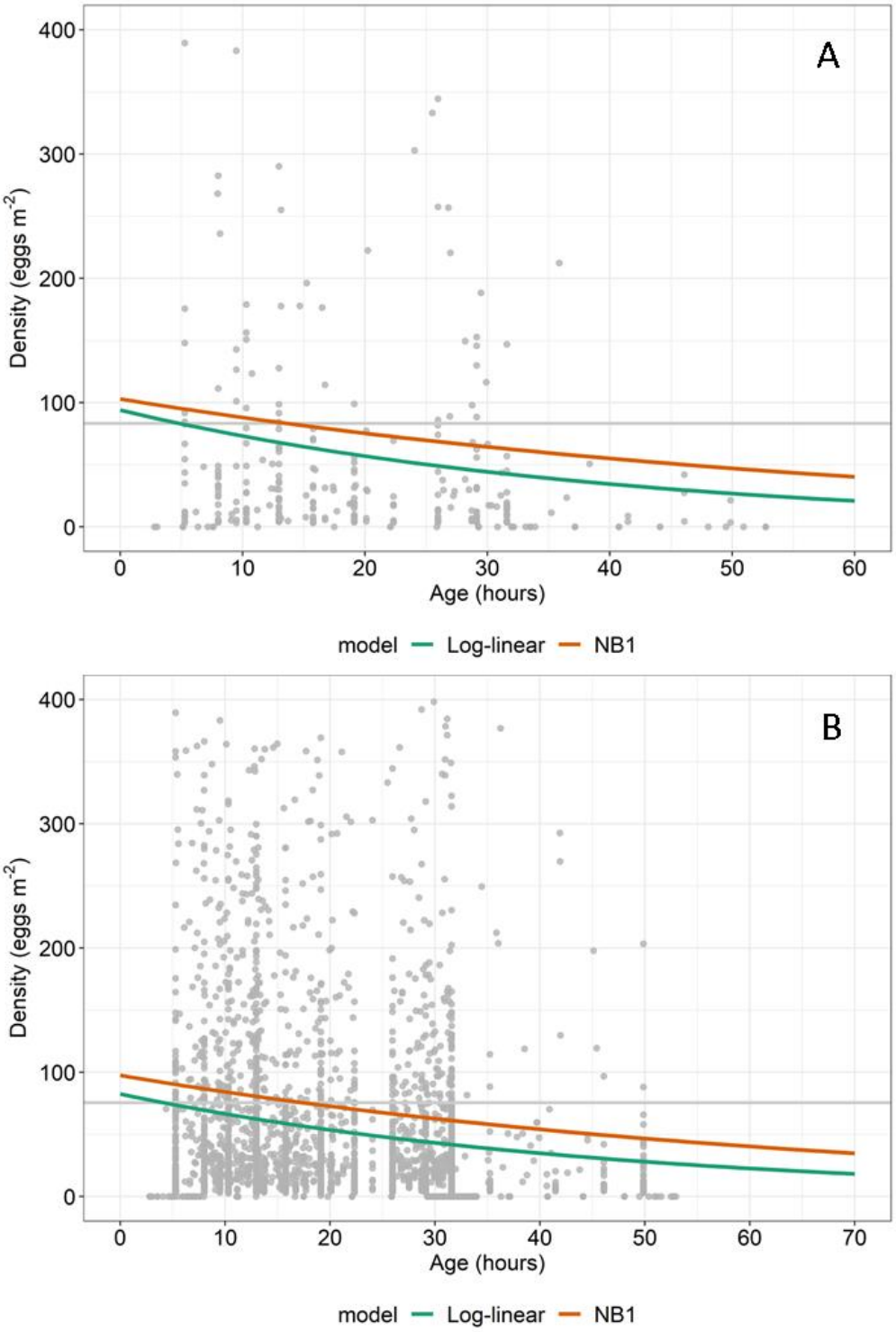


Figure 7. Models fitted to egg densities (eggs.m$^{-2}$) and egg age (hours) of Sardine cohorts in 2020 (**A**) and all years combined (1998 to 2020; **B**). Grey horizontal line: mean egg density.

## 2.5. Adult parameters

Adult parameters used to estimate spawning biomass in 2020 were derived from samples collected off South Australia between 1998 and 2018 (see Appendix 1).

### 2.5.1. *Mean female weight*

The mean weight of mature females (*W*, 95% CI) estimated from 16,995 fish (255 samples) collected between 1998 and 2018 was 58.4 (23.1–93.7) g (Table 4). Estimates of *W* for individual years ranged between 46.5 g in 1998 and 78.7 g in 2004 (Appendix 1).

### 2.5.2. *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). Estimates of *R* for individual years ranged from 0.36 in 2009 to 0.70 in 2018 (Appendix 1).

### 2.5.3. *Batch fecundity*

Between 1998 and 2018, 1,099 females with hydrated oocytes were collected (Fig. 8). The fecundity-weight relationship estimated from these samples was: Batch Fecundity = 335 × 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 17,816 (3,819–31,813) oocytes (Table 4).

The overall estimate of F/W was 305.0 (303.8–306.3) eggs.g$^{-1}$ (Table 4). Estimates of *F/W* for individual years ranged from 292.5 eggs.g$^{-1}$ in 2000 to 312.9 eggs.g$^{-1}$ 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 0.108 (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 0.41 in 2014 to 0.79 in 2018 (Appendix 1).

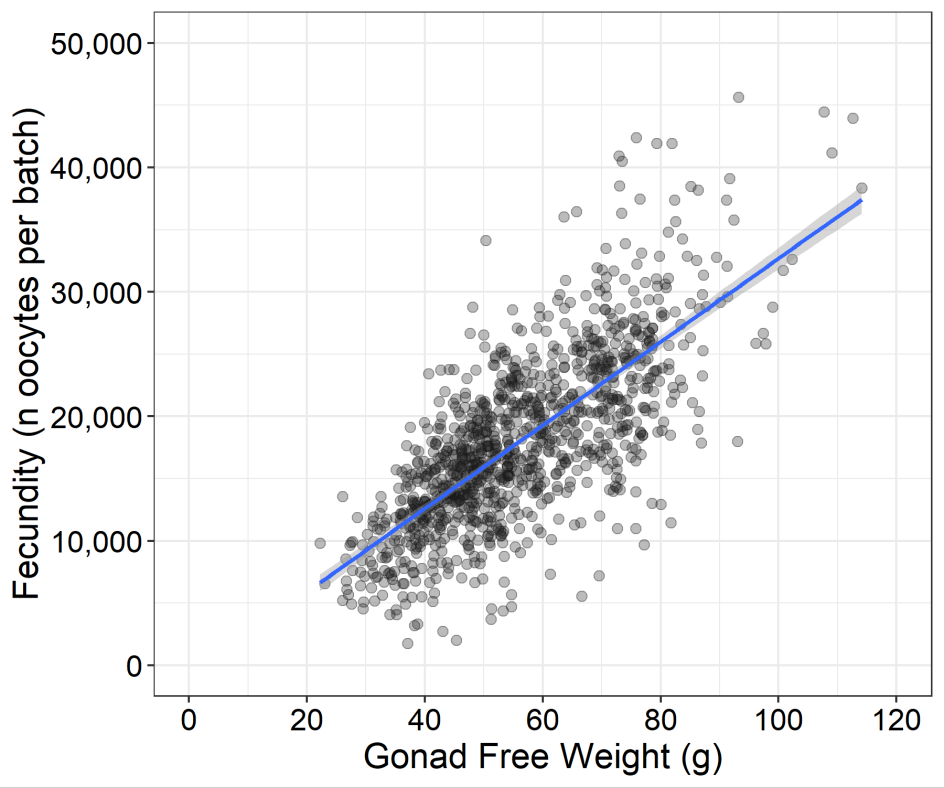


Figure 8. Relationship between gonad-free weight and batch fecundity (*F*) for all hydrated Sardine collected from 1998 to 2018 (blue shading = 95% CI). F = 335*Gonad Free Weight - 797, ($R^2$ = 0.53).

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

| Parameter | All Years | 95% CI | CV | Range (among years) |
|---|---|---|---|---|
| Egg Production ($P_o$, eggs.day$^{-1}$.m$^{-2}$) | 82.6 | 74.2–91.7 | 0.05 | 39.0–145.3 |
| Sex Ratio (R) | 0.55 | 0.52–0.58 | 0.03 | 0.36–0.70 |
| Fecundity (F, eggs.female$^{-1}$) | 17,816 | 3,819–31,813 | 0.40 | 14,107–23,601 |
| Spawning Fraction (S) | 0.108 | 0.100–0.123 | 0.05 | 0.041–0.179 |
| Female Weight (W, g) | 58.4 | 23.1–93.7 | 0.31 | 46.5–78.7 |
| Relative Fecundity (F' eggs.g$^{-1}$) | 305.0 | 303.8–306.3 | 0.00 | 292.5–312.9 |
| Spawning Area (A, km$^2$) | - | - | - | 15,637–82,627 |

## 2.6. Spawning biomass

The estimate of spawning biomass (95% CI) calculated using the estimate of *A* obtained from the survey conducted in 2020, and the all-years estimates of $P_0$ (log-linear model), *S, R,* and *F/W* was 378,923 (318,777–439,068) t (Fig. 9). The estimate calculated using the value of *A* without the stations added in the 2020 survey was 347,056 t.

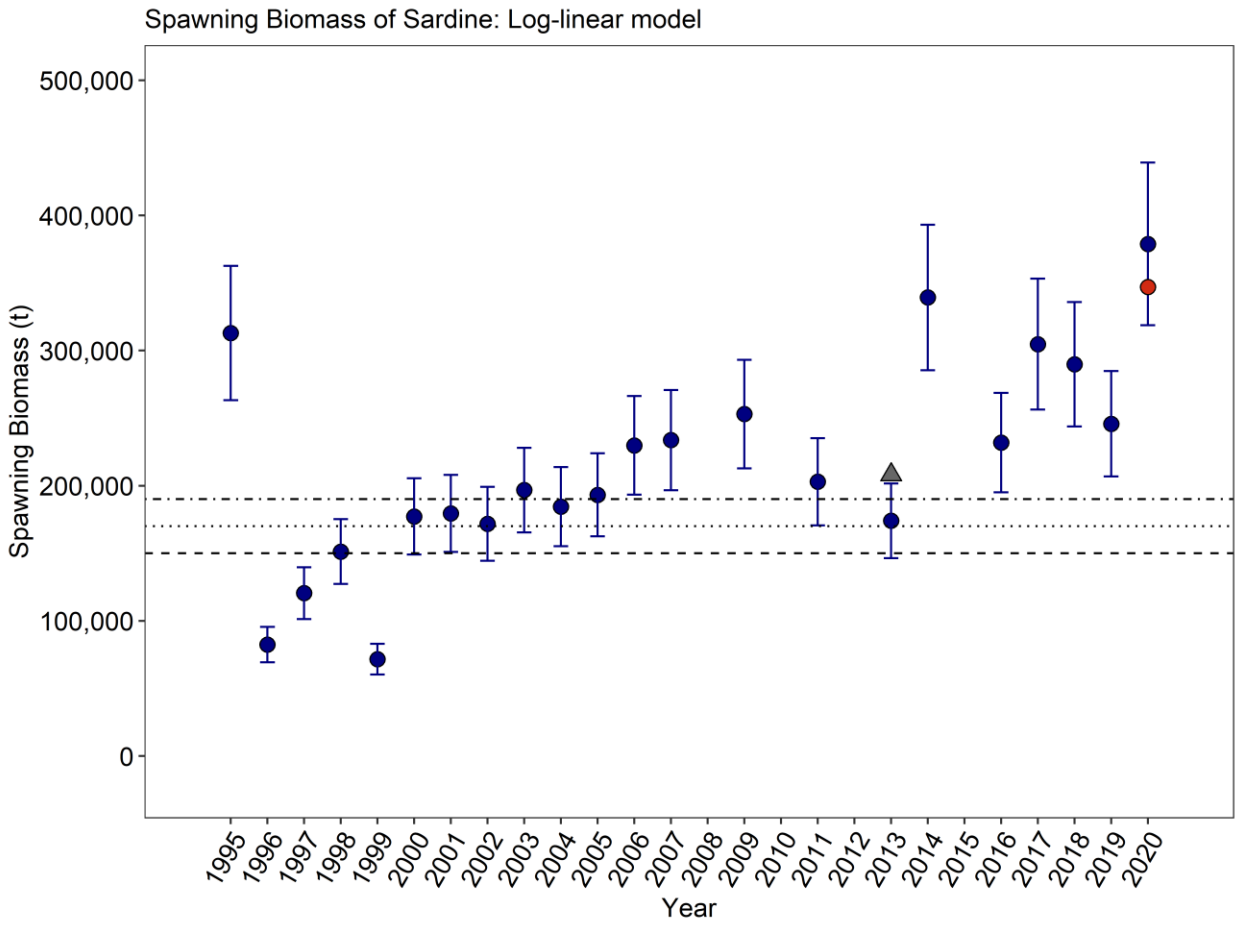


Figure 9. Estimates of spawning biomass (95% CI) for Sardine in South Australian waters from 1995 to 2020 using the log-linear egg production model and all-years data for all parameters, except for spawning area (*A*). The red circle for 2020 is the estimate of spawning biomass obtained using estimate of *A* without the additional stations added in 2020. 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$^2$). 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).

## 2.7. Sensitivity analysis

The sensitivity analysis shows the effects of inter-annual variability in parameters (i.e. $P_0$, *R, S, F, W* and *F'*) on the estimate of spawning biomass for 2020 (Table 4, Fig. 10, Appendix 1).

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. 178,000 to 666,000 t, Fig. 10). The estimate of spawning biomass obtained using the $P_0$ estimated from 2020 survey was higher 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 2019 (Fig. 10, Table 4).

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 2020 (i.e. 295,000 to 583,000). 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 is *S* had the strong influence of all of the parameters on the estimate of spawning biomass (i.e. 228,000 to 985,000 t). The sensitivity analysis demonstrated the benefit of using all available data to estimate *S*.

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* ~ 301,000 to 510,000 t; *F* ~ 286,000 to 479,000 t). However, *F'* was similar among years and inter-annual variation in this combined parameter had a much smaller effect on spawning biomass (i.e. 363,000 to 395,000 t, Fig. 10).

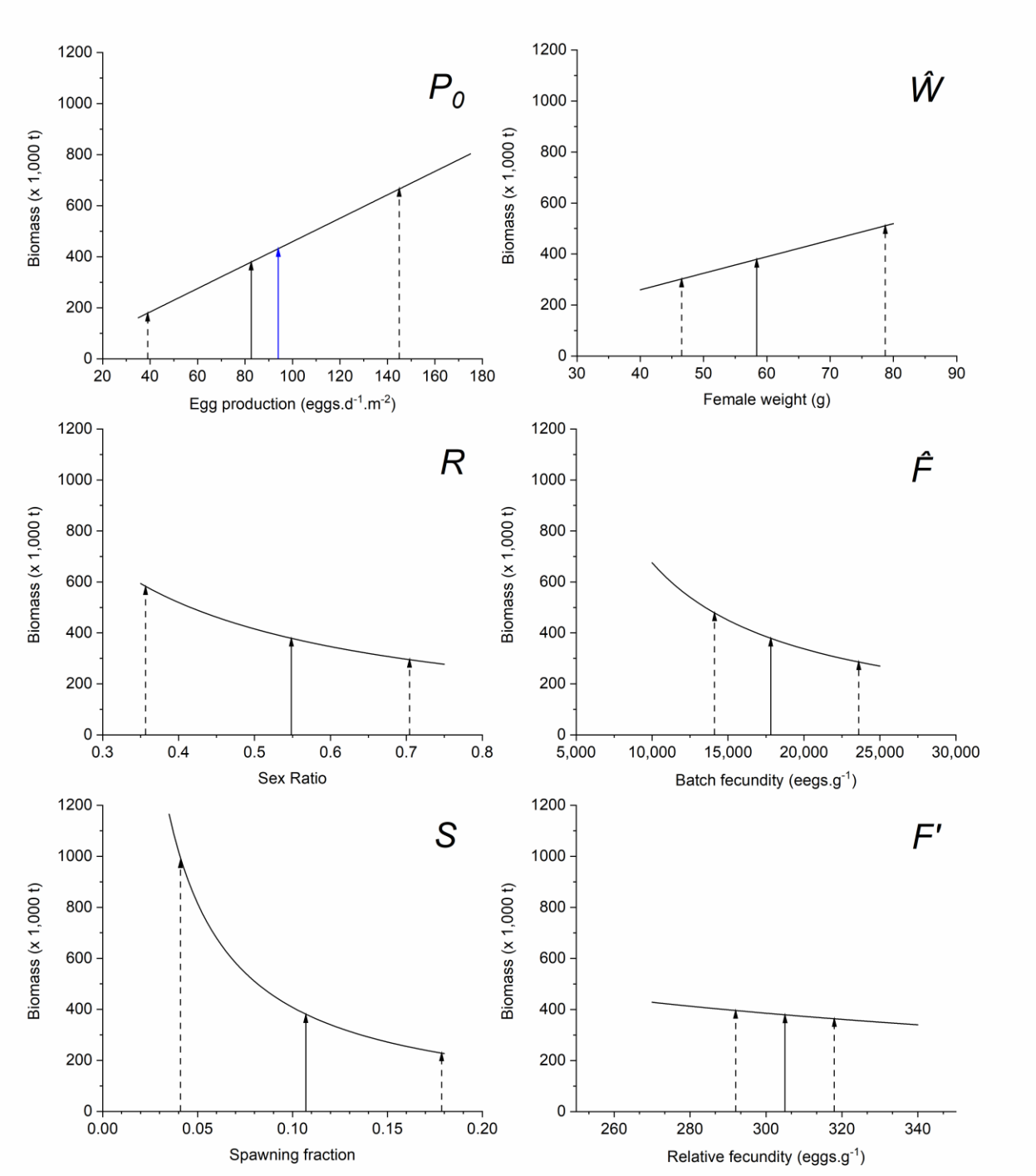


Figure 10. Sensitivity plots showing effects of variability in adult parameters and egg production on estimates of spawning biomass. Solid black arrows: parameter estimates for all years combined; Dashed arrows: range of values recorded between 1998 and 2018; Blue arrow: $P_0$ estimate using only data collected in 2020.

# 3. DISCUSSION

## 3.1. Egg distribution

The distribution of Sardine eggs off South Australia in 2020 was broadly similar to previous years. However, in 2020 Sardine eggs occurred in almost all of the areas where large numbers eggs have been collected historically (Ward et al 2019, 2020). In contrast, in most previous years there were some areas where few eggs were collected, such as off southern Eyre Peninsula in 2019 and the central GAB in 2018 (Ward et al 2019, 2020). In 2020, 40 additional sites were sampled to the south-east of the traditional survey area. This additional sampling was undertaken in response to recent observations that Sardine eggs had become more common south of Kangaroo Island (e.g. Ward et al. 2019) and that commercial catches were increasing in the area south-east of Kangaroo Island (Paul Watson, pers. comm). The widespread occurrence of Sardine eggs in the new sampling sites provided evidence that the expansion of the survey area was warranted and should be maintained in future years.

## 3.2. Spawning area and mean daily egg production

The spawning area in 2020 was estimated to be 82,627 $km^2$, which is the largest on record. Excluding the additional sites that were sampled in 2020 reduced the spawning area to 75,678 $km^2$, which is still larger than previous largest area of 73,981 $km^2$ recorded 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 evidence that Sardines were widespread and abundant off South Australia in 2020.

Recent studies (e.g. Ward et al. 2019, Ward et al. 2020) have shown that for Sardine off South Australia inter-annual variability in estimates of $P_0$ is low compared to statistical uncertainty. In the present study, we addressed this issue by estimating $P_0$ from data obtained from all years between 1998 and 2020. The estimate of $P_0$ obtained using this approach was more precise (SD = 4.5) than the estimate obtained using data from 2020 only (SD = 18.4). This approach prevents 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.

### 3.3. Adult parameters

Evidence compiled in this report and elsewhere (e.g. Ward et al. 2019, 2020) suggest that 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 (e.g. Ward et al. 2019, 2020) suggest that individual adult reproductive 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 obtained in a given year, with values of *R* for individual years ranging between 0.36 (male dominated) in 2009 and 0.70 (female dominated) 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 of 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 (e.g. Ward et al. 2020). 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). Samples obtained in years when estimates of *R* were low (e.g. 0.36) 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. 2014). This dominance of males and females in samples has previously been interpreted to be the result of differential sampling of spawning and non-spawning schools, respectively (Ganias et al. 2014). 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. 2014). Like *R*, the all-years values of *S* is likely to be a better approximation of spawning fraction in any individual year than the estimate obtained using data collected in that year alone.

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 the high levels of variability in *F* and *W* among years, the estimates of *F'* obtained in individual years are remarkably similar (i.e. range 293–313 eggs.g$^{-1}$). This low variability among years in *F'* means that this combined parameter has minimal influence on estimates of spawning biomass. For this reason, there is limited benefit in estimating *F* and *W* annually. *F'* rather than *F* and *W* estimated separately should be used to calculate spawning biomass as this approach improves precision. Like *R* and *S*, the all-years values of *F'* is likely to be a better approximation of this parameter than estimates obtained using data collected in that year alone.

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.

### 3.4. Spawning biomass

The estimate of spawning biomass for 2020 of 378,993 (318,777–439,064) 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 2019 (Ward et al. 2019), the stock assessment report for 2019 (Ward et al. 2020) 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 ($km^2$), *A*: spawning area ($km^2$); $P_0$: mean daily egg production ($egg \cdot m^{-2} \cdot day^{-1}$); *S*: spawning fraction; *R*: sex ratio; *W*: mean female weight (g); *F*: batch fecundity ($oocytes \cdot batch^{-1}$); *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* | $P_0$ | $P_0$ SD | N.$P_0$ | *S* | *S* SD | N.S | n.S | *R* | *R* SD | N.R | n.R | *W* | *W* SD | N.W | n.W | *F* | *F* SD | N.F | n.F | *F*/*W* | *F*/*W* SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **All Years** | - | - | 82.6 | 4.5 | 6051 | 0.108 | 0.006 | 247 | 16334 | 0.55 | 0.01 | 210 | 27931 | 58.4 | 18.0 | 255 | 16995 | 17816 | 7141 | 255 | 16995 | 305.1 | 0.6 |
| 1998 | 48379 | 32980 | 99.0 | 30.8 | 164 | 0.139 | 0.015 | 12 | 530 | - | - | - | - | 46.5 | 11.2 | 12 | 554 | 14107 | 5093 | 12 | 554 | 303.1 | 3.8 |
| 1999 | 65956 | 15637 | 50.0 | 14.9 | 213 | 0.169 | 0.021 | 15 | 763 | - | - | - | - | 52.4 | 13.0 | 16 | 785 | 15704 | 5592 | 16 | 785 | 299.9 | 2.9 |
| 2000 | 102198 | 38658 | 52.9 | 12.7 | 290 | 0.158 | 0.012 | 15 | 1012 | 0.52 | 0.05 | 15 | 2179 | 49.2 | 12.2 | 16 | 1071 | 14378 | 5420 | 16 | 1071 | 292.5 | 2.5 |
| 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 | 15743 | 5182 | 11 | 1002 | 310.3 | 2.6 |
| 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 | 18992 | 7598 | 22 | 1841 | 307.3 | 1.8 |
| 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 | 16087 | 5268 | 7 | 435 | 307.1 | 4.3 |
| 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 | 23602 | 6958 | 10 | 413 | 299.8 | 3.8 |
| 2005 | 122831 | 42142 | 59.5 | 14.3 | 334 | 0.100 | 0.019 | 32 | 2223 | 0.51 | 0.04 | 32 | 4827 | 73.9 | 16.0 | 33 | 2234 | 22415 | 7164 | 33 | 2234 | 303.4 | 1.6 |
| 2006 | 119038 | 50121 | 102.4 | 26.5 | 341 | 0.095 | 0.018 | 20 | 1332 | 0.59 | 0.05 | 20 | 2445 | 63.1 | 21.8 | 21 | 1337 | 19190 | 8165 | 21 | 1337 | 304.0 | 2.2 |
| 2007 | 119036 | 50972 | 104.9 | 27.1 | 341 | 0.130 | 0.019 | 20 | 1084 | 0.54 | 0.07 | 20 | 2244 | 71.1 | 16.8 | 21 | 1086 | 21514 | 7505 | 21 | 1086 | 302.8 | 2.3 |
| 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 | 18032 | 6350 | 19 | 1537 | 301.1 | 2.0 |
| 2011 | 119449 | 44245 | 51.5 | 15.4 | 340 | 0.044 | 0.006 | 14 | 1169 | 0.65 | 0.05 | 13 | 1798 | 46.8 | 12.3 | 15 | 1181 | 14510 | 5484 | 15 | 1181 | 310.2 | 2.5 |
| 2013 | 119297 | 37953 | 39.0 | 8.7 | 340 | 0.072 | 0.016 | 9 | 703 | 0.69 | 0.03 | 9 | 1089 | 51.3 | 12.3 | 9 | 723 | 15924 | 5567 | 9 | 723 | 310.7 | 3.0 |
| 2014 | 125249 | 73981 | 92.7 | 20.0 | 355 | 0.041 | 0.006 | 16 | 886 | 0.57 | 0.02 | 16 | 1574 | 47.9 | 13.9 | 16 | 886 | 14945 | 6145 | 16 | 886 | 312.1 | 3.0 |
| 2016 | 122598 | 50551 | 47.7 | 9.9 | 350 | 0.088 | 0.012 | 9 | 656 | 0.65 | 0.03 | 9 | 1088 | 49.7 | 17.3 | 9 | 681 | 15395 | 6444 | 9 | 681 | 309.5 | 3.2 |
| 2017 | 119661 | 66453 | 136.3 | 25.8 | 343 | 0.120 | 0.019 | 8 | 504 | 0.52 | 0.05 | 9 | 1042 | 59.6 | 12.3 | 9 | 511 | 18634 | 6528 | 9 | 511 | 312.9 | 3.8 |
| 2018 | 120043 | 63215 | 112.4 | 24.6 | 343 | 0.054 | 0.009 | 9 | 714 | 0.70 | 0.03 | 9 | 1026 | 46.5 | 7.2 | 9 | 718 | 14496 | 4661 | 9 | 718 | 311.6 | 3.3 |
| 2019 | 119369 | 53600 | 68.1 | 16.3 | 339 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2020 | 129700 | 82627 | 94.0 | 18.4 | 379 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |