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Spawning biomass of Sardine, *Sardinops sagax*, in waters off South Australia in 2023

Grammer, G. L. and Ivey, A. R.

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Report to PIRSA Fisheries and Aquaculture

Government of South Australia
Department of Primary Industries and Regions

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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The South Australian Research and Development Institute respects Aboriginal people as the state's first people and nations. We recognise Aboriginal people as traditional owners and occupants of South Australian land and waters. We pay our respects to Aboriginal cultures and to Elders past, present and emerging.

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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 2023. 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 stock of Australian Sardine.

An ichthyoplankton survey was conducted during February/March 2023. The survey area was expanded in 2023 to include 22 new sites south-east of Kangaroo Island and on the end of the transects in the central Great Australia Bight (GAB). This was in addition to the 40 sites added in 2020. The total survey area was 136,471 km^2. Live Sardine eggs were collected at 189 of 403 (46.9%) sites. The total spawning area (A) in 2023 was 66,248 km^2. Removing the additional sites added since 2020 for comparison to previous years, reduced the spawning area to 60,300 km^2.

Mean daily egg production (P_0, 95% CI) was 84.0 (75.9–93.0) eggs.day^{-1}.m^{-2}. P_0 was estimated using the linear version of the exponential mortality model and all data combined from 1998 to 2023.

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): 0.108 (0.100–0.119); and relative fecundity (F'): 305.4 (304.2–306.6) eggs.g^{-1}.

Sensitivity analyses showed the effects of inter-annual variability in parameters (i.e. P_0, R, S and F') on the estimate of spawning biomass for 2023 and demonstrated the benefits of using parameter estimates obtained from historical data to estimate spawning biomass.

The estimate of spawning biomass (95% CI) of Sardine for 2023 was 307,881 (260,468–412,113) t, which was above the target reference point of 200,000 t set in the harvest strategy for the South Australian Sardine Fishery. On this basis, the southern stock of Australian Sardine is classified as **Sustainable**. This classification is consistent with the findings of the spawning biomass report for 2022, the stock assessment report for 2023, and the most recent report in the Status of Australian Fish Stocks (2023).

Keywords: Sardine, Spawning Biomass, South Australia, Daily Egg Production Method.

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 more than 20 species of small to medium-sized pelagic fishes (e.g. Stratoudakis et al. 2006, Dimmlich et al. 2009, Neira et al. 2009, Grammer et al. 2022). 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 the product of mean sex ratio (by weight, R), mean spawning fraction (proportion of mature females spawning each day/night, S) and mean relative fecundity (number of eggs produced per gram of total female weight ($\hat{F}/W$, F'). Spawning biomass (SB) is calculated according to the equation:

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

The DEPM, as applied to Sardine off South Australia, underwent a comprehensive review in 2020 (Ward et al. 2021). This review reanalysed data collected between 1995 and 2019 for South Australian Sardine and identified several ways to increase the precision of estimates of spawning biomass: 1) increase the precision of total daily egg production (P_0*A) by using the estimate of P_0 obtained from all historical data rather than annual estimates of P_0; 2) continue to use the log-linear model to estimate P_0 for the southern stock of Australian Sardine; 3) increase the precision of mean daily fecundity ($R*S*F'$) by using the estimates of sex ratio (R), spawning fraction (S) and relative fecundity (F') obtained from all historical data rather than annual estimates; and 4) combine batch fecundity (F) and female weight (W) into a single parameter: relative fecundity ($F' = \hat{F}/W$). These recommendations were implemented in 2022 and continue for this report to ensure that estimates of spawning biomass are, and continue to be, as accurate and precise as possible.

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, 2021). The estimate of spawning biomass obtained using the DEPM is the key performance indicator for determining the status of the southern stock of Australian Sardine (PIRSA 2023). The objective of this report is to estimate the spawning biomass of Sardine in waters off South Australia in 2023. Annual estimates of P_0 and A were obtained from an ichthyoplankton survey conducted in 2023. Adult parameters were derived from all adult samples collected off South Australia between 1998 and 2018; an estimate of P_0 was also obtained from all historical data. 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 2023.

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 15–26 February and 10–19 March 2023 (Fig. 1). Plankton samples were collected from 403 sites along 40 transects between Kingston and the Head of Bight (Fig. 1). The 2023 survey included 40 sites that were added in 2020 and was further expanded southeast of the traditional survey area and along the outer end of transects in the GAB to include an additional 22 sites (Fig. 1). These sites were added in response to the expansion of the fishery into this region and recent observations that Sardine eggs had become more common in the area (Ward et al. 2020; Fig. 1).

An adaptive approach to egg sampling has been applied since 2014 to ensure that each survey covers as much of the spawning area as possible. Adaptive sampling was implemented in response to the incomplete coverage of the spawning area in 2013 (see Grammer et al. 2021). Under the protocol, extra samples are taken at sites located outside the area covered by the pre-determined sampling sites (Fig. 1). Decisions about whether (or not) to take extra samples are based on the presence/absence of eggs in samples taken using the Continuous Underway Fish Egg Sampler (CUFES) at sites located on the seaward end of transects. Sampling at additional sites continues until Sardine eggs are not present in the CUFES samples. In 2023, two extra sites were sampled on the seaward ends of two transects.

2.1.2. 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 available, 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 (µg.L^{-1}). Spatial plots of sea surface temperature (SST) and chlorophyll-*a* concentration were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

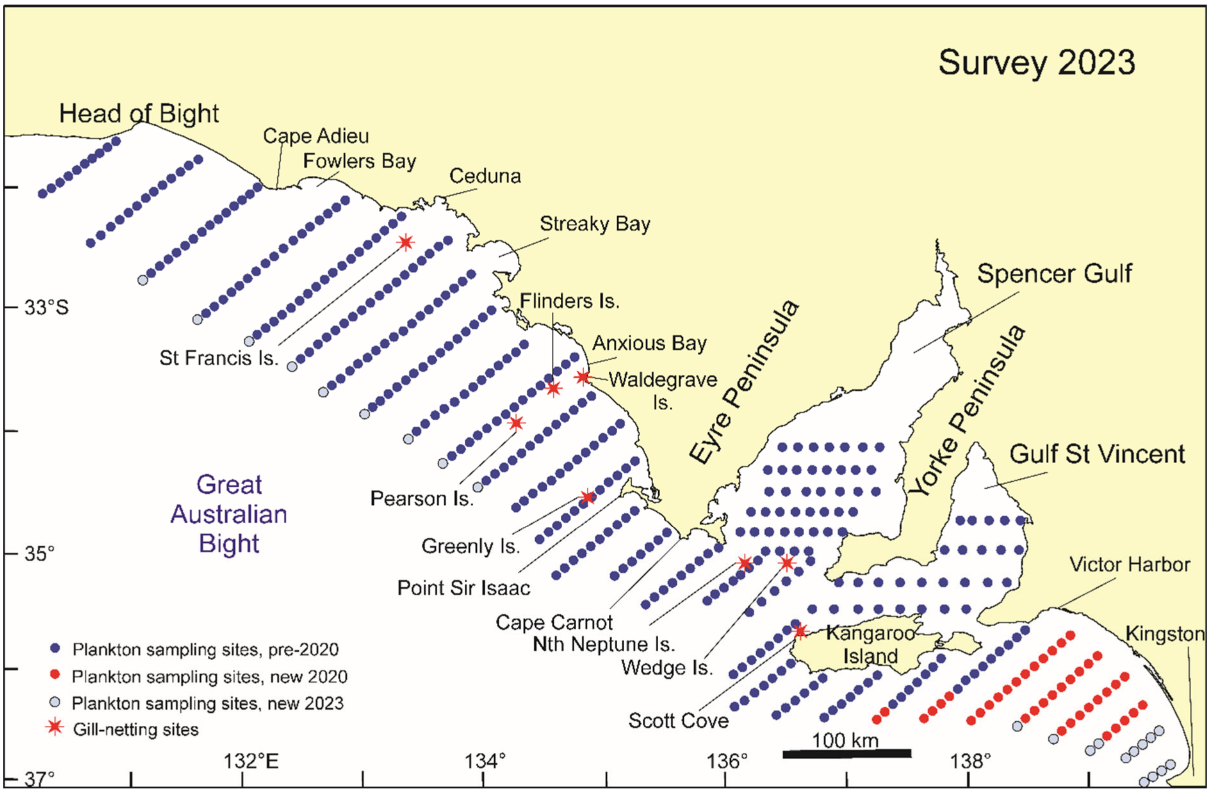

Figure 1. Map of South Australia showing sites where plankton samples were collected during 2023 and where adult samples were collected with gill-nets between 1998 and 2018. Red dots: new sites added to the survey in 2020, grey dots: new sites added in 2023.

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 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 the two cod-ends were rinsed with seawater, combined into a 1 L container, and fixed in 5% buffered formaldehyde and seawater 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 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 can be under- and over-represented in samples, respectively (Ward et al. 2018a).

2.2.3. Egg ageing and treatment of zero count egg samples

The development rate of Sardine eggs is dependent on ambient water temperature (Picquelle and Stauffer 1985, Pauly and Pullin 1988). Based on the temperature data from the CTD, egg samples were allocated to one of three temperature bins that covered the range of temperatures encountered during surveys (14–18°C, 18–22°C, and 22–26°C). The temperature bins were comparable to those used by Le Clus and Malan (1995) to describe the developmental rates of Sardine eggs. These published development rates were used to assign a mean age to each egg in each sample (Ward et al. 2018a).

After each egg was assigned an age, the eggs in each sample were grouped into daily cohorts. This was done because a sample usually included eggs spawned on more than one night. The total number of eggs in each daily cohort was calculated by summing the number of eggs of each stage assigned to a spawning day (i.e. day 0, day 1, day 2). The age of a daily cohort was calculated from the average age of each stage within the daily cohort, weighted by the number of eggs in each stage.

Samples with eggs could contain several possible combinations of daily cohorts depending on water temperature, spawning time (peak around 2:00 am) and sampling time. Zero counts were allocated for daily cohorts where the cohort was expected to be present but was not found within the sample (Ward et al. 2018a). Samples with no eggs were excluded from the analyses and not considered part of the spawning area.

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:

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

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

2.2.5. Spawning area (A)

The spawning area (*A*, Lasker 1985, Somarakis et al. 2004) was estimated using the Voronoi natural neighbour method (Watson 1981). The survey area was divided into a series of contiguous polygons approximately centered on each site using the 'deldir' package in the statistical program R (Fig. 2; R Core Team 2023, Turner 2023). The area represented by each site (km^2) was calculated. *A* was defined as the total area of the polygons where live Sardine eggs were present in the plankton sample (see Fletcher et al. 1996).

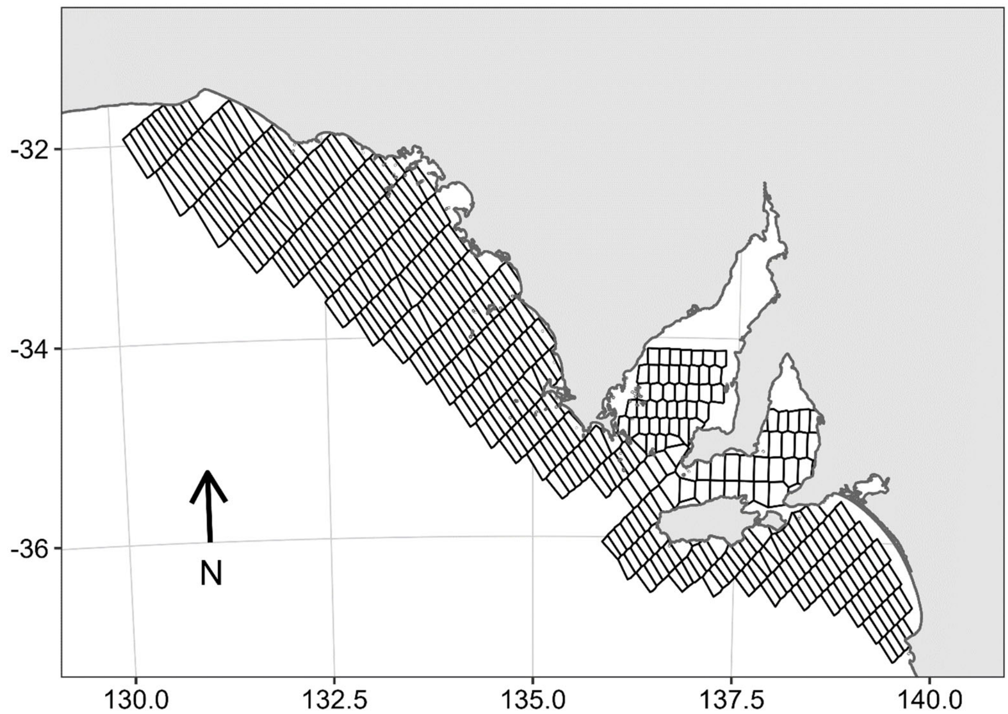

Figure 2. Voronoi nearest neighbour polygons used to estimate the total spawning area in 2023.

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:

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

where $P_{i,t}$ 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 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, σ^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) 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 ε is the error term. The negative binomial error structure used is considered suitable for over-dispersed data, such as egg density by age (e.g. Ward et al. 2011, 2018a, 2021). For NB1, variance increased linearly with the mean ($\sigma = \mu*(1+ \mu + \varphi)$), where μ is the model estimate, σ is the model variance and φ is the over-dispersion parameter. The GLM used a log-link function (Wood 2006) and was fit using the glmmTMB 'R' package (Brooks et al. 2017).

Following the recommendations of Ward et al. (2021), the value of P_0 from the log-linear model was used to estimate spawning biomass for Sardine. P_0 was calculated using data collected solely in 2023, as well as with data from all years (combined) between 1998 and 2023. 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. 2021).

2.3. Adult reproductive parameters

Adult parameters used to estimate spawning biomass were derived from all adult samples of Sardine collected for DEPM surveys off South Australia between 1998 and 2018 (see Ward et al. 2021)

2.3.1. Sampling methods

From 1998 to 2018, samples of mature Sardine were collected from sites located in the eastern Great Australian Bight, southern Spencer Gulf and Investigator Strait using a gillnet (Fig. 1). In the late afternoon, a dual frequency echo sounder (60 and 180 KHz) was used to search areas where schools of adult Sardine were known to aggregate. A gillnet comprised of three panels, each with a different multi-filament nylon mesh size (*Double Diamond*: 210/4 ply meshes 25, 28 and 32 mm) was deployed from the port side of the *RV Ngerin* at protected locations where schools were encountered. 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 Sardine collected were counted and sexed. Mature males and immature fish were frozen. Mature females were fixed in 10% buffered formaldehyde seawater solution.

2.3.2. Female weight (W) and Male weight

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:

$$W = \overline{\left[\overline{W}_i * \frac{n_i}{N} \right]}$$ 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.

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

2.3.3. 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:

$$R = \overline{\left[\overline{R}_i * \frac{n_i}{N} \right]}$$ 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:

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

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

2.3.4. 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:

$$S = \overline{\left[\overline{S_i} * \frac{n_i}{N} \right]}$$ 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:

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

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

2.3.5. Batch fecundity (F)

Batch fecundity (*F*) 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. Methods to estimate the batch fecundity for mature females without hydrated ovaries ($\hat{F}$) followed those outlined in Ward et al. (2021).

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*).

2.4. Spawning biomass

Spawning biomass was calculated using the all-years estimate of P_0 (1998 to 2023) obtained from the log-linear model, spawning area (*A*) in 2023 and estimates of *R*, *S* and *F'* obtained from adult samples collected between 1998 and 2018.

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* and *F'*, were calculated from the all-years adult data. A ratio estimator was used calculate the variance for *S, R,* and *F'* (Rice 1995). The variance for the spawning biomass estimates were calculated by summing the squared CVs for each parameter and multiplying by the square of the estimate of spawning biomass (Parker 1985). Uncertainty estimates presented for all parameters are 95% CIs. Data analyses were done in the R programming environment (R Core Team 2023).

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 year between 1998 and 2023 on the estimate of spawning biomass for 2023.

3. RESULTS

3.1. Distribution and abundance of eggs

A total of 6,378 live Sardine eggs were collected at 189 of 403 (46.9%) sites on 40 transects between the Kingston and Head of Bight between February and March 2023 (Fig. 3). Sites with the highest egg densities were in the mouth of Spencer Gulf and on the outer shelf in the eastern Great Australian Bight (GAB). Eggs were also widespread throughout the central GAB, Investigator Strait and southeast of Kangaroo Island.

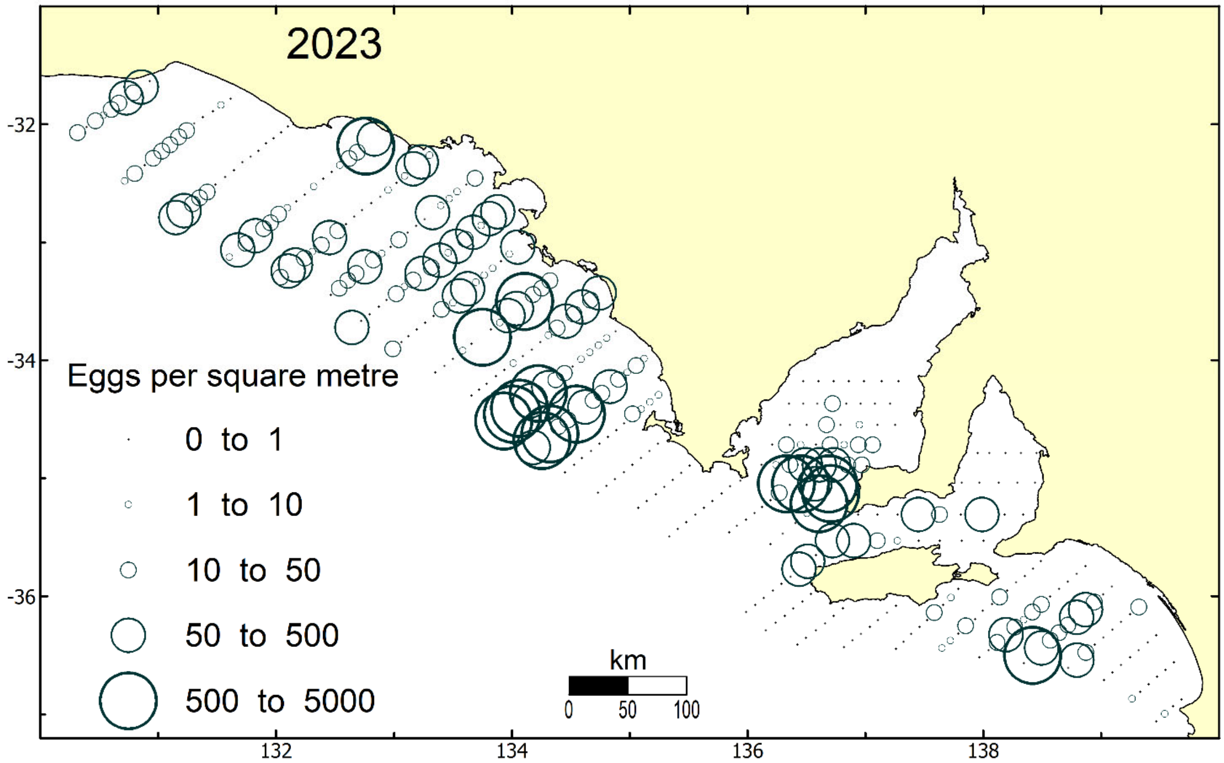

Figure 3. Densities of live Sardine eggs at sites sampled during February and March 2023.

3.2. Biophysical variables

3.2.1. Sea surface temperature

Sea surface temperatures (SSTs) ranged from 17.0 to 22.1˚C (Fig. 4) between February and March 2023. High SSTs (>20˚C) were recorded in Spencer Gulf, Gulf St. Vincent and throughout the central GAB. Cooler, upwelled water (<19˚C) was less common throughout the eastern GAB compared to previous years, although cooler water occurred southeast of Kangaroo Island and off the Bonney coast to the east of the survey area.

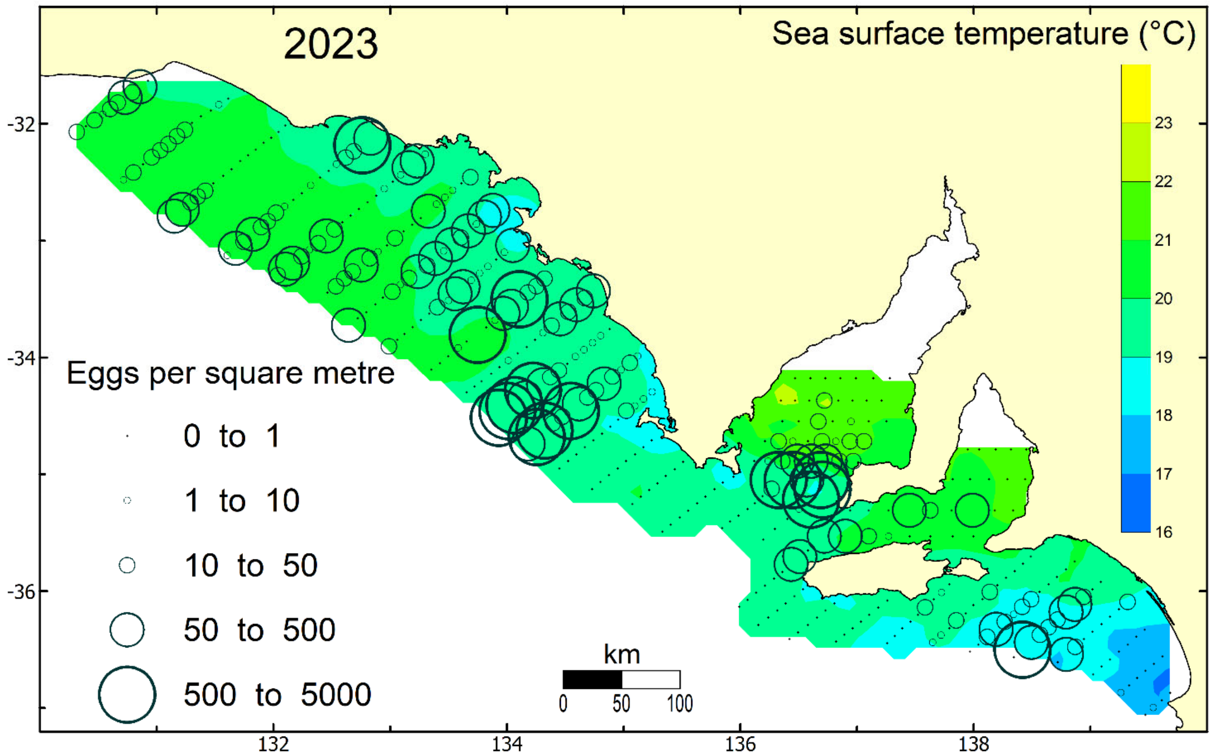

Figure 4. Sea surface temperatures overlaid with densities of live Sardine eggs at sites sampled during February and March 2023.

3.2.2. Fluorescence

Surface chlorophyll-*a* concentration at each site ranged between 0.3 and 6.2 µg.L^{-1} (Fig. 5) between February and March 2023. The highest values were recorded in the Gulfs, Investigator Strait, off Cape Adieu, the southern Eyre Peninsula and in the southeast of the survey. The remainder of coastal and shelf waters mainly had chlorophyll-*a* concentrations <1.0 µg.L^{-1}.

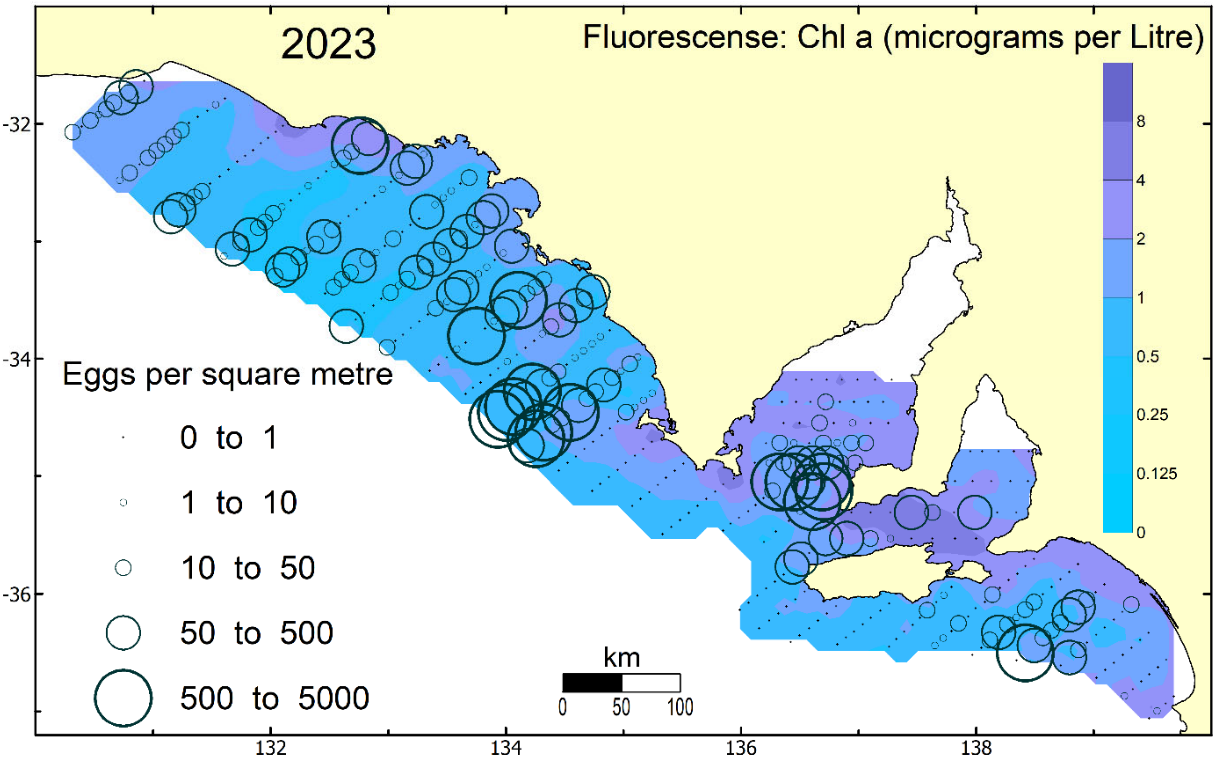

Figure 5. Surface concentration of chlorophyll-*a* overlaid with densities of live Sardine eggs at sites sampled during February and March 2023.

3.2.3. Zooplankton abundance

Total densities of zooplankton ranged between 0.43 and 95.5 ml.m^{-3} (Fig. 6) between February and March 2023. The highest densities occurred off Kingston in the southeast, south of Kangaroo Island, the mouth of Spencer Gulf in the Gulfs, and south of the Eyre Peninsula, and were mainly composed of salps (pelagic tunicates).

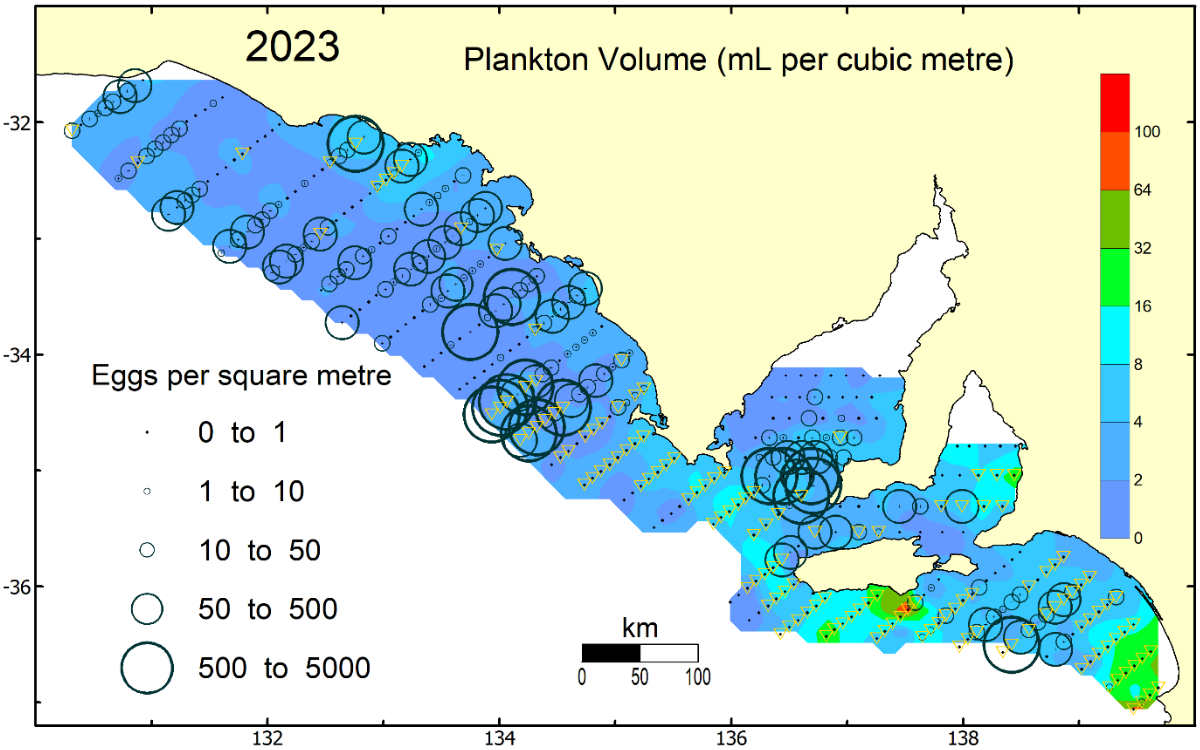

Figure 6. Distribution and abundance of zooplankton (ml.m^{-3}) overlaid with densities of live Sardine eggs at sites sampled during February and March 2023. Yellow triangles indicate the presence of salps in a sample.

3.2.4. Salinity

Surface salinity at each site ranged from 33.7 to 37.6 parts per thousand (Figure 7). Higher salinities were observed in the Gulfs and at the Head of the Bight. The influence of freshwater outflow from floodwaters from the mouth of the Murray River is evident in the southeast of the survey.

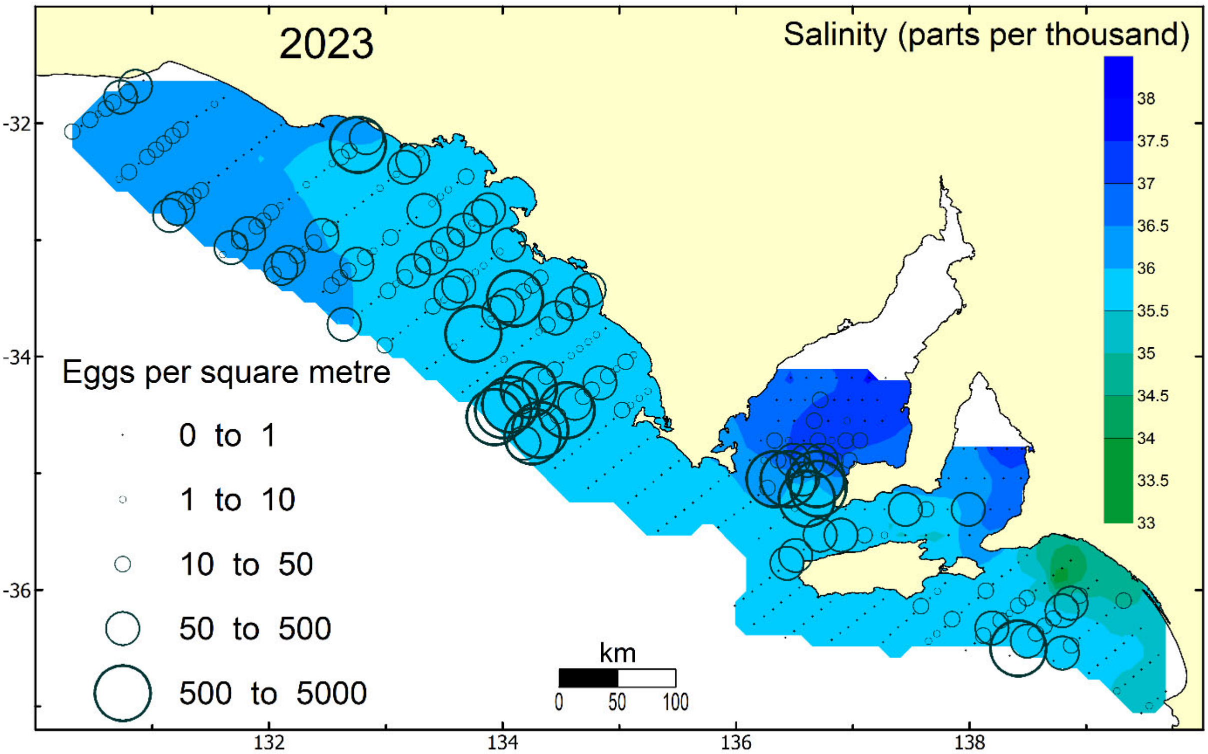

Figure 7. Surface salinity overlaid with densities of live Sardine eggs at sites sampled during February and March 2023.

3.3. Spawning area

The estimated spawning area was 66,248 km^2 and comprised 48.5% of the total area sampled (136,471 km^2, Table 1, Fig. 3). If the additional stations added to the main survey since 2020 were not included, the spawning area would have been 60,300 km^2 (Figs. 1 and 8, Table 1).

Table 1. Total area surveyed and spawning area (*A)* estimated in 2023.

	Area sampled (km^2)	Spawning area, *A* (km^2)	Spawning area percentage
Pre-2020 survey	121,531	60,300	49.6
With new stations	136,471	66,248	48.5

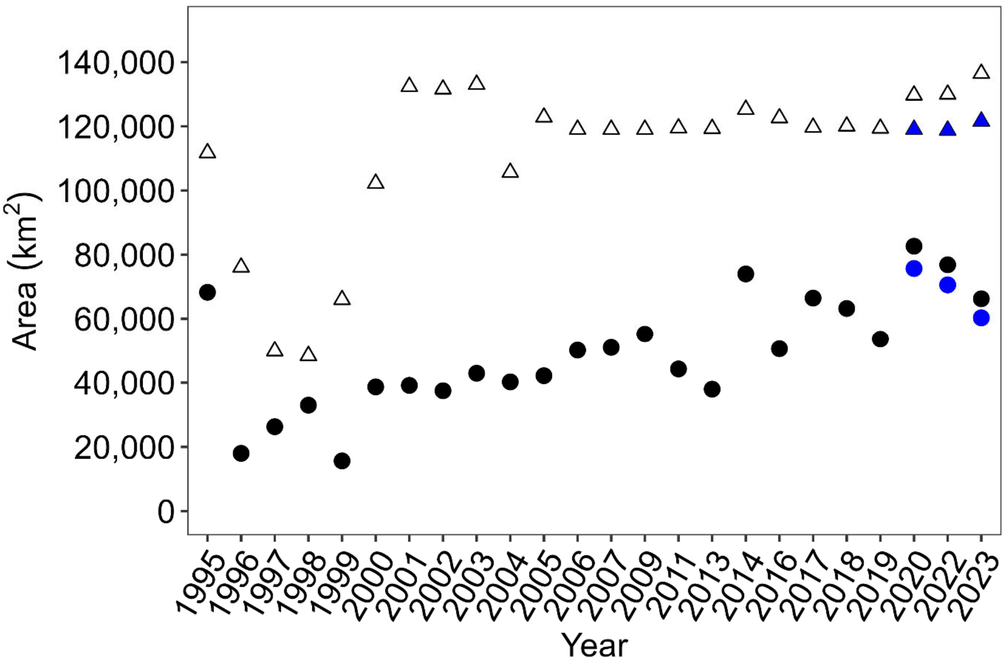

Figure 8. Sampling area (open triangles) and spawning area (closed circles) over the history of DEPM surveys in South Australia. For comparison: blue points for 2020, 2022 and 2023 exclude additional sites added since 2020.

3.4. Mean daily egg production (P_0)

The estimate of P_0 (95% CI) obtained by fitting the log-linear model (Eq. 3) to all data from 1998 to 2023 was 84.0 (75.9–93.0) eggs.day^{-1}.m^{-2} (Table 2, Fig. 9). The estimate of P_0 obtained by fitting the GLM NB1 (Eq. 5) to all data from 1998 to 2023 was 99.3 (84.9–116.9) eggs.day^{-1}.m^{-2} (Table 2, Fig. 9).

Table 2. Mean daily egg production (P_0) and instantaneous daily mortality (Z) estimated by fitting the log-linear model and GLM NB1 to all data collected from 1998 to 2023.

Model fit	P_0 (eggs·day^{-1}·m^{-2}) (95% CI)	Z
Log-linear model	84.0 (75.9–93.0)	0.52 (0.44–0.60)
GLM, Negative Binomial	99.3 (84.9–116.9)	0.35 (0.29–0.41)

The estimate of P_0 (95% CI) obtained by fitting the log-linear model (Eq. 3) to data solely obtained in 2023 was 93.8 (55.6–157.0) eggs.day^{-1}.m^{-2} (Fig. 8, Table 3). The estimate of P_0 obtained by fitting the GLM NB1 (Eq. 5) to data solely obtained in 2023 was 126.4 (72.1–202.4) eggs.day^{-1}.m^{-2} (Fig. 8, Table 3). Estimates of P_0 for individual years ranged from 39.0 in 2013 to 145.3 in 2004 (Appendix 1).

Table 3. Mean daily egg production (P_0) and instantaneous daily mortality (Z) estimated by fitting using the log-linear model and GLM NB1 to data solely collected in 2023.

Model fit	P_0 (eggs·day^{-1}·m^{-2}) (95% CI)	Z
Log-linear model	93.8 (55.6–157.0)	0.56 (0.14–1.00)
GLM, Negative Binomial	126.4 (72.1–202.4)	0.31 (0.05–0.59)

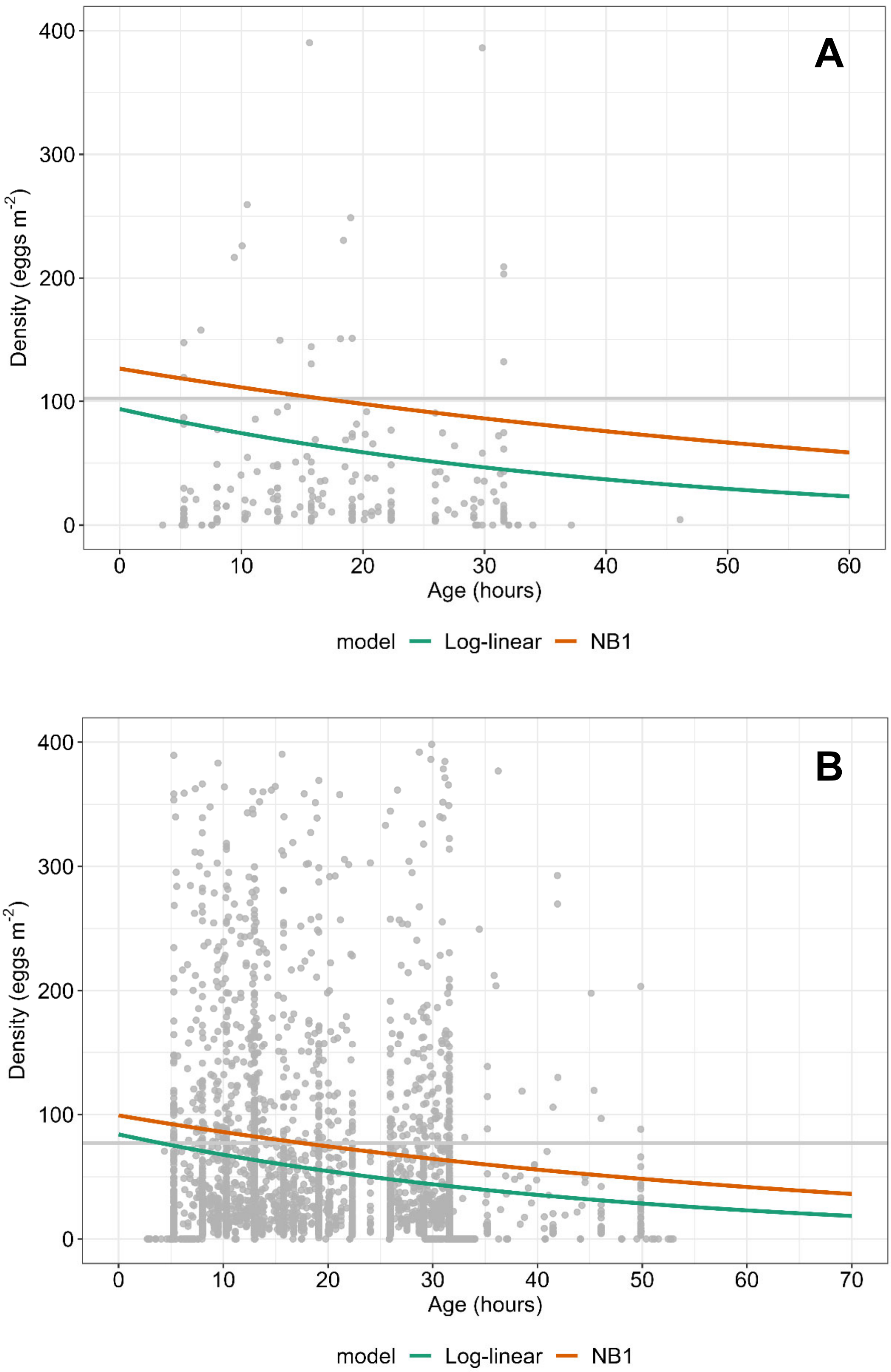

Figure 9. Models fitted to egg densities (eggs.m^{-2}) and egg age (hours) of Sardine cohorts in 2023 (**A**) and all years combined (1998 to 2023; **B**). Grey horizontal line: mean egg density.

3.5. Adult parameters

3.5.1. 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 g (23.1–93.7) (Ward et al. 2021). Estimates of *W* for individual years ranged between 46.5 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) (Ward et al. 2021, Table 4). 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, 1,099 females with hydrated oocytes were collected (Ward et al. 2021). The fecundity-weight relationship estimated from these samples was: Batch Fecundity = 335 × Gonad Free Female Weight – 797 ($R^2 = 0.53$) (Ward et al. 2021). Mean gonad free female weight between 1998 and 2018 was 55.5 g and ranged between 43.2 and 75.0 g (Appendix 1). Overall mean batch fecundity ($\hat{F}$, 95% CI) was 17,835 (3,790–31,880) oocytes (Appendix 1).

The overall estimate of *F’* was 305.4 (95% CI: 304.2–306.6) eggs.g^{-1} (Ward et al. 2021, Table 4). Estimates of *F’* for individual years ranged from 295.9 eggs.g^{-1} in 2000 to 312.9 eggs.g^{-1} in 2011 (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 (0.100–0.119) (Ward et al. 2021, Table 4). A total of 16,334 ovaries were examined; 2,578 had day-0 POFs or hydrated oocytes, 1,540 had day-1 POFs and 1,046 day-2 POFs (Ward et al. 2021). Estimates of *S* for individual years ranged from 0.041 in 2014 to 0.179 in 2001 (Appendix 1).

Table 4. Parameter estimates used in the calculations of spawning biomass. Estimates of adult parameters (*R, S, F'*) are calculated from all historical data from 1998 to 2018 (Ward et al. 2021).

Parameter	All Years	95% CI	CV	Range (among years)
Egg Production (P_0, eggs.day^{-1}.m^{-2})	84.1	75.9–93.0	0.051	39.0–145.3
Sex Ratio (*R*)	0.55	0.52–0.58	0.026	0.36–0.70
Spawning Fraction (*S*)	0.108	0.100–0.119	0.053	0.041–0.179
Relative Fecundity (*F'*, eggs.g^{-1})	305.4	304.2–306.6	0.002	295.9–312.9
Spawning Area (*A*, km^2)	-	-	-	15,637–82,627

3.6. Spawning biomass

The estimate of spawning biomass (95% CI) calculated using the estimate of *A* obtained from the survey conducted in 2023, and the all-years estimates of P_0 (log-linear model), *S, R,* and *F'* was 307,881 (260,468–412,113) t (Fig. 10). The estimate calculated using the value of *A* without the additional stations added since 2020 was 280,239 t.

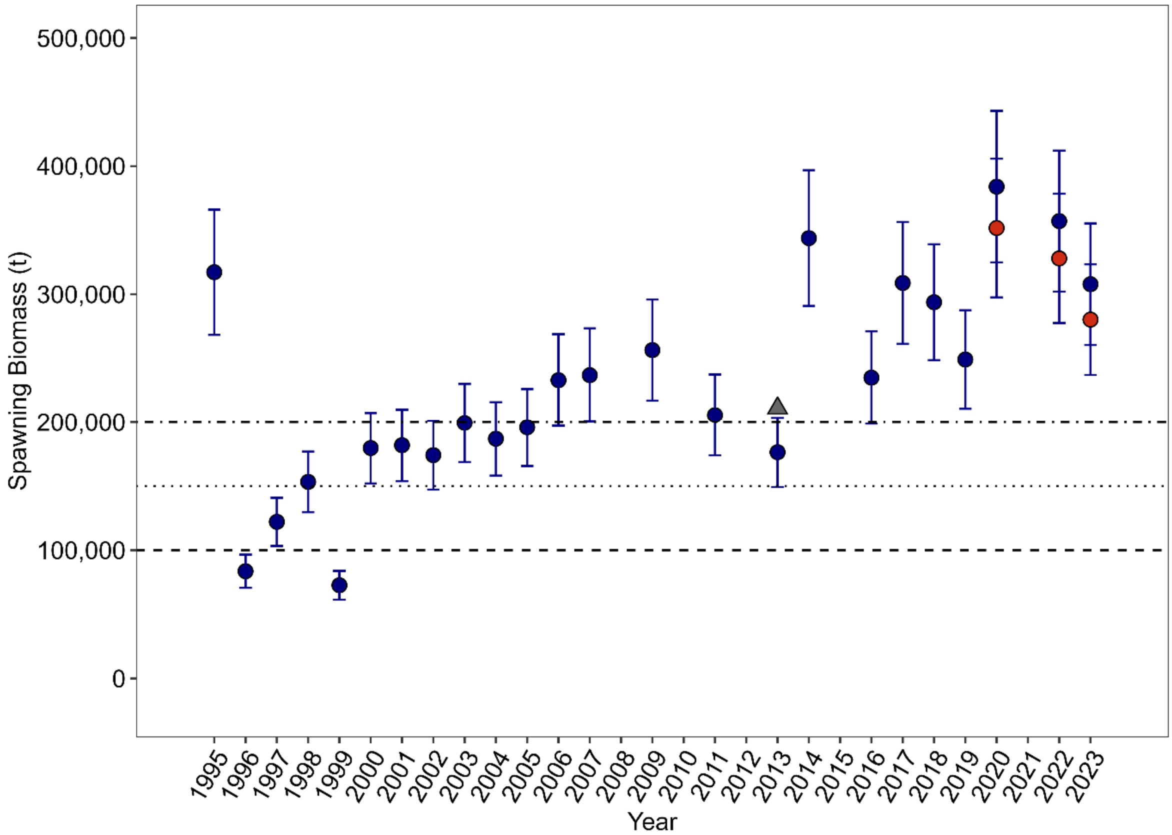

Figure 10. Estimates of spawning biomass (95% CI) for Sardine off South Australia from 1995 to 2023 using the log-linear egg production model and all-years data for all parameters, except for spawning area (*A*). The red circles for 2020, 2022 and 2023 are the estimate of spawning biomass obtained using estimate of *A* without the additional stations added since 2020. The 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 100,000 t (dash), 150,000 t (dotted) and 200,000 t (dash/dot) reference points in the harvest strategy (PIRSA 2023).

3.7. Sensitivity analysis

The sensitivity analysis shows the effects of inter-annual variability in parameters (i.e. P_0, *R, S* and *F'*) on the estimate of spawning biomass for 2023 (Table 4, Fig. 11, Appendix 1).

The high level of inter-annual variability in the estimates of P_0 reflects the high level of statistical uncertainty associated with annual estimates of this parameter (Fig. 11, Appendix 1) (see Ward et al. 2021). This range of variability had a strong influence on the estimate of spawning biomass (i.e. 143,000 to 532,000 t; Fig. 11). The estimate of spawning biomass obtained using the P_0 estimated from 2023 survey was higher than the estimate obtained using all-years data. Both estimates of P_0 were within the range of values obtained in individual years between 1998 and 2022 (Fig. 11, Table 4).

The estimates of *R* obtained in individual years were variable (Table 4; Appendix 1). The extreme values (i.e. 0.36 and 0.70) are likely due to the limitations of the adult sampling program rather than the relative abundance of sexes in the population (see Ward et al. 2021). The variations in *R* had a large influence on the estimate of spawning biomass for 2023 (i.e. 240,000 to 474,000), which 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). 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 (see Ward et al. 2021). Inter-annual variability in *S* had the strongest influence of all the parameters on the estimate of spawning biomass (i.e. 185,000 to 803,000 t), which demonstrates the benefits of using all-years data to estimate this parameter.

The estimate of relative fecundity (*F'*) was similar among years and inter-annual variation in this combined parameter had a much smaller effect on spawning biomass (i.e. 300,000 to 317,000 t, Fig. 11). Inter-annual variations in *W* and *F* between 1998 and 2018 were large and have a strong influence on the estimate of spawning biomass (Appendix 1; see Ward et al. 2021). Estimating *F'* as a single parameter, as done in the original formulation of the DEPM (Parker, 1980), from all historical data greatly increases the precision of estimates of spawning biomass for Sardine (Ward et al. 2021).

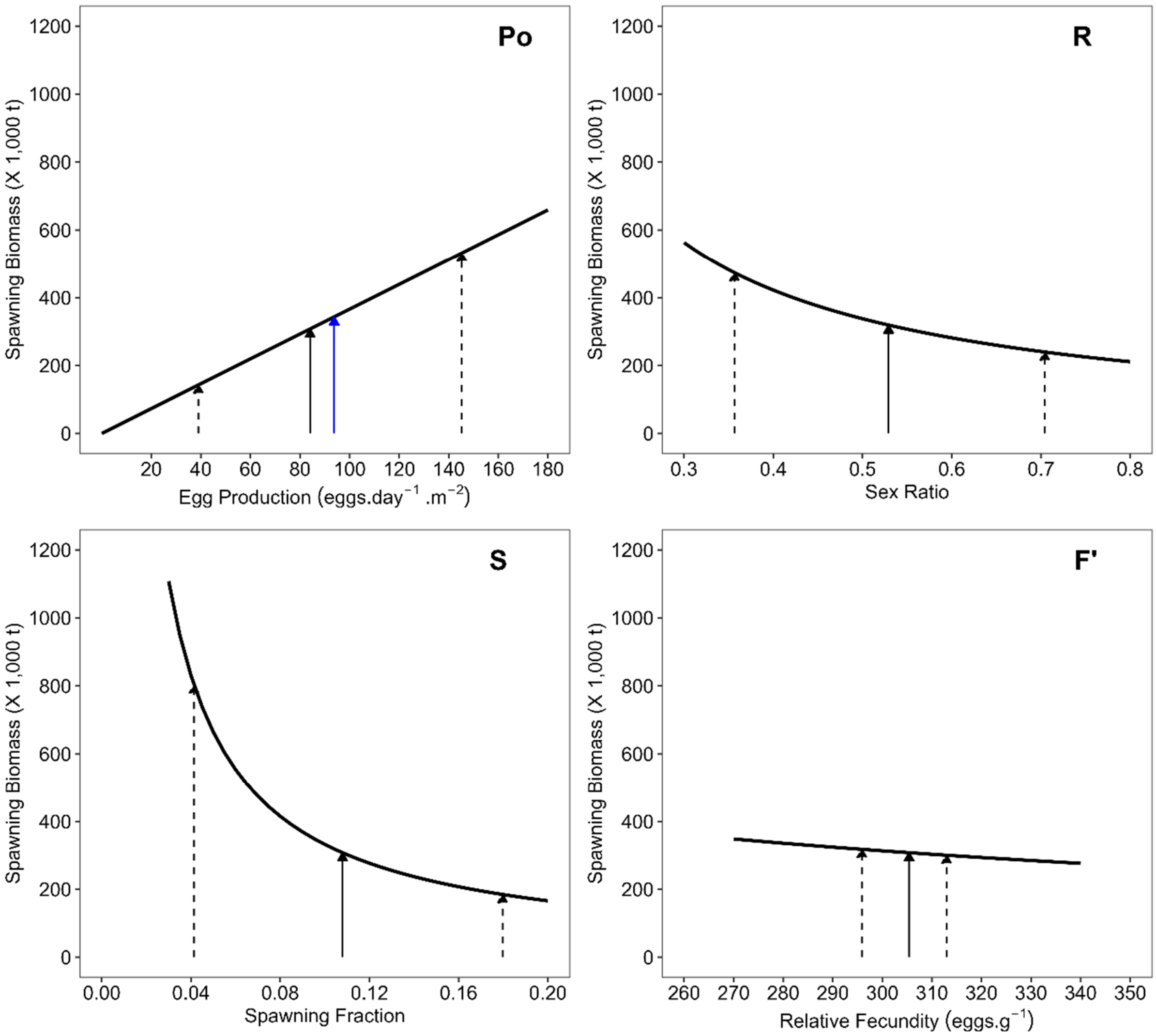

Figure 11. Sensitivity plots showing effects of variability in adult parameters and egg production on estimates of spawning biomass. Solid black arrows: parameter estimates for all years combined; Dashed arrows: range of values recorded between 1998 and 2018; Blue arrow: P_0 estimate using only data collected in 2023.

4. DISCUSSION

4.1. Egg distribution and environmental factors

The overall distribution of Sardine eggs off South Australia in 2023 was similar to previous years, with eggs occurring throughout the GAB, southern Spencer Gulf, Investigator Strait and south-east of Kangaroo Island (e.g. Grammer et al. 2021, Grammer and Ivey 2022). The egg distribution was more sparsely distributed over the continental shelf of the eastern GAB in 2023 than in 2020 and 2022, but similar to the distributions observed in 2018 and 2019 (Grammer et al. 2021, Grammer and Ivey 2022). In contrast to 2020 and 2022, eggs were absent in a large area south of the Eyre Peninsula and Kangaroo Island. This pattern has been observed in previous years (e.g. Ward et al. 2005, 2016, 2017).

In 2023, warm sea-surface water (19–22°C) was widespread over the continental shelf of the eastern GAB, which contrasts with 2020 and 2022, when the inner and mid-shelf waters were cooler (SST:16–19°C; Ward et al. 2020, Grammer and Ivey 2022). This pattern of warmer water across the GAB shelf has also been observed in other years (e.g. 2005, 2013, 2018c, 2019) with sparse egg distributions over this region (Ward et al. 2013, 2018c, 2019). In the Kangaroo Island region, seasonal upwelling (Jan-Mar) was lower and seasonal SST higher in 2013, 2018 and 2019 compared to adjacent years (Shute et al. 2022). The signal of seasonal upwelling off Kangaroo Island in 2023 appeared weak, with bottom temperatures (Jan-Mar) recorded at the IMOS National Reference Station (NRSKAI) generally warmer compared to 2020 and 2022 (per. comm., M. Doubell, SARDI).

The absence of eggs south of the Eyre Peninsula and Kangaroo Island may be correlated with high densities of zooplankton. In 2023, high densities of zooplankton were evident in this region during the survey, and many samples were mainly composed of salps. Salps are pelagic tunicates that can form intermittent swarms and rapidly become the most dominant zooplankton in an area (Henschke 2014). The lack of sardine eggs in this area could be because: 1) Sardine may avoid areas of salp swarms as the mucus nets they produce may clog fishes' gills (Kashkina, 1986); 2) salps are highly efficient, non-selective suspension feeders consuming food from <1 µm to 1 mm in size (Vargas and Madin 2004, Gibbons and Richardson 2013) and therefore may inadvertently ingest fish eggs present in the area; or 3) grazing pressure created by salp swarms can quickly remove the daily primary production (Dubischar and Bathmann 1997), stripping the area of food for other planktivorous, such as Sardine. Similar patterns of egg absence south of the Eyre Peninsula and Kangaroo Island have been seen in other years along with high densities of zooplankton (e.g. Ward et al. 2005, 2016).

The abundance of Sardine eggs in the southeast portion of the survey was lower compared to 2022 (see Grammer and Ivey 2022). There were both high densities of zooplankton in this area (salps) and an area of low salinity east of the mouth of the River Murray due to freshwater inflow from the flood event in 2022/23 (DEW 2023). It is interesting to note that there was a strong spawning response from Australian Anchovy (*Engraulis australis*) observed in this area with some of the highest anchovy egg abundances recorded compared to previous years (see Appendix 2).

Since 2020, additional sites in the southeast have been added to the traditional survey area. This additional sampling was undertaken in response to observations that Sardine eggs had become more common south of Kangaroo Island (e.g. Ward et al. 2018b, 2019) and that commercial catches were increasing in the area south-east of Kangaroo Island (Grammer et al. 2021). Eleven additional sites were added in 2023 to cover the entire southeast region to Kingston. The continued occurrence of Sardine eggs in the southeast provides evidence that the expansion of the survey area is warranted and should be maintained in future years.

4.2. Spawning area and mean daily egg production

The spawning area in 2023 was estimated to be 66,248 km^2, which was lower than the spawning areas in 2020 of 82,627 km^2 and in 2022 of 76,842 km^2 (Ward et al. 2020, Grammer and Ivey 2022). However, these two years were the highest estimates of spawning area recorded since DEPM surveys began being conducted in South Australia in 1995. The estimate for 2023 remains relatively high (6th highest *A*) in the time series available for South Australian Sardine, although it is important to note that the survey area was expanded by 22 sites in 2023. Excluding the additional sites added since 2020 results in a spawning area of 60,300 km^2, which is the 7th highest on record for South Australian Sardine.

Spawning area is strongly correlated with Sardine abundance (Mangel and Smith 1990, Gaughan et al. 2004), and Ward et al. (2021) confirmed that spawning area is a good proxy for the abundance of adult Sardine off South Australia. Warmer SSTs over the survey area, the presence of high densities of zooplankton (salps) south of Kangaroo Island and the Eyre Peninsula, and the freshwater influence of the recent River Murray flood event provide some explanation for the reduction of spawning area in 2023 compared to the two previous estimates from 2020 and 2022. The spawning area observed in this study provides evidence that Sardine were widespread and abundant off South Australia in 2023.

Using historical data to estimate all DEPM parameters except spawning area means that fluctuations in estimates of spawning biomass are driven almost entirely by changes in the measure of spawning area. Adaptive sampling to ensure plankton samples are collected from the entire spawning area was implemented after the marine heat wave in 2013 (Roberts et al. 2019), when Sardine appeared to move further offshore past the outer extent of the survey area (Ward et al. 2013, 2014). Adaptive sampling has been regularly applied since 2014 and was continued in 2023. Future surveys must cover as much of the spawning area as possible and should continue to involve the adaptive approach to sampling.

Inter-annual variability in estimates of P_0 is low compared to statistical uncertainty (imprecision) for Sardine off South Australia (Ward et al. 2021). The estimate of P_0 obtained by adding the 2023 data to all historical data was more precise (SD = 4.3) than the estimate obtained using data from 2023 only (SD = 26.1). Using historical data to estimate of P_0 prevents large inter-annual fluctuations in estimates of spawning biomass driven by variations in the annual estimates of this parameter caused by statistical uncertainty. However, it is critical that P_0 continue to be monitored at a yearly timescale to look for changes in the parameter over time to reduce the risk of over- or under-estimating spawning biomass (Ward et al. 2021). In future applications of the DEPM to Sardine off South Australia, P_0 should continue to be estimated using data obtained in all years since 1998, as well as within each year.

4.3. Adult parameters

Re-analysis of adult samples of Sardine 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) (Ward et al. 2021). Large variations among years observed in the estimates of the adult parameters 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 (Ward et al. 2021). Therefore, adult parameters used to calculate the spawning biomass of Sardine off South Australia were estimated from data obtained in adult surveys conducted between 1998 and 2018.

While no adult parameter was correlated with spawning area (Ward et al. 2021)—correlation would suggest density dependent changes had occurred—detecting changes in the adult population over time due to environmental conditions or density dependent effects is needed. Adult sampling is costly and logistically challenging, yet necessary. In recent years, completing a successful ichthyoplankton survey that covers the entire spawning area has taken precedence over adult sampling. Detecting any changes in key adult parameters of Sardine, such as spawning fraction (*S*), is critical since errors when estimating these parameters have major implications for estimates of spawning biomass (Ward et al. 2021). To mitigate this risk, an extensive adult sampling program is planned to occur alongside the DEPM in 2024 to determine if changes in key adult parameters have occurred. The adult sampling program should be repeated periodically (e.g. every 3-5 years) to continue to monitor for changes.

4.4. Spawning biomass

The estimate of spawning biomass for 2023 of 307,881 (260,468–412,113) t is above the target reference point of 200,000 t in the harvest strategy for the South Australian Sardine Fishery (PIRSA 2023). On this basis, the southern stock of Australian Sardine is classified as **Sustainable**. This classification is consistent with recent assessments provided in the spawning biomass report for 2022 (Grammer et al. 2022), the stock assessment report for 2023 (Grammer et al. in prep) and the most recent report on the Status of Australian Fish Stocks (SAFS 2023).

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APPENDIX 1: ANNUAL AND ALL-YEARS PARAMETERS FOR ESTIMATES OF SPAWNING BIOMASS

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); $\hat{F}$: batch fecundity ($oocytes \cdot batch^{-1}$); *F'*: Fecundity / Female Weight. Errors around the estimates are standard deviation (SD). N: number of samples; n: number of individuals. *F'* was calculated using the all-years $\hat{F}$ relationship with *W* from that year. Data sources for table: Ward et al. (2021), Grammer and Ivey (2022).

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	$\hat{F}$	$\hat{F}$ SD	N. $\hat{F}$	n. $\hat{F}$	*F'*	*F'* SD
All Years	-	-	84.1	4.3	6835	0.108	0.006	247	16334	0.55	0.01	210	27931	58.4	18.0	255	16995	17835	7166	255	16995	305.4	0.6
1998	48379	32980	99.0	30.8	164	0.139	0.015	12	530	-	-	-	-	46.5	11.2	12	554	14070	5295	12	554	302.3	3.8
1999	65956	15637	50.0	14.9	213	0.169	0.021	15	763	-	-	-	-	52.4	13.0	16	785	15743	5616	16	785	300.7	3.0
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	14543	5416	16	1071	295.9	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	15614	5069	11	1002	307.7	2.7
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	19093	7664	22	1841	309.0	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	16179	4720	7	435	308.9	3.9
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	23995	7607	10	413	304.8	3.7
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	22426	7367	33	2234	303.5	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	19163	8194	21	1337	303.6	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	21723	7266	21	1086	305.7	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	17886	6183	19	1537	298.7	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	14640	5487	15	1181	312.9	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	15842	5761	9	723	309.1	3.2
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	14896	5983	16	886	311.1	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	15399	6774	9	681	309.6	3.3
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	18331	6250	9	511	307.8	3.7
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	14466	4430	9	718	311.0	3.0
2019	119369	53600	68.1	16.3	339	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2020	129700	82627	94.0	18.4	379	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2022	129982	76842	97.6	19.0	381																		
2023	136471	66248	93.8	26.1	403																		

APPENDIX 2: DISTRIBUTION AND ABUNDANCE OF AUSTRALIAN ANCHOVY EGGS FROM 2019 TO 2023

Densities of live Australian Anchovy eggs at sites sampled during the DEPM surveys for Sardine in February and March of 2019, 2020, 2022 and 2023 overlaid on sea surface temperatures for those years.