Spawning biomass of the eastern component of the south-eastern stock of Sardine (*Sardinops sagax*) in 2019

Tim M. Ward, Gretchen L. Grammer and Alex R. Ivey

February 2022

Ward, T.M. Grammer, G.L., Ivey, A.I., 2022. Spawning biomass of the eastern component of the south-eastern stock of Sardine (*Sardinops sagax*) in 2019. Report prepared for The Tasmanian Department of Primary Industries, Parks, Water and Environment by the Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, 28p.

Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49,

Hobart TAS 7001

Enquires should be directed to:

Dr Tim Ward

Institute for Marine and Antarctic Studies

University of Tasmania

Private Bag 49, Hobart, Tasmania 7001, Australia

timothy.ward@utas.edu.au

Ph. 0401 126 388

ACKNOWLEDGEMENTS

The information on the distribution and abundance of eggs of Sardine presented in this report were collected during a survey designed to apply the Daily Egg Production Method to Jack Mackerel (*Trachurus declivis*) that was funded by the Australian Fisheries Management Authority (AFMA). The South Australian Research and Development (SARDI), which led the AFMA project, is acknowledged for making these data available to IMAS and for supporting the involvement of Dr Gretchen Grammer and Mr Alex Ivey as co-authors in this study. The efforts of Dr Stephen Mayfield (SARDI) in facilitating the provision of these data are gratefully acknowledged. *Springer* is acknowledged for allowing us to adapt a figure from Izzo *et al.* (2017, *Reviews in Fish Biology and Fisheries*) to produce Figure 1. SARDI is acknowledged for providing permission to reproduce images from Ward et al. (2015) and Ward et al. (2018) that were used to produced Figure 2. Oxford University Press is acknowledged for permission to adapt figures from Ward et al. (2021, *ICES Journal of Marine Science*) to produce Figures 6 and 7. The report was reviewed by Dr Alyssa Marshell (IMAS) and approved for release by Professor Caleb Gardner (Director, Sustainable Marine Resources Collaborative Agreement, Fisheries and Aquaculture, IMAS).

TABLE OF CONTENTS

LIST OF FIGURES

HIGHLIGHTS

1. The spawning biomass of Sardine of the eastern component of the South-eastern stock in 2019 was estimated to be approximately 185,000 t.
2. A previous study that did not cover the entire spawning area suggested that the spawning biomass of the western component of this stock was at least 30,000 t.
3. The total spawning biomass of the South-eastern stock is likely to be greater than 200,000 t, and may be greater than 250,000 t.
4. Future applications of the DEPM to the South-eastern stock should cover all of the potential spawning area between southern New South Wales and the Victorian-South Australian border.
5. The South-eastern stock of Sardine has the capacity to sustain substantial fisheries.

NON-TECHNICAL SUMMARY

The South-eastern stock of Australian Sardine (*Sardinops sagax*, herein referred to as Sardine) occupies continental shelf waters off Tasmania, Victoria and southern New South Wales. In this study, the Daily Egg Production Method (DEPM) was used to estimate the spawning biomass of the eastern component of this stock, which extends from southern New South Wales to south-eastern Tasmania, and includes Bass Strait as far west as 146°30'E.

Using the DEPM, spawning biomass is estimated by dividing the mean number of 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).

Total daily egg production of Sardine was calculated from estimates of mean daily egg production and spawning area obtained from an ichthyoplankton survey conducted for Jack Mackerel in 2019. The Jack Mackerel survey covered continental shelf and inner continental slope waters between central New South Wales and south-eastern Tasmania, including Bass Strait as far west as 146°30'E. The survey coincided with the spawning season of the South-eastern stock of Sardine and covered the entire known spawning habitat of the eastern component of this stock.

Mean daily fecundity was calculated from estimates of sex ratio (i.e. 0.55, 95% CI: 0.52–0.58), spawning fraction (i.e. 0.11, 95% CI: 0.10–0.12) and relative fecundity (i.e. 305 eggs.g^{-1}, 95% CI: 304–306) obtained from samples of Sardine collected off South Australia between 1998 and 2018. Using data from adults collected off South Australia to estimate mean daily fecundity is justified because these parameters are stable for *S. sagax* and other species of Sardine worldwide. However, if a substantial fishery is established on the South-eastern stock, it is recommended that representative adult samples are collected from across the entire stock to assess the validity of this assertion.

The estimates of mean daily egg production obtained in this study using the log-linear version of the exponential mortality model and Generalized Linear Model (GLM) with a negative binomial distribution (i.e. 95 and 82 eggs·day^{-1}·m^{-2}, respectively) were similar to those obtained off South Australia using the same methods (i.e. 81 and 97 eggs·day^{-1}·m^{-2}, respectively). Future DEPM studies of the Southern stock of Sardine should be designed to obtain further information on the levels of mean daily egg production across the entire stock.

The estimate of mean daily egg production obtained with the GLM was used to estimate the "base case" spawning biomass obtained in this study because it was more conservative (lower) than the estimates obtained using the log-linear model. The "base case" estimate of spawning biomass for the eastern component of the South-eastern stock was 184,721 t (95%CI: 86,282–283,158 t).

There is a strong linear relationship between the spawning area and spawning biomass of Sardine. In this study, the spawning area of the eastern component of this South-eastern stock was estimated to be approximately 41,000 km^2. A previous study indicated that the spawning area of the western component of the South-east stock (i.e. west of 146°30'E) was at least 16,000 km^2, but this estimate did not include unsurveyed parts of western Bass Strait where Sardines are likely to spawn. If the relationship between spawning area and spawning biomass estimated for the South-eastern stock is similar to that observed in the Southern stock (i.e. each square kilometre of spawning area supports approximately 4.5 t of spawning biomass), which is likely, then the spawning biomass of the South-eastern stock may be greater than 250,000 t.

This study provides compelling evidence that the spawning biomass of the eastern component of the South-eastern Sardine stock in 2019 was approximately 185,000 t. This estimate does not include spawning biomass west of 146°30'E, which has previously been estimated to be at least 30,000 t. It is

likely that the spawning biomass of the South-eastern stock is greater than 200,000 t. It may even be greater than 250,000 t. Future applications of the DEPM to the South-eastern stock should cover all of the potential spawning area between southern NSW and the Victorian-South Australian border and be explicitly designed to estimate the relationship between spawning area and spawning biomass for the Southern stock.

BACKGROUND

South-eastern stock of Australian Sardine

The distribution and stock structure of Australian Sardine *(Sardinops sagax*, herein referred to as Sardine*)* is shown in Figure 1. It is widely accepted that Sardine is a meta-population with effective isolation of four stocks: South-western; Southern; South-eastern and Eastern (Izzo et al. 2017). The South-eastern stock covers continental shelf waters off Tasmania, Victoria and southern New South Wales. Izzo et al. (2017) found that the South-eastern stock was clearly separated from the Southern stock, perhaps because seasonal upwelling provided an effective barrier to mixing, but that the South-eastern stock mixed with the Eastern stock off central New South Wales.

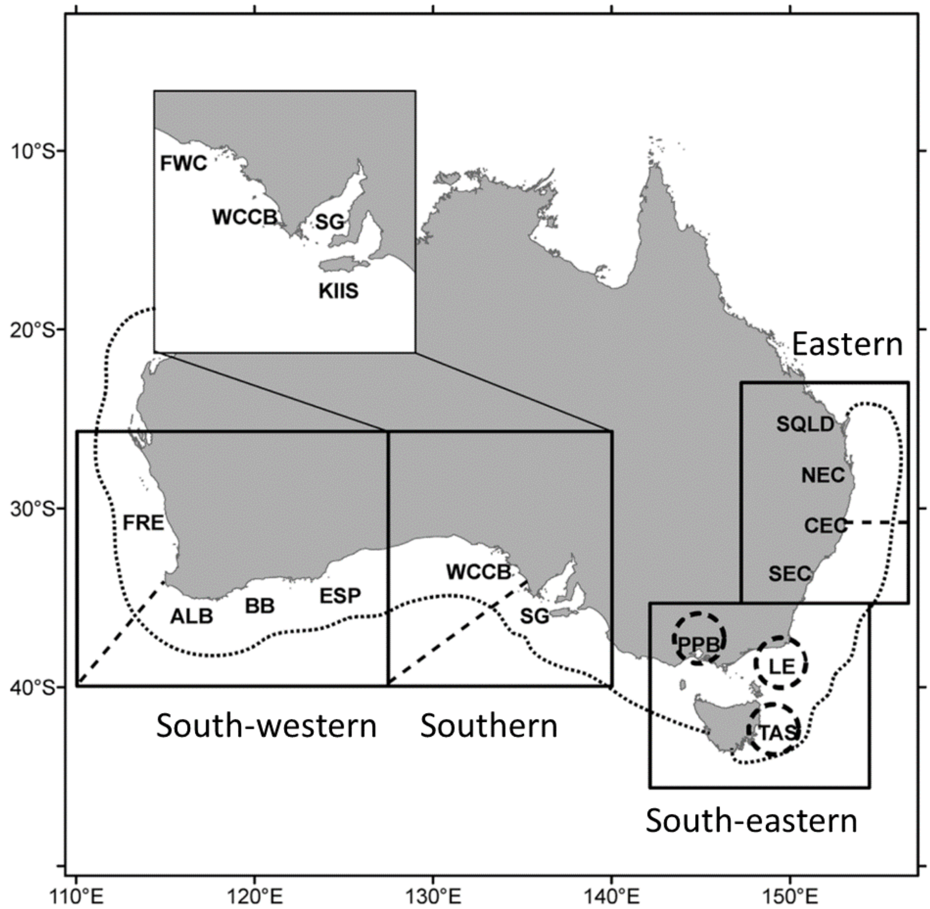

Figure 1. The four stocks of Sardine, *Sardinops sagax* found in Australian waters (South-western, Southern, South-eastern, Eastern). The dotted line is the approximate distribution of the meta-population. Regional codes show locations where data were available to use in the analysis: SQLD Southern Queensland, NEC North East Coast, CEC Central East Coast, SEC Southern East Coast, LE Lakes Entrance, TAS Tasmania, PPB Port Philip Bay, SG Spencer Gulf, WCCB West Coast SA and Coffin Bay, ESP Esperance, BB Bremer Bay, ALB Albany, FRE Fremantle. The inset figure shows the key sardine fishing regions in South Australia; FWC Far West Coast, KIIS Kangaroo Island and Investigator Strait (Adapted from Izzo et al. 2017 with permission of Springer).

Over the last decade, most of the catch from the South-eastern stock has been taken from eastern Victoria, where annual catches have reached up to 2,628 t (https://www.fish.gov.au/report/311-Australian-Sardine-2020). Sardine fishing in Victoria's Port Phillip Bay is scheduled to cease by 2022. Over the last decade, catches from southern New South Wales averaged approximately 120 t. A developmental fishery was established in Tasmania in 2015 but catches have been limited.

Preliminary applications of the Daily Egg Production Method (DEPM) to the western and eastern component of the South-eastern stock were conducted in 2016-17 (Ward et al. 2018) and 2014 (Ward et al. 2015), respectively (Figure 2). Data used in those studies were obtained from DEPM surveys for Jack Mackerel, which spawns at the same time as Sardine in this region (Ward et al. 2015, 2018). The spawning biomass of the western component (i.e. not including the area west of Victorian-South Australia border) was estimated to be at least 30 000 t (Ward et al. 2018, Figure 2A). The spawning biomass of the eastern component was at least 11 000 t (Ward et al. 2015, Figure 2B). However, neither of these surveys covered the entire spawning area, including parts of Bass Strait where sardines are known to spawn. Both surveys under-estimated the spawning area and total spawning biomass of the South-eastern stock (Figure 2).

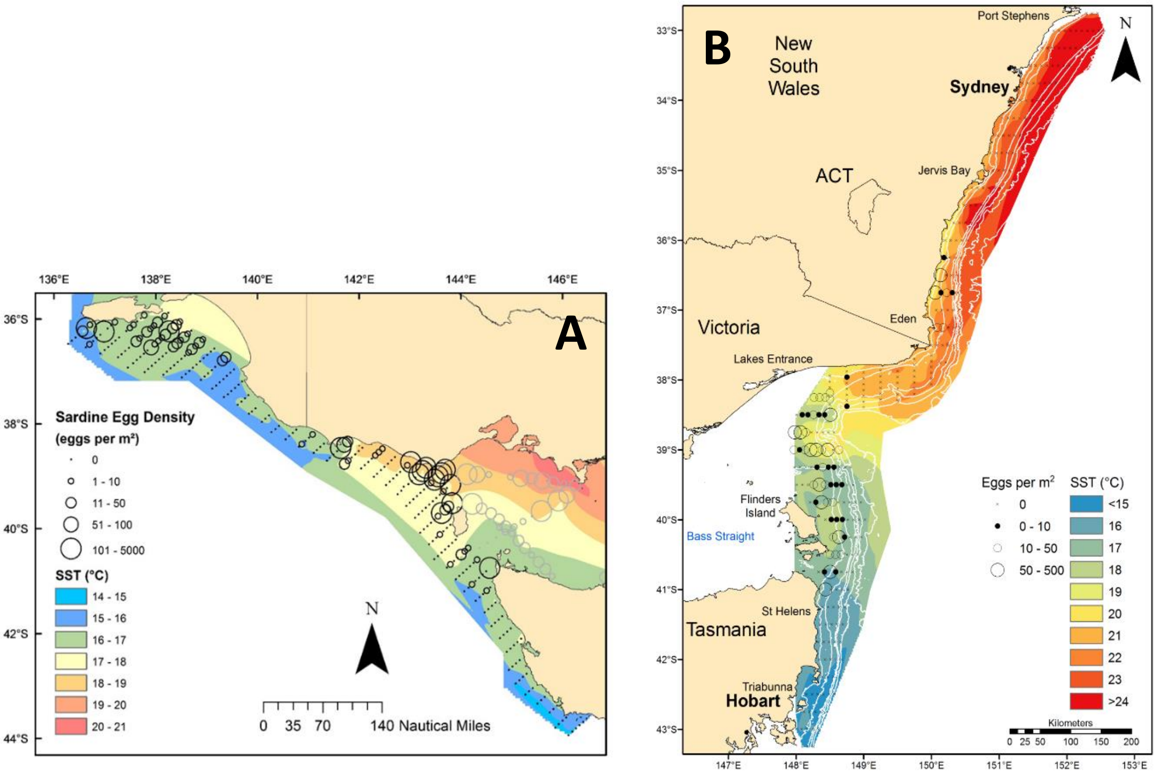

Figure 2. Sea surface temperatures and densities of sardine eggs at sites sampled in the ichthyoplankton surveys of conducted in the western (A) and eastern (B) components of the South-eastern stock in 2016-17 and 2014, respectively. (Source Ward et al. 2015; 2018). Images reproduced with the permission of the South Australian Research and Development Institute.

Daily Egg Production Method

The DEPM can be applied to fishes that spawn multiple batches of pelagic eggs over an extended spawning season (Lasker 1985; Alheit 1993; Stratoudakis et al. 2006; Bernal et al. 2012; Dickey-Colllas et al. 2012). The premise of the DEPM is that spawning biomass can be estimated by dividing the mean number of 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; Ward et al. 2021). Total daily egg production is calculated from estimates of mean daily egg production and spawning area obtained from ichthyoplankton surveys. Mean daily fecundity is estimated most precisely from three adult reproductive parameters, i.e. sex ratio, spawning fraction and relative fecundity (Parker 1980, Ward et al. 2021). The equation underpinning the DEPM is shown in Appendix 1 (Equation 1).

A recent study (Ward et al. 2021) showed that for Sardine off South Australia inter-annual variations in mean daily egg production were low in comparison to statistical uncertainty and this parameter could be estimated most precisely from data collected over multiple years. The study also showed that adult reproductive parameters are effectively stable among years and that values obtained for Sardine off South Australia were similar to those obtained for other species of Sardines in ecosystems around the world (Ganais 2014; Somarakis et al. 2019). These findings suggest that estimates of the spawning biomass of Sardine are driven primarily by interannual variations in spawning area. These results support the findings of other studies that have shown a strong linear relationship between the spawning area and spawning biomass of Sardine (Mangel and Smith, 1990; Zenitani and Yamada, 2000; Gaughan et al., 2004). For Sardine off South Australia, each square kilometre of spawning area supports approximately 4.5 t of spawning biomass (Ward et al. 2021).

AIMS AND OBJECTIVES

The aim of this study was to estimate the spawning biomass of Sardine (*Sardinops sagax*) in the eastern component of the South-Eastern Australian stock.

METHODS

Overview and rationale

The approach taken to applying the DEPM to the eastern component of the South-Eastern Australian stock in this study follows the methods outlined in Ward et al. (2021).

Total daily egg production of Sardine was calculated from estimates of mean daily egg production and spawning area obtained from an ichthyoplankton survey conducted as part of an application of the DEPM to Jack Mackerel (*Trachurus declivis*) in 2019 (Ward et al. 2020). The survey covered continental shelf and inner continental slope waters between central New South Wales and south-eastern Tasmania, including Bass Strait as far west as 146°30'E. The survey coincided with the spawning season of the South-eastern stock of Sardine and covered the known spawning habitat east of 146°30'E.

Mean daily fecundity was estimated from adult reproductive data obtained for Sardine off South Australia between 1998 and 2018 (Ward et al. 2021). Using data from adults collected off South Australia to estimate the spawning biomass of Sardine off Tasmania, Victorian and southern New South Wales is justified because these parameters are relatively stable for this species worldwide (Ganais 2014; Somarakis et al. 2019; Ward et al. 2021).

Ichthyoplankton survey and plankton sampling

Ichthyoplankton samples were collected at 205 sites on 29 transects in shelf waters between south-eastern Tasmania and central New South Wales (Figure 3). The survey was undertaken from 15 January to 7 February 2019.

Paired bongo nets (0.6 m internal diameter, 500 µm mesh, plastic cod-ends) were deployed to 10 m above the sea floor or to a maximum depth of 200 m and retrieved vertically at ~1 $m \cdot s^{-1}$ (Ward et al. 2020). Water temperature profiles were recorded with a Sea-Bird™ Conductivity-Temperature-Depth (CTD) attached to the nets. General Oceanics™ 2030 flowmeters and factory calibration coefficients were used to estimate the distance travelled during each tow. If there was >5%

difference between the paired flowmeters, then the relationship between wire length released and flow-meter units was used to determine which meter was more accurate, and that value was used for both nets. At each sampling site, plankton collected in the paired net cod-ends were combined into one sample and fixed in a 5% buffered formalin and seawater solution.

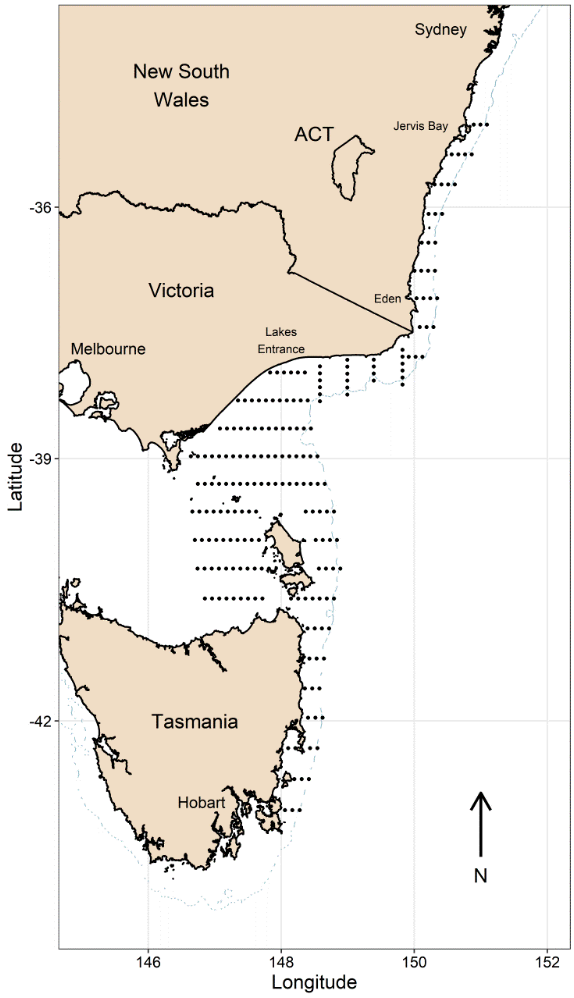

Figure 3. Locations between central New South Wales and south-eastern Tasmania where ichthyoplankton samples (black dots) were collected in January-February 2019. The dashed line is the 200 metre depth contour.

Egg identification, staging and density

Sardine eggs were identified and staged using the descriptions of White and Fletcher (1998). The number of eggs of each stage in each sample was counted. The first and last stages were excluded from the statistical analyses because they are under- and over-represented in plankton samples, respectively (Ward et al. 2021). Egg density was estimated as the number of eggs of each stage per m^2 using the method of Smith and Richardson (1977, Appendix 1, Equation 2).

Egg ageing and treatment of zero count egg samples

The development rate of sardine eggs is dependent on water temperature (Picquelle and Stauffer 1985, Pauly and Pullin 1988). Based on CTD data, egg samples were allocated to one of three temperature bins (14–18°C, 18–22°C, and 22–26°C) that were similar to those used by Le Clus and Malan (1995) to describe the developmental rates of sardine eggs. The development rates of Le Clus and Malan (1995) were used to assign a mean age to each egg (Ward et al. 2021). Model 2 of Le Clus and Malan (1995) was used to calculate the development rate at 24°C and estimate the age of eggs in the 22-26°C temperature bin. Eggs from each sample were then grouped into daily cohorts because samples usually included eggs spawned on more than one night.

The total number of eggs in a 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 each daily cohort was calculated from the average age of each stage within the cohort, weighted by the number of eggs in each stage. Samples with eggs could have contained several possible combinations of daily cohorts depending on water temperature, spawning time (peak around 2:00 am, Ward et al. 2001, 2011) 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. 2021). Samples with no eggs were excluded from the analyses and not considered part of the spawning area.

Spawning area

The spawning area (*A*, Appendix 1, Equation 1, 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 centred on each site using the 'deldir' package in the statistical program 'R (R Development Core Team 2019, Turner 2016). The area represented by each site (km^2) was calculated. Spawning area was defined as the total area of the polygons where one or more live sardine eggs were present in a sample (see Fletcher et al. 1996). Sites where eggs were not collected were not included in the spawning area because there was no evidence that spawning occurred at those sites.

Mean daily egg production

Estimating mean daily egg production (P_0, Appendix 1, Equation 3) and instantaneous egg mortality rate (z, day^{-1}) is difficult because egg density data include many low values and some very high values (Ward et al. 2011, 2021). The exponential egg mortality model of Lasker (1985) cannot be fitted using non-linear least squares because egg data are not normally distributed (e.g. Ward et al. 2011, 2021). Because the distribution of egg data is approximately log-normal, a log-linear version of the exponential egg mortality model (Appendix 1, Equation 4a) was fitted to estimates of egg age and density for each daily cohort at each site (Picquelle and Stauffer 1985). Estimates of mean daily egg production from the log-linear model have a negative bias (Picquelle and Stauffer 1985), so a bias correction factor was applied (Appendix 1, Equation 4b).

Mean daily egg production was also estimated using a generalized linear model (GLM, Appendix 1, Equation 5) with a negative binomial error structure where variance increased linearly with the mean ($\sigma = \mu^*(1+ \mu +\phi)$), and μ is the model estimate, σ is the model variance and ϕ is the over-dispersion parameter. The GLM was fitted using a log-link function (Appendix 1, Equation 5) and the glmmTMB 'R' package (Brooks et al. 2017).

Adult sampling

Samples of mature sardine were collected from sites located off South Australia using a gillnet and lights. Detailed descriptions of the sampling method are provided in Ward et al. (2001, 2011, 2021). Estimates of each adult parameter were obtained from data pooled across years (i.e. 1998 to 2018), because statistically significant interannual variation could not be detected for any parameter (Ward et al. 2021).

Sex Ratio

The mean sex ratio by weight (*R*, Appendix 1, Equation 1) of mature individuals in the population was calculated from the average of sample means weighted by sample size (Appendix 1, Equations 6a, b, Ward et al. 2021).

Spawning fraction

Ovaries of mature females were sectioned, stained with haematoxylin and eosin and examined for 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 *S*, Appendix 1, Equation 1) of each sample was calculated from females with (i) hydrated oocytes plus day-0 POFs (assumed to be spawning or have spawned on the night of capture), (ii) day-1 POFs (assumed to have spawned the previous night) and (iii) day-2 POFs (assumed to have spawned two nights prior). Estimates of spawning fraction calculated from each POF cohort were not significantly different. The mean spawning fraction of the population was calculated from the average of sample means (all POF cohorts) weighted by proportional sample size (Appendix 1, Equations 7a, b, Ward et al. 2021).

As adult samples were only collected over a discreet 10-hour period (2000 hours to 0600 hours) at night, which included the peak spawning time of 0200 hours, assigning POFs to a daily cohort was relatively straightforward because their ages and appearances were quite distinct. Calculating spawning fraction as the mean of all three daily cohorts also mitigated problems that could have arisen from assigning a POF to the wrong cohort (Ward et al. 2021)

Relative fecundity

Mature females from each sample were removed from formalin and weighed (± 0.01 g). The mean weight of mature females in the population (*W*) was calculated from the average of sample means weighted by sample size (Appendix 1, Equation 8, Ward et al. 2021).

Batch fecundity (*F*) was estimated using the methods of Hunter et al. (1985). Both ovaries were weighed and the number of hydrated oocytes in three ovarian sub-sections were counted and weighed. 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 linear relationship between female weight (ovaries removed) and batch fecundity was estimated from all females with hydrated oocytes. This relationship was used to estimate the batch fecundity ($\hat{F}$) for each mature female without hydrated ovaries. This was done by generating a predicted fecundity ($\hat{F}$) for each mature female based on the estimated fecundity-at-weight relationship and its level of variance given each fish's weight (Table 1, Equation 10). The standard deviation of the normal distribution was determined using weight-dependent variance terms that allowed variance to increase with weight and ensured that the precision of estimates of $\hat{F}$ was not over-estimated for larger fish. Predicted fecundities were estimated using the 'truncnorm' package (Mersman et al. 2018) which prevented estimates of batch fecundity falling below zero. Females <25g (i.e. below the size at 50% maturity) were excluded (Ward and Staunton-Smith 2002).

Relative fecundity (*F'*, Appendix 1, Equation 1), i.e. the number of eggs produced per gram of total female weight (*W*), was calculated for each female by dividing batch fecundity (i.e. F and $\hat{F}$) by total female weight (*W*, Appendix 1, Equation 9a, Ward et al. 2021).

Estimates of uncertainty

The 95% Confidence Intervals (CIs) and Coefficients of Variation (CVs) for mean daily egg production were estimated using bootstrap resampling methods with 10,000 iterations (Ward et al. 2021). The 95% CIs and CVs of sex ratio, spawning fraction and relative fecundity were calculated using a ratio estimator (Rice 1995) as demonstrated for relative fecundity in Appendix 1, Equation 9b, Ward et al. 2021).

Spawning biomass

Spawning biomass was calculated with Equation 1 (Appendix 1) using spawning area, estimates of mean daily egg production obtained from both the log-linear model and the GLM and estimates of sex ratio, spawning fraction and relative fecundity obtained from adult samples collected off South Australia in 1998 to 2019 (Ward et al. 2021).

The variance around the estimates of spawning biomass were calculated by summing the squared CVs for each parameter and multiplying by the square of the estimate of spawning biomass. All analyses were done in the R programming environment (R Core Team, 2019).

Sensitivity analysis

Sensitivity analyses were conducted to assess the effects of potential variation of each parameter on the estimates of spawning biomass. The range of values used in the analysis were the highest and lowest annual estimates of each parameter obtained for Sardine off South Australia from 1998 to 2019 (Ward et al. 2021). These were spawning area (15,637 and 73,981 km^2), mean daily egg production (39 and 170 eggs.g^{-1}), sex ratio (0.36 and 0.70), spawning fraction (0.04 and 0.18) and relative fecundity (296 and 313 eggs.g^{-1}). The sensitivity analysis also showed the effect on spawning biomass of estimating mean daily egg production using the log-linear model or the GLM.

RESULTS

Egg distribution and abundance

A total of 2,676 live Sardine eggs were collected from 124 (59.3%) of the 205 sites between Jervis Bay, New South Wales and south-eastern Tasmania (Table 1, Figure 4A). Sardine eggs were found at most sites and were highly abundant in shelf waters south of Lakes Entrance and in Bass Strait. Eggs were more sparsely distributed and less abundant off the east coasts of New South Wales and Tasmania.

Table 1. Number of sites and total area sampled. Proportion of sites and area sampled with live Sardine eggs present.

Survey	Total	With live eggs
Number of sites	205	124 (59.3%)
Survey area	68,295 km^2	41,017 (60.1%)

Spawning area

The spawning area of Sardine was estimated to be 41,017 km^2 and comprised 60.1% of the total area sampled (68,295 km^2, Table 1, Figure 4B).

Mean daily egg production

The estimates of mean daily egg production (P_0) obtained using the log-linear model and GLM were 95.0 eggs·day^{-1}·m^{-2} (95% CI: 46.7-186.7) and 81.5 eggs·day^{-1}·m^{-2} (95% CI: 47.5-131.4), respectively (Table 2, Figure 5). The instantaneous daily egg mortality rates (z) were 1.2 (95% CI: 0.6-1.9) day^{-1}and 0.91 (95% CI: 0.5-1.4) day^{-1}, respectively (Table 2, Figure 5).

Table 2. Estimates of mean daily egg production (P_0, eggs·day^{-1}.m^{-2}) and instantaneous daily egg mortality (z, day^{-1}) for Sardine (*Sardinops sagax*) in the eastern component of the South-eastern stock in 2019

Egg Production Model	Egg Production eggs·day^{-1}·m^{-2} (95% CI)	Daily Egg Mortality day^{-1} (95% CI)
Log-linear model	95.0 (46.7-186.7)	1.2 (0.6-1.9)
GLM Negative Binomial	81.5 (47.5-131.4)	0.9 (0.5-1.4)

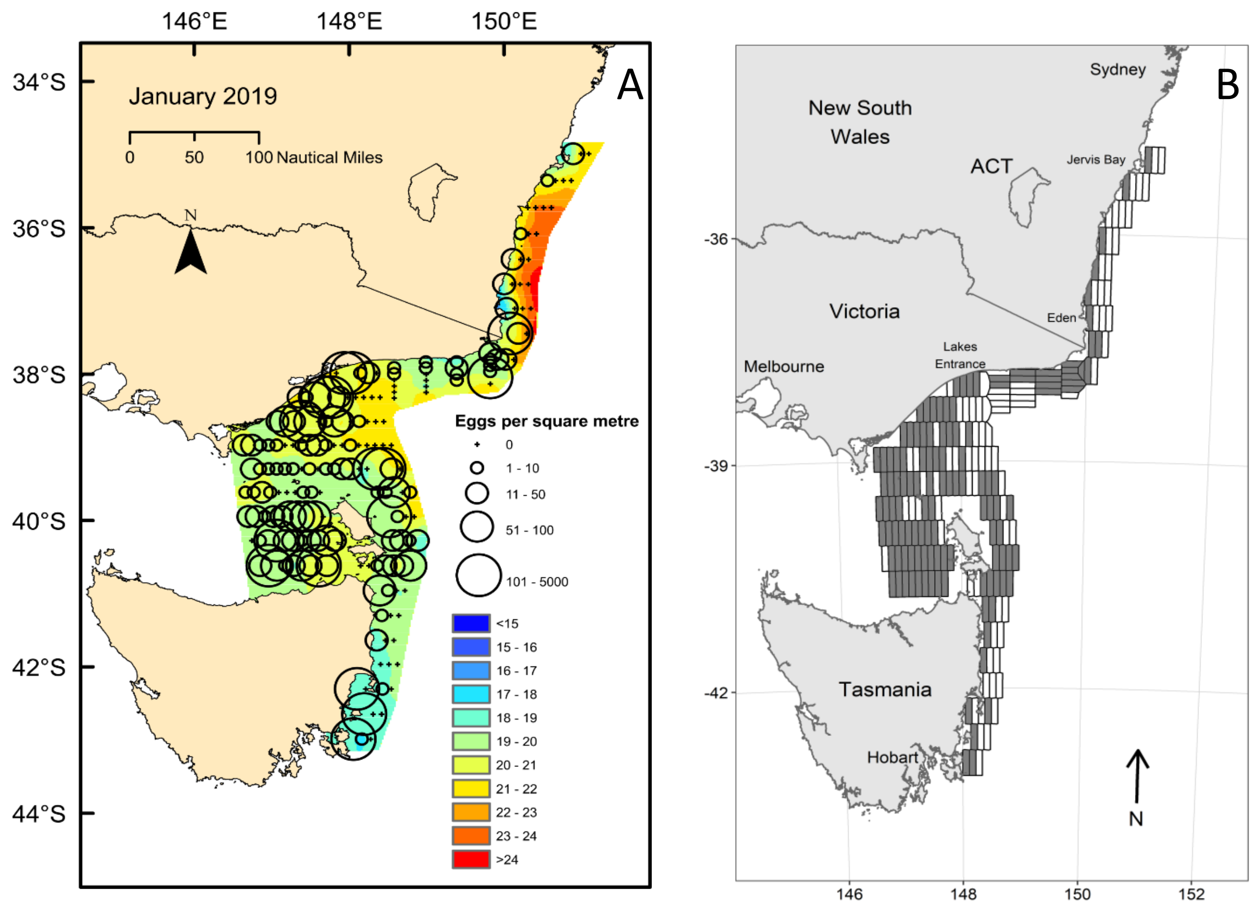

Figure 4. (A) Sea surface temperatures (SST; °C) and distribution and densities (eggs·m^{-2}) of live Sardine (*Sardinops sagax*) eggs collected between central New South Wales and south-eastern Tasmania during January to February 2019. (B) Polygons used to estimate the spawning area, with polygons where Sardine eggs were collected shaded grey.

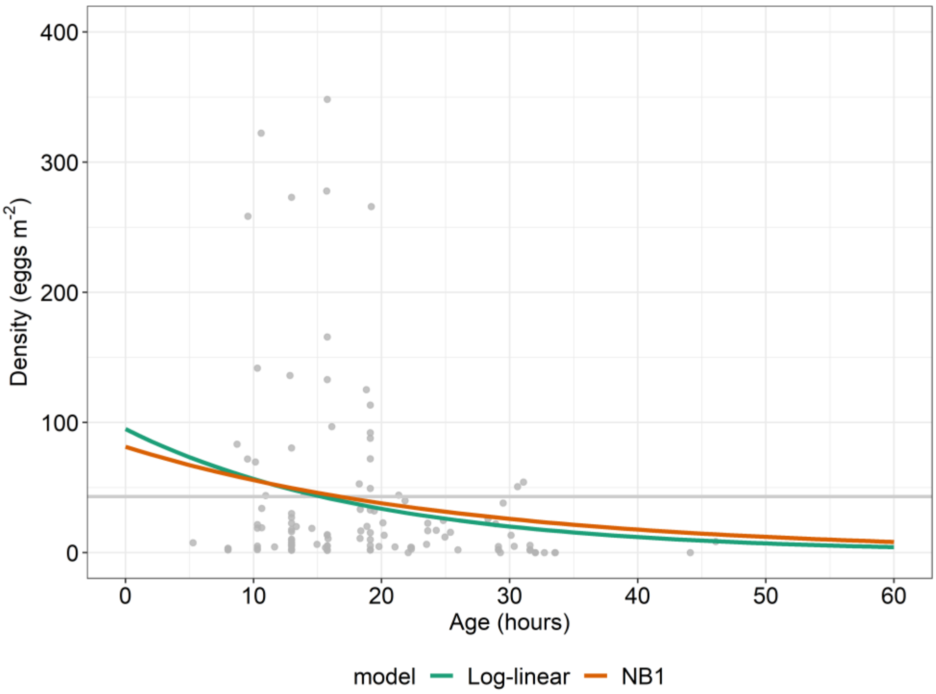

Figure 5. Plot of egg age and density for each daily cohort of Sardine at each site sampled during the ichthyoplankton survey off eastern Australia in January-February 2019. Curves used to estimate mean daily egg production (*P0*, egg·day-1·m-2) and instantaneous daily mortality (*z,* day 1) were fitted using the log-linear version of the exponential mortality model and a Generalised Linear Model with a negative binomial error structure. The grey horizontal line is the mean egg density for the survey.

Sex ratio

Annual estimates of sex ratio ranged from 0.36 (95% CI = 0.28–0.43) in 2009 to 0.70 (95% CI = 0.64–0.77) in 2018 (Figure 6, Ward et al. 2021). The mean sex ratio from data combined across years was 0.55 (95% CI = 0.52–0.58).

Spawning fraction

Annual estimates of spawning fraction ranged from 0.04 in 2014 (95% CI: 0.03–0.05) in 2014) to 0.18 (95% CI: 0.15–0.21 in 2001 (Figure 6, Ward et al. 2021). The mean spawning fraction from data combined across years was 0.11 (95% CI: 0.10–0.12).

Relative fecundity

The batch fecundity relationship for females with hydrated oocytes was Batch Fecundity = 335 × Gonad Free Female Weight – 797 (R^2 = 0.53). Annual estimates of $\hat{F}$ ranged from 14,070 oocytes (95% CI: 2,609–24,619) in 1998 to 23,995 oocytes (95% CI:10,091–37,472) in 2004 (Figure 6, Ward et al. 2021). The estimate of batch fecundity calculated for all mature females was 17,835 oocytes·batch^{-1} (95% CI: 3,819–31,813).

Annual values of relative fecundity ranged from 295.9 to 312.9 egg.g^{-1}. The overall estimate of relative fecundity was 305.4 eggs.g^{-1} (95% CI: 303.8–306.3) and was approximately constant across the range of weights of mature females obtained in samples (Figure 7, Ward et al. 2021).

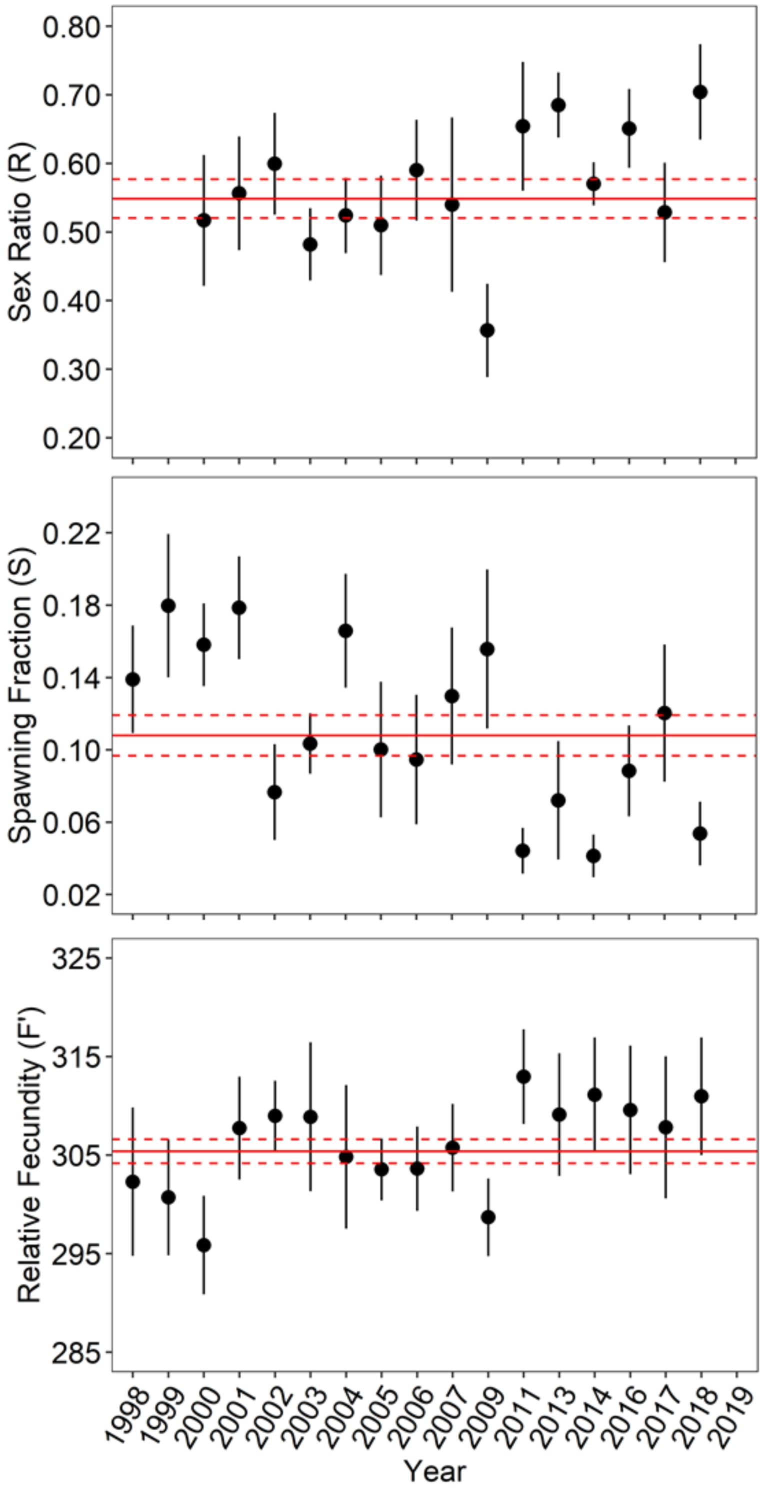

Figure 6. Estimates of sex ratio (top), spawning fraction (middle) and relative fecundity (bottom) of Sardine for collected off South Australia each year (black) and for all years combined (red). Errors bars and red dotted lines are 95% Confidence Intervals. Adapted from Ward et al. (2021). Reproduced with permission of Oxford University Press.

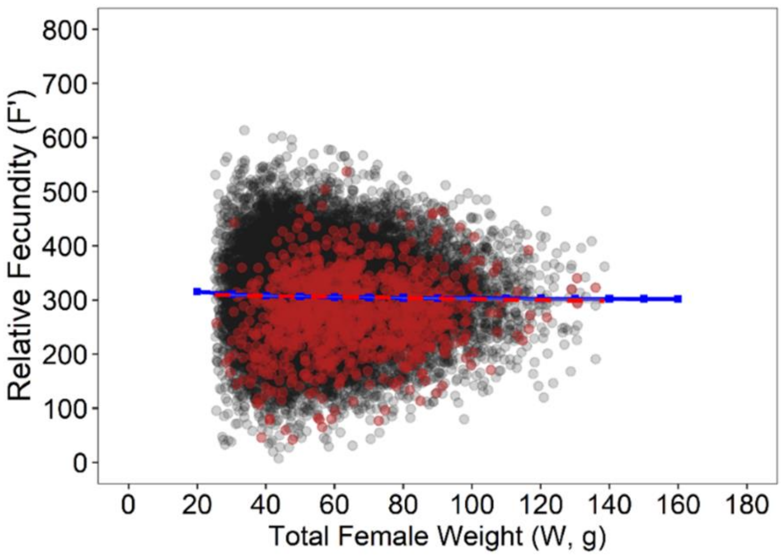

Figure 7. Relationship (blue line, red dotted line) between relative fecundity (*F′*, egg.g^{-1}) and female weight (*W, g*) of Sardine collected off South Australia between 1998 and 2018. Red: measured *F′* values; Black: estimated *F′* values. Adapted from Ward et al. (2021). Reproduced with permission of Oxford University Press.

Spawning biomass

The estimate of spawning biomass calculated using the estimate of the spawning area of 41,017 km^2 obtained from the survey, the estimate of mean daily egg production obtained using the General Linear Model (i.e. 81.5 eggs·day^{-1}·m^{-2}) and the mean values of sex ratio (0.55), spawning fraction (0.11) and relative fecundity (305.41 eggs.g^{-1}) obtained from Sardine collected off South Australia between 1998 and 2018 was 184,721 t (95%CI: 86,282–283,158 t). The estimate obtained using the log-linear version of the exponential model was 215,369 t (95%CI: 163,787–379,157 t).

Sensitivity analyses

The sensitivity analysis shows that potential variations in sex ratio and relative fecundity have relatively small effects on estimates of spawning biomass (Figure 8). In contrast, spawning biomass varies markedly in response to potential variations in spawning area, mean daily egg production and spawning fraction. Previous studies have shown that interannual variations in mean daily egg production and spawning fraction are driven by sampling error, whereas annual estimates of spawning area are considered more reliable (e.g. Ward et al. 2021). The estimate of spawning biomass calculated using the estimate of mean daily egg production obtained with the GLM was lower than the estimate obtained using the log-linear model. Because the estimate of spawning biomass obtained using the estimate of mean daily egg production from the GLM was the most conservative, it was adopted as the "base case" estimate in this report.

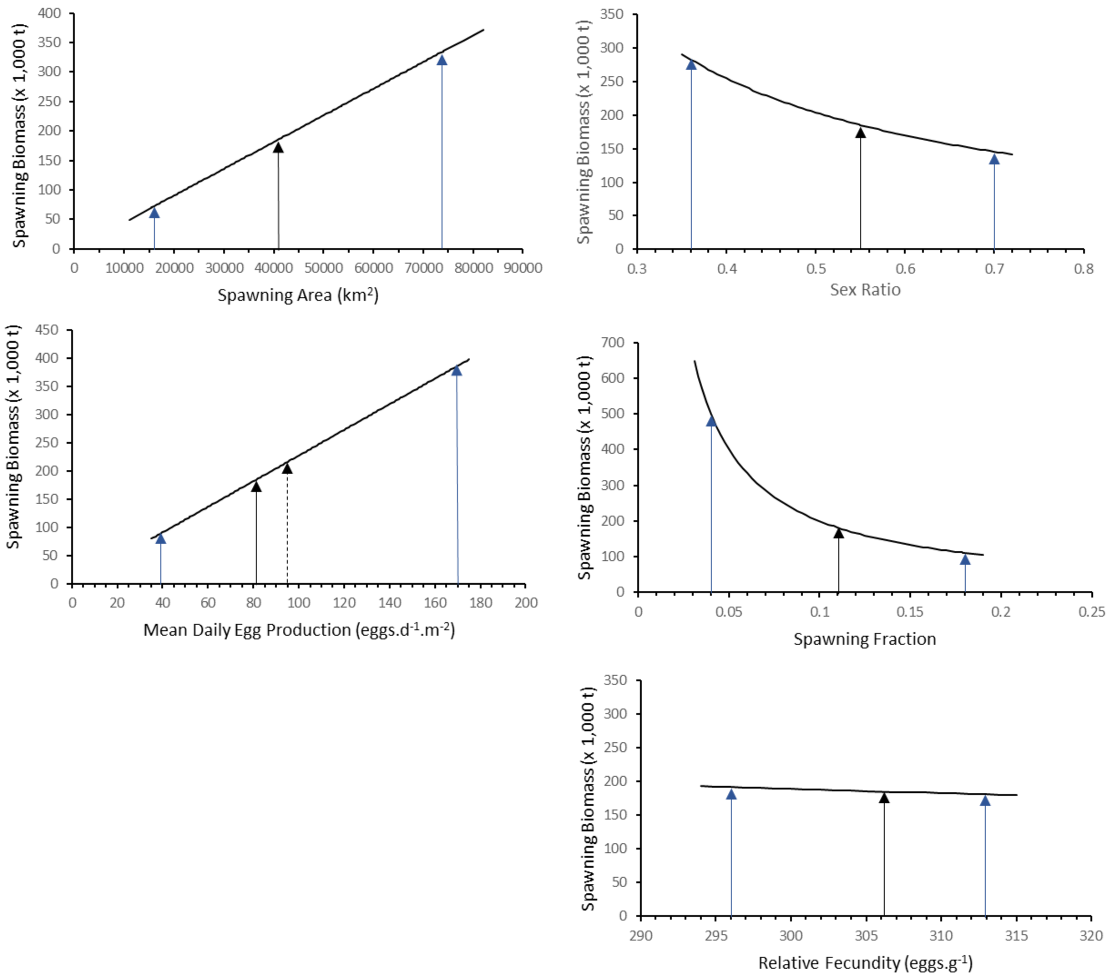

Figure 8. Sensitivity plots showing effects of variability in each parameter on estimates of spawning biomass. Solid black lines are the estimates of each parameter used in the current study. For mean daily egg production the solid black line is the estimate obtained using the Generalised Linear Model with a negative binomial error structure and the dotted line is the estimate obtained using the log-linear version of the exponential mortality model. Blue lines are the range of values for each parameter recorded for Sardine off South Australia between 1998 and 2018 (from Ward et al. 2021).

DISCUSSION

The results of this study suggest that the spawning biomass of the eastern component of the South-eastern stock of Sardine in 2019 was 184,721 t (95%CI: 86,282–283,158 t), which is an order of magnitude larger than the preliminary estimate of the minimum spawning biomass in this region obtained in a previous study (i.e. 11,000 t, Ward et al. 2015). The large increase in the estimate of spawning biomass obtained in the present study reflects the expansion of the ichthyoplankton survey to include parts of eastern Bass Strait where Sardine eggs were widely distributed and abundant (Figure 4). The survey conducted in the present study is likely to have covered most of the spawning habitat of Sardine in the eastern component of the South-eastern stock (i.e. east of 146°30'E). However, the survey did not cover spawning habitat west of 146°30'E and is therefore an under-estimate of the total spawning biomass of the South-eastern stock.

This study indicates that the spawning area of Sardine in the eastern component of the South-eastern stock covers more than 41,000 km^2. A previous study suggested that the western component of the South-eastern stock (i.e. between 146°30'E and the Victorian-South Australian border) covered at least 16,000 km^2 (Ward et al. 2015), but that study did not include likely spawning habitat of Sardine in some western parts of Bass Strait. Together, the findings of Ward et al. (2015) and the present study suggest that the total spawning area covered by the South-eastern stock of Sardine is at least 57,000 t, which is similar to the average spawning area recorded for the Southern stock between 2005 and 2020 (i.e. ~56,000 t, Grammer et al. 2021). This finding is important because numerous studies have shown that there is a strong linear relationship between the spawning area and spawning biomass of Sardine (Mangel and Smith, 1990; Zenitani and Yamada, 2000; Gaughan et al., 2004; Ward et al. 2021). If this relationship for the South-eastern stock is similar to that observed for the Southern stock (i.e. each square kilometre of spawning area supports approximately 4.5 t of spawning biomass), then the total spawning biomass of the South-eastern stock would be similar to that of the Southern stock (i.e. average of ~253,000 t between 2005 and 2020, Grammer et al. 2021), which supports Australia's largest fishery, the South Australian Sardine Fishery.

There are two reasons that suggest the relationship between spawning area and spawning biomass for the South-eastern stock may be similar to that for the Southern stock. Firstly, the estimates of mean daily egg production obtained for the eastern component of the South-eastern stock using the log-linear model and GLM (i.e. 95 and 82 $eggs \cdot day^{-1} \cdot m^{-2}$, respectively) are remarkably similar to those obtained for the Southern stock (i.e. 81 and 97 $eggs \cdot day^{-1} \cdot m^{-2}$, respectively, Ward et al. 2021). The similarity of these values may be because mean egg density, which together with egg mortality drives levels of mean daily egg production, is relatively stable for sardines, and perhaps some other planktivorous species, even when spawning biomasses vary by orders of magnitude (Zweifel, 1973; Smith and Richardson, 1977; Wolf et al. 1987; Mangel and Smith, 1990). It has been suggested that egg density may be relatively stable for these planktivorous pelagic species because successful fertilization requires a minimum density of spawning adults (e.g. Mangel and Smith, 1990) and the need to minimize cannibalism of eggs and larvae places an upper limit on adult density (e.g. Hunter and Kimbrell 1980; Somarakis et al., 2019). Future DEPM studies of the Southern stock of Sardine should be designed to obtain further information on the levels of mean daily egg production across the entire stock.

The second reason is that the adult reproductive parameters of the two stocks are likely to be similar. This is because adult reproductive parameters of *Sardinops sagax* and several other species of sardines are remarkably stable across several ecosystems worldwide (see Ganias et al. 2009;

Somarakis et al., 2019; Ward et al. 2021). For example, even though estimates of sex ratio can be highly variable among years (mainly due to sampling error), there is no evidence to suggest that the sex ratio (by numbers) of Sardine or any other sardine species is consistently skewed towards either males or females (e.g. Ganias et al. 2009, Ganias 2014). However, during the spawning season females are often slightly heavier than males at any given length so the sex ratio by weight can be higher than 0.5. On this basis it seems likely that the sex ratio by weight for the South-eastern stock is similar to the estimate of 0.55 obtained by Ward et al. (2021) for the Southern stock. The spawning fraction for the Southern-eastern stock of Sardine is also likely to be similar to the estimate of 0.11 reported for the Southern stock (Ward et al. 2021). This is because the mean value of spawning fraction for the Southern stock is similar to both the global mean for this species of 0.12 (e.g. Ganias et al. 2009; Ganias 2014) and estimates available for several of other species of Sardines in other ecosystems (e.g. Somarakis et al., 2019). The relative fecundity for the Southern-eastern stock of Sardine may also be similar to that reported for the Southern stock (i.e. 305 eggs.g^{-1}) because Somarakis et al. (2006) showed that this parameter is relatively constant across two different genera of sardine (*Sardinop*s, *Sardina*) in several different ecosystems, including *S. sagax* off southern Australia. Although it is likely that the adult reproductive parameters of the South-eastern and Southern stocks are similar, if a substantial fishery is established on the South-eastern stock, it is recommended that representative adult samples are collected from across the entire stock to assess the validity of this assertion.

The evidence indicating that mean daily egg production and adult parameters of the Southern-eastern stock of Sardine are likely to be similar to those of the Southern stock suggests that the relationship between spawning area and spawning biomass in the two stocks may also be similar. The present study provides strong evidence that the spawning biomass of the eastern component of the South-eastern stock of Sardine is approximately 185,000 t. This estimate does not include spawning biomass in the area west of 146°30’E and is therefore a conservative estimate of the spawning biomass of the total South-eastern stock of Sardine. A previous study suggested that the spawning biomass of the western component of the South-eastern stock of Sardine was at least 30,000 t (Ward et al. 2018), which suggests that the spawning biomass of the entire South-eastern stock is at least 200,000 t. If relationship between spawning area and spawning biomass for the South-eastern stock is similar to that observed in the Southern stock, which is likely, then the total spawning biomass of the South-eastern stock may be 250,000 t, or greater. Future applications of the DEPM to the South-eastern stock of Sardine should cover all of the potential spawning area between southern New South Wales and the Victorian-South Australian border, especially parts of western Bass Strait that have not yet been surveyed and be explicitly designed to estimate the relationship between spawning area and spawning biomass for the Southern stock.

REFERENCES

Alheit, J. 1993. Use of the Daily Egg-Production Method for Estimating Biomass of Clupeoid Fishes - a Review and Evaluation. *Bulletin of Marine Science* 53:750-767.

Bernal, M., Somarakis, S., Witthames P.R., van Damme, C.J.G. Uriarte, A., Lo, N.C.H. and Dickey-Collas, M. 2012. Egg production methods in marine fisheries: An introduction. *Fisheries Research* 117–118:1-5.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J. et al. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9: 378–400.

Dickey-Collas, M., Somarakis, S., Witthames, P R., van Damme, C.J.G. Uriarte, A. Lo, N.C.H. and Bernal, M. 2012. Where do egg production methods for estimating fish biomass go from here? *Fisheries Research* 117–118:6-11.

Ganias, K., Somarakis, S., Machias, A., and Theodorou, A. 2009. Pattern of oocyte development and batch fecundity in the Mediterranean sardine. *Fisheries Research*, 67: 13–23.

Ganias, K. 2014. Biology and Ecology of Sardines and Anchovies. Apple Academic Press Inc. Oakville, Canada.

Gaughan, D.J., Leary, T.I., Mitchell, R.W. and Wright, I.W. 2004. A sudden collapse in distribution of Pacific sardine (*Sardinops sagax*) off southwestern Australia enables an objective re-assessment of biomass estimates. *Fishery Bulletin* 102:617-633.

Hunter, J.R. and Goldberg, S.R. 1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. *Fisheries Bulletin* 77:641-652.

Hunter, J.R. and Kimbrell, C.A. 1980. Egg cannibalism in the northern anchovy, *Engraulis mordax*. *Fisheries Bulletin*, 78: 811–816.

Hunter, J.R. and Macewicz, B. J. 1985. Measurement of spawning frequency in multiple spawning fishes. n 'An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*'. (Ed. R. Lasker.) pp. 79–94. Technical Report 36. National Marine Fisheries Services. National Oceanic and Atmospheric Administration. South West Fisheries Science Center, La Jolla, California

Izzo, C., Ward, T.M., Ivey, A.R., Suther, I.M., Stewart, J., Sexton, S.C. and Gillanders, B.M. (2017). Integrated approach to determining stock structure: implications for fisheries management of sardine, *Sardinops sagax*, in Australian waters. *Reviews in Fish Biology and Fisheries*. 1:267-284

Lasker, R. 1985. An egg production method for estimating spawning biomass of pelagic fish: Application to the Northern Anchovy, *Engraulis mordax*. National Marine Fisheries Services. Technical Report 36. National Marine Fisheries Services. National Oceanic and Atmospheric Administration. South West Fisheries Science Center, La Jolla, California

Mangel, M. and Smith, P.E. 1990. Presence-absence sampling for fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences* 47:1875-1887.

Mersmann, O., Trautmann, H., Steuer, D., and Bornkamp, B. 2018. truncnorm: Truncated Normal Distribution. R package version Increasing the precision of the DEPM 1193 Downloaded from https://academic.oup.com/icesjms/article/78/4/1177/6199409 by guest on 22 September 2021 1.0-8. https://CRAN.R-proj

Parker, K. 1980. A direct method for estimating northern anchovy, *Engraulis mordax*, spawning biomass. *Fisheries Bulletin* 84:541-544.

Pauly, D., and Pullin, R.S.V. 1988. Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental Biology of Fishes* 22:261-271.

Picquelle, S., and Stauffer. G. 1985. Parameter estimation for an egg production method of anchovy biomass assessment. In 'An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*'. (Ed. R. Lasker.) pp. 7–17. Technical Report 36. National Marine Fisheries Services. National Oceanic and Atmospheric Administration. South West Fisheries Science Center, La Jolla, California.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rice, J. A. 1995. Mathematical Statistics and Data Analysis. Duxbury Press, Belmont, California.

Somarakis, S., Tsoukali, S., Giannoulaki, M. Scismenou, E., and Nikolioudakis, N. 2019. Spawning stock, egg production and larval survival in relation to small pelagic fish recruitment. *Marine Ecology Progress Series* 617-618: 113-136.

Smith, P. E., and Richardson, S. L. 1977. Standard Techniques for Pelagic Fish Egg and Larva Surveys. FAO Fisheries Technical Paper 175, 100 p. Rome, Italy.

Somarakis, S., Palomera, I., Garcia, A., Quintanilla, L., Koutsikopoulos, C., Uriarte, A., and Motos, L. 2004. Daily egg production of anchovy in European waters. *ICES Journal of Marine Science* 61: 944–958.

Somarakis, S., Tsoukali, S., Giannoulaki, M., Scismenou, E., and Nikolioudakis, N. 2019. Spawning stock, egg production and larval survival in relation to small pelagic fish recruitment. *Marine Ecology Progress Series* 617–618: 113–136

Stratoudakis, Y., Bernal, M., Ganias, K. and Uriarte, A. 2006. The daily egg production method: recent advances, current applications and future challenges. *Fish and Fisheries* 7:35-57.

Turner, R. 2015. deldir: Delaunay Triangulation and Dirichlet (Voronoi) Tessellation. R package version 0.1-9. http://CRAN.R-project.org/package=deldir.

Ward, T. M., Burch, P.. McLeay, L.J. and Ivey, A.R. 2011. Use of the Daily Egg Production Method for stock assessment of sardine, *Sardinops sagax*; lessons learned over a decade of application off southern Australia *Reviews in Fisheries Science* 19:1-20.

Ward, T. M., O. Burnell, A. Ivey, J. Carroll, J. Keane, J. Lyle, and S. Sexton. 2015. Summer spawning patterns and preliminary daily egg production method survey of Jack Mackerel and Sardine off the East Coast. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.

Ward, T. M., Grammer, G.L., Ivey, A.R., Smart, J. and Keane, J. 2018. Spawning biomass of Jack Mackerel (*Trachurus declivis*) and Sardine (*Sardinops sagax*) between western Kangaroo Island, South Australia and south-western Tasmania. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2018/000174-1. SARDI Research Report Series No. 983. 51p.

Ward, T. M., Grammer, G. L. Ivey, A. R. and Keane, J. 2020. Spawning biomass of Jack Mackerel (*Trachurus declivis*) in the East sub-area of the Small Pelagic Fishery during summer 2019. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2020/000206-01. 42pp.

Ward, T. M., Grammer, G. L., Ivey, A. R., Smart, J. J., and McGarvey, R. 2021. Increasing the precision of the daily egg production method; 2020's remix of a 1980's classic. *ICES Journal of Marine Science*, 78(4): 1177-1195 https://doi.org/10.1093/icesjms/fsab015

Ward, T. M., Hoedt, F., McLeay, L., Dimmlich, W. F., Kinloch, M., Jackson, G., and McGarvey, R. 2001. Effects of the 1995 and 1998 mass mortality events on the spawning biomass of *Sardinops sagax* in South Australian waters. *ICES Journal of Marine Science*, 58: 865–875.

Ward, T.M. and Staunton-Smith, J. 2002. Comparison of the spawning patterns and fisheries biology of the sardine, *S. sagax*, in temperate South Australia and sub-tropical southern Queensland. *Fisheries Research* **56**: 37-49.

Watson, D. F. 1981. Computing the n-dimensional Delaunay tessellation with application to Voronoi polytopes. *The Computer Journal*. Pages 167-172.

Wolf, P., Smith, P. E., and Scannell, C. L. 1987. The relative magnitude of the 1986 Pacific sardine spawning biomass off California. CalCOFI Report, XXVIII. pp. 21–26

Zenitani, H., and Yamada, S. 2000. The relation between spawning area and biomass of Japanese pilchard, *Sardinops melanostictus*, along the Pacific coast of Japan. *Fishery Bulletin*, 98: 842–848

Zweifel, J. R. 1973. A non-parametric approach to the estimation of relative change in fish population size from egg and larval surveys. *Rapports et procès-verbaux des réunions Conseil international pour l'exploration de la mer* 164: 276–281.

Appendix 1. Equations used in the application of the Daily Egg Production Method to estimate the spawning biomass of sardine off South Australia in 1998-2018. Adapted from Ward et al. (2021). Reproduced with permission of Oxford University Press.

Calculation	Equation	Eq. No.	Parameters	Reference
Spawning Biomass	$SB = \frac{P_0\, A}{R\, S\, F'}$	1	P_0: mean daily egg production A: total spawning area R: mean sex ratio F': mean relative fecundity S: mean spawning fraction	Parker 1980
Egg Density (sample)	$P_s = \frac{C\, D}{V}$	2	P_s: density of eggs in a sample C: number of eggs of each age in each sample V: volume of water filtered (m^3) D: depth (m) of net cast	Smith and Richardson (1977)
Exponential egg mortality model (P_0)	$P_t = P_0 e^{-z\, t}$	3	P_t: egg density at age t z: the instantaneous rate of daily egg mortality	Lasker (1985)
Negatively biased estimate (P_b)	$\ln P_{i,t} = \ln P_b - z\, t$	4a	P_b: negatively biased P_0 $P_{i,t}$: density of eggs of age t at site i z: instantaneous rate of daily egg mortality	Picquelle and Stauffer (1985)
Bias corrected (P_0)	$P_0 = e^{\ln P_b + \sigma^2/2}$	4b	P_b: negatively biased estimate of daily egg production σ^2: variance of P_b estimate	
Generalised Linear Model (GLMs) with error structure of negative binomial	$E[P_0] = g^{-1}(-zt + \varepsilon)$	5	E[P_0]: expected value of P_0 g^{-1}: inverse-link function zt: the instantaneous rate of daily egg mortality at age t ε: error term	Ward et al. (2021)
Sex Ratio: sample ($\overline{R_i}$)	$\overline{R_i} = \frac{F_i}{F_i + M_i}$	6a	F_i: total weight of mature females in each sample i M_i: total weight of mature males in each sample i	Lasker (1985)
Sex Ratio: population (R)	$R = \overline{\left[\frac{\overline{R_i}\, n_i}{N}\right]}$	6b	$\overline{R_i}$: mean sex ratio of each sample n: number of fish in each sample N: total number of fish collected in all samples and	Lasker (1985)

Spawning Fraction: sample ($\overline{S_i}$)	$\overline{S_i} = \frac{d0 + d1 + d2}{3\, n_i}$	7a	*d*0, *d*1 and *d*2: the number of mature females with POFs aged day 0, 1 or 2 in each sample n_i: is the total number of females within a sample.	Lasker (1985)
Spawning Fraction: population (S)	$S = \overline{\left[\frac{\overline{S_i}\, n_i}{N}\right]}$	7b	$\overline{S_i}$: mean spawning fraction of each sample *n*: number of fish in each sample *i* *N*: total number of fish collected in all samples	Lasker (1985)
Female Weight (W)	$W = \overline{\left[\frac{\overline{W_i}\, n_i}{N}\right]}$	8	$\overline{W_i}$: mean female weight of each sample *i*; *n*: number of fish in each sample *N*: total number of fish collected in all samples	Lasker (1985)
Relative Fecundity (F')	$F' = \bar{F}/\bar{W}$	9a	*F'*: Relative Fecundity $\bar{F}$: Mean Fecundity $\bar{W}$: Mean Weight	Parker (1980)
Ratio estimator (e.g. *F'*)	$Var(F') = \frac{1}{n_{fish}} \cdot \frac{1}{\bar{W}^2} \cdot \{(F')^2 \cdot \sigma_W^2 + \sigma_F^2 - 2 \cdot F' \cdot cov(F,W)\}$	9b	*F'*: Relative Fecundity $\bar{F}$: Mean Fecundity $\bar{W}$: Mean Weight σ_F^2 and σ_W^2: variances of $\bar{F}$ and $\bar{W}$ $cov(F,W)$: covariance of *F* and *W*	Rice (1995)