Spawning biomass of sardine, *Sardinops sagax*, in waters off South Australia in 2013

Ward, T.M., Ivey, A.R. and Burch, P.

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SARDI Aquatics Sciences
PO Box 120 Henley Beach SA 5022

October 2013

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South Australian Research and Development Institute
SARDI Aquatic Sciences
2 Hamra Avenue
West Beach SA 5024

Telephone: (08) 8207 5400
Facsimile: (08) 8207 5406
http://www.sardi.sa.gov.au

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Author(s): Ward, T.M., Ivey, A.R. and Burch, P.
Reviewer(s): Mayfield, S. and Steer, M.
Approved by: Mayfield, S. Science Leader - Fisheries

Signed: [Signature]
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PREFACE

The Daily Egg Production Method (DEPM) has been used to assess the stock status of sardine, *Sardinops sagax*, in South Australian waters since 1995. The estimate of spawning biomass obtained using this method is the key biological performance indicator for the South Australian Sardine Fishery (SASF). Up until 2007, spawning biomass was estimated annually; since then the DEPM has been applied biennially. Since 2008, the Fishery Assessment Report, which integrates all information available for the SASF, has been produced in the alternate year to the Spawning Biomass Report. The present report provides an estimate of the spawning biomass of sardine in waters off South Australia in February-March 2013.
EXECUTIVE SUMMARY

- This report provides an estimate of the spawning biomass of sardine, *Sardinops sagax*, in South Australian waters in 2013.
- Data were obtained from research surveys conducted from the *RV Ngerin* during February and March 2013. The total survey area was 114,425 km².
- Sea surface temperatures (SSTs) during the surveys ranged from 19.1 to 22.8°C. No areas of cold water associated with seasonal upwelling were observed and chlorophyll-a concentrations and zooplankton densities were low compared to previous years. SSTs in shelf waters of the eastern GAB were higher than those recorded in previous years.
- A total of 2,159 live sardine eggs were collected from 340 stations. High densities of eggs were recorded south of Spencer Gulf and in western Investigator Strait, near the coast of southern Eyre Peninsula and the mid-shelf out to the shelf break.
- A total of nine samples comprising 1,089 mature fish was collected at sampling locations in Investigator Strait, southern Spencer Gulf and the eastern Great Australian Bight.
- The estimated spawning area (*A*) of 36,549 km² was lower than in recent years. This may be because the 2013 survey did not cover the entire area over which spawning occurred.
- Mean daily egg production (*P₀*), calculated using the log-linear version of the egg mortality model, was 44.3 eggs.day⁻¹.m⁻² (95% CI = 30.3 – 67.1 eggs.day⁻¹.m⁻²).
- Estimates of mean adult reproductive parameters were for 2013: female weight, *W* = 51.1 g (95% CI = 46.1 – 55.4); batch fecundity, *F* = 16,902 hydrated oocytes (95% CI = 14,983 – 18,616); sex ratio, *R* = 0.68 (95% CI = 0.63 – 0.74); and spawning fraction, *S* = 0.070 (95% CI = 0.037 – 0.101).
- Spawning biomass for 2013 was calculated using the average sex ratio for samples collected from 1998 to 2011.
- The mean spawning biomass for 2013 was estimated between 135,438 and 162,645 t. Maintaining the Total Allowable Commercial Catch (TACC) at 34,000 t for 2014 would result in an exploitation rate above the reference point of 20% identified in the current harvest strategy. This reference point was identified at the low risk level associated with biennial application of the DEPM.
1. INTRODUCTION

1.1 Daily Egg Production Method

The Daily Egg Production Method (DEPM) was developed for stock assessment of the northern anchovy, *Engraulis mordax* (Parker 1980; Lasker 1985), and has been applied to at least 18 species of small pelagic fishes worldwide (Stratoudakis *et al.* 2006; Neira and Lyle 2008; Dimmlich *et al.* 2009; Ward *et al.* 2009a). The method is widely used because it is often the most practical option available for stock assessment of small pelagic species. In many circumstances the only real alternative to the DEPM is acoustic surveys, which can produce biased estimates of biomass and require more sophisticated and expensive infrastructure, higher levels of technical support and expertise and have a longer developmental phase than the DEPM. A good example of these constraints comes from South Africa, where estimates of anchovy spawner biomass obtained using the DEPM over a decade were used to scale-up negatively-biased acoustic estimates, while methods were being developed for *in situ* estimation of acoustic target strength (Hampton 1996).

The DEPM relies on the premise that the biomass of spawning adults can be calculated by dividing the mean number of pelagic eggs produced per day throughout the spawning area (i.e. total daily egg production) by the mean number of eggs produced per unit mass of adult fish (i.e. mean daily fecundity; Lasker 1985). Total daily egg production is the product of mean daily egg production (*P*₀) and total spawning area (*A*). Mean daily fecundity is calculated by dividing the product of mean sex ratio (by weight, *R*), mean batch fecundity (number of oocytes in a batch, *F*) and mean spawning fraction (proportion of mature females spawning each day/night, *S*) by mean female weight (*W*). Spawning biomass (*SB*) is calculated according to the equation:

\[ SB = \frac{P_0 \cdot A}{(R \cdot F \cdot S / W)}. \]  

*Equation 1*
The DEPM can be applied to fishes that spawn multiple batches of pelagic eggs over an extended spawning season (e.g. Parker 1980). Data used to estimate DEPM parameters are typically obtained during fishery-independent surveys involving vertical plankton tows at sites located at regular intervals along parallel cross-shelf transects. Adult samples are often taken opportunistically during the survey and may be complemented by samples collected concurrently from commercial vessels (Stratoudakis et al. 2006). The key assumptions of the DEPM are that: 1) surveys are conducted during the main (preferably peak) spawning season; 2) the entire spawning area is sampled; 3) eggs are sampled without loss and identified without error; 4) levels of egg production and mortality are consistent across the spawning area; and 5) representative samples of spawning adults are collected during the survey period (Parker 1980; Alheit 1993; Hunter and Lo 1997; Stratoudakis et al. 2006).

Although the DEPM is used widely, a range of problems have been encountered, and estimates of spawning biomass are generally considered to be accurate (unbiased) but relatively imprecise (e.g. Alheit 1993; Hunter and Lo 1997; Stratoudakis et al. 2006). This imprecision is mainly due to uncertainties associated with the estimation of total daily egg production, i.e. $P_0$ and $A$ (Fletcher et al. 1996; McGarvey and Kinloch 2001; Ward et al. 2001a, b; Gaughan et al. 2004). A range of analytical methods have been used to calculate these parameters and these have the potential to significantly affect estimates of $SB$. For example, egg age has been estimated using a range of models that combine information on daily spawning synchronicity and mean egg developmental rates in relevant temperature ranges (e.g. Lo 1985; Piquelle and Stauffer 1985; Ibaibarriaga 2007). Perhaps most importantly, $P_0$ has been determined by fitting the exponential decay model to estimates of the mean age of daily cohorts and their density in each sample using non-linear regression, a log-linear model of ln-transformed data (e.g. Piquelle and Stauffer 1985), generalised linear models (GLMs) with appropriate link functions and generalised additive models (GAMs) (ICES 2004). The delta method (Seber 1982) and parametric (Borchers et al. 1997) and non-parametric bootstraps (Jackson and Chen 2001; ICES 2004) have been used to estimate confidence intervals of $P_0$. $A$ has been estimated by dividing the survey into stratified grids using a subjective ‘manual’ method (Lasker 1985) and objectively using nearest neighbour methods (Watson 1981; Ward et al. 2009b). Confidence intervals for $A$ have been estimated using GAMs (Stratoudakis et al. 2006).
Many DEPM studies have been impeded by difficulties associated with obtaining representative samples of adults to estimate reproductive parameters (see Stratoudakis et al. 2006). Estimating $R$ and $W$ is relatively straightforward if representative samples can be collected. $F$ has been estimated by calculating the relationship between fish weight (ovary-free) and batch fecundity for females with gonads containing hydrated ooocytes using either linear regression (Picquelle and Stauffer 1985) or a gamma or negative binomial GLM with an identity link function (ICES 2004); this relationship has then been applied to the mean gonad free weight of all mature female fish. $S$ is often the most difficult DEPM parameter to estimate for clupeoids. Obtaining representative samples of adults is difficult because during the spawning period spawning females are over-represented in ephemeral spawning aggregations and under-represented in the remainder of the population (Stratoudakis et al. 2006). Much of the uncertainty surrounding estimates of $S$ is associated with determining whether imminent or recent spawners or both should be used in calculations.

At least two reviews have concluded that the DEPM may be better tailored to anchovies (*Engraulis* spp.) than sardine, *Sardinops sagax* (Alheit 1993; Stratoudakis et al. 2006). The main argument used to support this assertion is that because a higher proportion of anchovies are actively spawning during the peak spawning season, daily fecundity can be estimated more precisely for anchovy than sardine (e.g. Alheit 1993; Stratoudakis et al. 2006). Despite these apparent limitations of the DEPM for stock assessment of sardine, the method is a critical component of the assessment of this species in several locations. For example, the DEPM has been used for stock assessment of *S. sagax* off the west coast of North America (e.g. Lo et al. 2005) and the western and southern coasts of Australia (Fletcher et al. 1996; Gaughan et al. 2004; Ward et al. 2009b). However, there are important differences between locations in the manner in which egg and adult samples are collected, data are analysed and how estimates of spawning biomass are used to support fisheries management. A Continuous Underway Fish Egg Sampler (CUFES) has been used routinely in surveys off California but not Australia (e.g. Lo et al. 2001). In addition, adult samples have usually been collected by mid-water trawling off California, purse-seining off Western Australia (Gaughan et al. 2004) and gill-netting off South Australia (Ward et al. 2009b). Furthermore, estimates of $SB$ obtained from DEPM surveys are used directly for fisheries management in South Australia, and are incorporated into age-structured stock assessment models in California and Western Australia.
1.2 Application of the DEPM off South Australia

The DEPM has been used to estimate the spawning biomass of sardine, *Sardinops sagax*, in South Australian (SA) waters since 1995 (Ward *et al.* 1998; 2009b). Application of this method has facilitated the rapid and sustainable development of the South Australian Sardine Fishery (SASF), despite the effects of two mass mortality events that both killed over 70% of the adult population of sardine in SA waters (e.g. Ward *et al.* 2001a; 2008). The current harvest strategy indicates that a baseline Total Allowable Commercial Catch (TACC) of 30,000 t will be maintained while the latest estimate of spawning biomass remains between 150,000 and 300,000, which corresponds to exploitation rates of 20% and 10%, respectively. Since 2010, an additional 4,000 t has been allocated to be caught outside traditional fishing areas. A recent review that re-analysed data collected since 1998 using the various statistical methods identified the optimal approach for applying the DEPM in SA waters (Ward *et al.* 2011). That review, and a study that investigated the potential for utilising a CUFES in future studies (Ward and Ivey 2009), also identified options for improving the methods currently used to estimate key DEPM parameters.

1.3 Aim and Objectives

This report provides an estimate of the spawning biomass of sardine in gulf and shelf waters of SA during February-March 2013. The objectives of the report are:

1. To describe the distribution and abundance of sardine eggs in relation to environmental variables;
2. To estimate DEPM parameters \( (A, P, W, R, F, S) \); and
3. To use the DEPM to estimate the spawning biomass in 2013.
2. METHODS

2.1 Study Area and Biophysical Variables

2.1.1 Study area
Two surveys were conducted aboard the RV Ngerin in shelf and gulf waters of South Australia between February and March 2013. Plankton samples were collected at 340 stations on 34 transects between Victor Harbor and Head of Bight (Fig. 1).

Figure 1. Map of South Australia showing stations where plankton and adult samples were collected during the 2013 DEPM surveys.
2.1.2 Water temperature and primary production
At each station (Fig. 1), a Sea-Bird Conductivity-Temperature-Depth (CTD) recorder fitted with a fluorometer was lowered to a depth of 70 m, or to 10 m from the bottom in waters less than 80 m deep. Estimates of water temperature and fluorescence at a depth of 3 m were extracted from each profile. Where CTD temperature was absent a correction factor was applied to the on-board temperature measurement (average difference between CTD and on-board temperature). Fluorescence is an indicator of primary production and gives an un-calibrated measure of chlorophyll-a concentration (μg.L⁻¹). Spatial plots of SST and chlorophyll-a concentration were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

2.1.3 Secondary production – zooplankton abundance
An index of zooplankton abundance at each station was estimated by dividing the displacement volume of zooplankton (ml) collected during plankton tows by the total volume of water filtered (m³). Spatial plots of zooplankton abundance were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

2.2 Daily Egg Production and Spawning Area

2.2.1 Plankton sampling
Plankton samples were collected at each station using paired Californian Vertical Egg Tow (CalVET) plankton nets. Each CalVET net had an internal diameter of 0.3 m, 330 μm mesh and plastic cod-ends. During each tow the CalVET nets were deployed to within 10 m of the seabed at depths <80 m or to a depth of 70 m at depths >80 m and retrieved vertically at a speed of ~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 500 units between flow-meters, the relationship between wire length released and flow-meter units was used to determine which was correct and that value repeated. Upon retrieval of the nets the samples from each of the two cod-ends were washed into a sample container. Plankton samples were fixed using 5% buffered formaldehyde and seawater.
2.2.2 Laboratory analysis
Sardine eggs and larvae were identified in each sample using published descriptions (White and Fletcher 1996; Neira et al. 1998). Eggs in each sample were counted, staged and assigned approximate ages based on descriptions and temperature-development keys in White and Fletcher (1996).

2.2.3 Egg density
The number of eggs of each stage under one square metre of water \( (P_i) \) was estimated at each site according to Equation 2:

\[
P_i = \frac{C \cdot D}{V}
\]

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\textsuperscript{®} (Ver. 8).

2.2.4 Spawning time and density weightings
The development time of sardine eggs is dependent on water temperature (Picquelle and Stauffer 1985). Kernel density methods were used to estimate the modal time of egg abundance for three categories of SST recorded in SA waters (Ward et al. 2011a). A peak spawning time of 2:00 am was established based on the assumption that Stage 2 eggs are approximately 3-4 hours old. In waters <19.0\(^\circ\)C, 19.0-20.0\(^\circ\)C and >20.0\(^\circ\)C, Stages 1-6, 1-7 and 1-8 were less than 24 hours old, respectively, and Stage 7-12, 8-12 and 9-12 eggs were 24-48 hours old. Ages were assigned to day-1 eggs (i.e. 0 – 24 hours old) by subtracting the estimated spawning time from the sampling time. Ages of day-2 eggs were assigned similarly, but an additional 24 hours were added to their ages. Densities of day-1 and day-2 eggs were weighted according to the relative size of the area from which they were taken.
2.2.5 Spawning area

The Voronoi natural neighbour (VNN) method (Watson 1981) in Mapinfo® (Vers. 8) was used to generate a polygon around each sampling site with the boundary as the midpoint equidistant between each sampling site (Fig. 2). The area represented by each station (km²) was then determined. The spawning area \((A)\) was defined as the total area of grids where live sardine eggs were found.

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![Figure 2. Voronoi nearest neighbour polygons generated in Mapinfo® (vers. 8) used to estimate the total spawning area in 2013.](image-url)

2.2.6 Daily egg production \((P_0)\) and egg mortality

Mean daily egg production \((P_b)\) was calculated by fitting the linear version of the exponential egg mortality model to estimates of egg age and density at each station (Picquelle and Stauffer...
1985). To allow the inclusion of data from stations where either day 1 or day 2 eggs were absent, one egg was added to the counts of day 1 or day 2 eggs at every positive station. The linear version of the exponential egg mortality model is:

\[ \ln P_b = \ln(P_i) - Zt \]  
\[ \text{Equation 3} \]

where, \( P_i \) is the density of eggs of age \( t \) at site \( i \) and \( Z \) is the instantaneous rate of egg mortality.

Estimates of \( P_b \) obtained using the linear version of the exponential mortality model have a strong negative bias, therefore a bias correction factor was applied following the equation of Picquelle and Stauffer (1985):

\[ P_0 = e^{\left(\ln P_b + \sigma^2 / 2\right)} \]  
\[ \text{Equation 4} \]

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

### 2.3 Adult Reproductive Parameters

#### 2.3.1 Sampling methods

Each afternoon areas where sardine schools were known to aggregate and suitable for gillnetting (i.e. adequately protected from the swell) were searched using a dual frequency echo sounder (Furuno - 60 and 180 KHz) (Fig. 1). The RV Ngerin was then anchored where several schools were observed. Samples of adults were collected using a gillnet comprising three panels, each with a different multi-filament nylon mesh size (double diamond: 210/4 ply meshes – 25, 28 and 32 mm). Surface and sub-surface lights (500 W) were illuminated near the net after
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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. Mature and immature males and females were counted. Mature females were fixed in 5% buffered formaldehyde solution. Immature females and males were frozen. Calculations of female weight, sex ratio, batch fecundity and spawning fraction were based on samples collected from Scotts Cove in Investigator Strait, North Neptune Island in southern Spencer Gulf and Pearson Island in the eastern Great Australian Bight.

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

\[
W = \frac{\sum W_i * n_i}{N}
\]

where, \(W_i\) is the mean female weight of each sample \(i\); \(n\) is the number of fish in each sample and \(N\) is the total number of fish collected in all samples.

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

2.3.4 Sex ratio (R)
The mean sex ratio of mature individuals in the population was calculated from the average of sample means weighted by sample size:
\[ R = \left[ \overline{R}_i \times \frac{n_i}{N} \right] \]  

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 \( i \). The mean sex ratio was also calculated for all years data from 1998 – 2011.

2.3.5 Batch fecundity (F)

Batch fecundity was estimated from ovaries containing hydrated oocytes using the methods of Hunter et al. (1985). Both ovaries were weighed and the number of hydrated oocytes in three ovarian sub-sections were counted and weighed. The total batch fecundity for each female was calculated by multiplying the mean number of oocytes per gram of ovary segment by the total weight of the ovaries. The relationship between female weight (ovaries removed) and batch fecundity was determined by linear regression analysis and used to estimate the batch fecundities of mature females in all samples.

2.3.6 Spawning fraction (S)

Ovaries of mature females were sectioned and stained with haematoxylin and eosin. Several sections from each ovary were examined to determine the presence/absence of post-ovulatory follicles (POFs). POFs were aged according to the criteria developed by Hunter and Goldberg (1980) and Hunter and Macewicz (1985). The spawning fraction of each sample was estimated as the mean proportion of females with hydrated oocytes plus day-0 POFs \((d0)\) (assumed to be
spawning or have spawned on the night of capture), day-1 POFs ($d1$) (assumed to have spawned the previous night) and day-2 POFs ($d2$) (assumed to have spawned two nights prior). The mean spawning fraction of the population was then calculated from the average of sample means weighted by proportional sample size.

\[ S = \left[ \frac{\sum S_i \cdot n_i}{N} \right] \quad \text{Equation 8} \]

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

\[ \overline{S_i} = \frac{[(d0 + d1 + d2 \text{POFs}) / 3]}{n_i} \quad \text{Equation 9} \]

where, $d0$, $d1$ and $d2$ POFs are the number of mature females with POFs in each sample and $n_i$ is the total number of females within a sample.

2.4 Spawning Biomass and Bootstrapping Procedures

2.4.1 Spawning biomass estimates

Spawning biomass was calculated according to Equation 1 using the estimate of $P_0$ obtained via the log-linear model and adult reproductive parameters collected during the 2013 survey, except that the mean value obtained between 2002 and 2013 was used as the estimate of sex ratio.
(R). Spawning biomass was also calculated using the spawning area obtained from the 2013 survey and the mean value obtained between 2002 and 2013.

2.4.2 Bootstrapping procedures and confidence intervals
To account for the covariance of adult parameters within individual samples, confidence intervals for all four adult parameters were calculated using a two stage bootstrap with 100,000 bootstrap iterations (Efron and Tibshirani 1993). For each iteration, the individual samples were resampled with replacement to obtain the bootstrapped samples. For each of the bootstrapped samples, the fish were resampled with replacement to generate a complete survey. The adult parameters W, S and R were calculated from the bootstrapped survey using the method described above. Batch fecundity (F) was calculated from the mean gonad-free weight using the batch relationship obtained by bootstrapping with replacement from females with hydrated oocytes. For each bootstrap iteration the value \( W / R . F . S \) was used in the calculation of bootstrapped confidence intervals for spawning biomass. The 95% confidence intervals of spawning biomass were estimated by calculating the spawning biomass 100,000 times from \( A \) and the 100,000 bootstrapped estimates of \( P_0 \) and \( \frac{W}{R . F . S} \) using the percentile method. Parameter estimates were calculated independently in Excel 2007 and R 2.9.2 with confidence intervals estimated with R 2.9.2.

2.5 Sensitivity Analysis
Sensitivity analyses were conducted by calculating spawning biomass across the range of values obtained for that parameter since 2002, when the population had recovered from the mass mortality events of the 1990s, while using all other parameters determined for 2013. Fecundity and mean female weight are correlated so these parameters were combined to form the parameter “relative fecundity”.
3. RESULTS

3.1. Biophysical Variables

3.1.1 Sea surface temperature

Sea surface temperatures (SSTs) ranged from to 19.1 to 22.8°C (Fig. 3) during February and March 2013. High SSTs (>19°C) were recorded in Spencer Gulf, Gulf St Vincent, south of Kangaroo Island and throughout the central Great Australian Bight.

Figure 3. Sea surface temperature profile across the 2013 February – March survey, showing stations where sardine eggs were collected (●).
3.1.2 Fluorescence (chlorophyll-a)

Chlorophyll-a concentration at each station ranged between 0.002 and 0.34 μg.L⁻¹ (Fig. 4) during February and March 2013. The highest values were recorded in western Spencer Gulf, eastern Gulf St Vincent and off Cape Adieu. The remainder of coastal and shelf waters mainly had chlorophyll-a concentrations ranging between 0.01 and 0.1 μg.L⁻¹.

Figure 4. Surface concentration of chlorophyll-a inferred from fluorescence readings across the 2013, February - March survey area, showing stations where sardine eggs were collected (●).
3.1.3 Zooplankton abundance
Total densities of zooplankton ranged between 0.05 and 8.8 ml.m$^{-3}$ (Fig. 5) during February and March 2013. The patches of zooplankton taxa observed on the outer shelf off western Eyre Peninsula were comprised mostly of salps.

Figure 5. Distribution and abundance (ml.m$^{-3}$) of zooplankton across the 2013, February - March survey area, showing stations where sardine eggs were collected (●).
3.2 Distribution and Abundance of Eggs and Larvae

3.2.1 Distribution and abundance of eggs

A total of 2,156 live sardine eggs were collected at 108 of 340 (31.8%) stations on 34 transects between the Head of Bight and Victor Harbor (Fig. 6) during February and March 2013. The stations with the highest egg densities were located in the mouth of Spencer Gulf, north of Coffin Bay Peninsula, and on the mid/outer shelf region of the eastern part of the Great Australian Bight. Egg densities up to 5,987 eggs m⁻² were recorded in these regions.

Figure 6. Spatial patterns of live sardine egg distribution and abundance during February and March 2013.
3.2.2 Larval abundance and distribution
A total of 1,867 sardine larvae were collected at 125 of 340 stations (36.7%) between the Head of Bight and Victor Harbor (Fig. 7) during February and March 2013. The spatial distribution of larvae was similar to that of sardine eggs. Densities were highest southwest of Anxious Bay, along the shelf break in the eastern Great Australian Bight, in southern Spencer Gulf, and south of Kangaroo Island, and ranged between 5 and 1,685 larvae.m\(^{-2}\).

Figure 7. Spatial patterns of sardine larval distribution and abundance during February and March 2013.
3.3 Spawning Area

The estimated spawning area for the entire survey area was 36,549 km$^2$, comprising 31.9% of the total area sampled (114,425 km$^2$, Table 1). The presence of high densities of eggs at stations on the seaward end of seven transects in the central and eastern GAB (Figure 6) suggests that the survey did not cover the entire area over which sardines spawned in 2013. The mean spawning area between 2002 and 2011 was 43,828 km$^2$ (Table 5) which suggests that the spawning area for 2013 could potentially have been under-estimated.

Table 1. Mean daily egg production ($P_0$, log-linear model), spawning area ($A$) and spawning biomass. Table shows the variance ($\sigma^2$) term used in estimate.

<table>
<thead>
<tr>
<th>Area sampled (km$^2$)</th>
<th>Spawning area A (km$^2$)</th>
<th>Percentage of area sampled</th>
<th>$\sigma^2 P_b$ (eggs.d$^{-1}$.m$^{-2}$)</th>
<th>$P_0$</th>
<th>Spawning biomass (t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total survey</td>
<td>114,425</td>
<td>36,549</td>
<td>31.9</td>
<td>1.15</td>
<td>44.3</td>
</tr>
</tbody>
</table>

3.4 Daily Egg Production ($P_0$)

The estimate of mean daily egg production, $P_0$ obtained using the linear version (Eq. 3) of the exponential egg mortality (recommended by Ward et al. 2011a) was 44.3 eggs.day$^{-1}$.m$^{-2}$ (95% CI = 30.3 – 67.1, Fig. 8, Table 1,5).
Figure 8. Linear regressions between ln-transformed sardine egg density (eggs.m$^{-2}$) and age (days) data in 2013.

$\ln \text{ Egg Density} = -0.346 \cdot \text{Age} + 3.219$

$R^2 = 0.115$
3.5 Adult Reproductive Parameters

A total of nine samples comprising 1,089 mature sardines were collected at Scotts Cove, Pearson Island and North Neptune Island during the 2013 survey (Table 2). Estimates of the adult female reproductive parameters used in calculations of spawning biomass are provided in Tables 3, 4 and 5. The means and ranges of adult parameters calculated from samples collected between 1998 and 2011 are provided in Table 5. Bootstrapped parameter estimates that provided 95% confidence intervals are shown in Table 5.

Table 2. Sampling details for adult sardine collected in Investigator Strait and the eastern Great Australian Bight during the 2013 DEPM surveys.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Survey</th>
<th>N samples</th>
<th>n fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>04/02/2011</td>
<td>Scotts Cove</td>
<td>1</td>
<td>3</td>
<td>489</td>
</tr>
<tr>
<td>07/03/2011</td>
<td>North Neptune Island</td>
<td>2</td>
<td>3</td>
<td>230</td>
</tr>
<tr>
<td>15/03/2011</td>
<td>Pearson Island</td>
<td>2</td>
<td>3</td>
<td>370</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>9</td>
<td>1089</td>
</tr>
</tbody>
</table>

3.5.1 Mean female weight

The mean weight of mature females in samples ranged from 43.5 to 58.9 g (Table 3). The weighted mean weight of mature females in 2013 was 51.2 g (95% CI = 46.1 – 55.4, Table 3, 5).

3.5.2 Sex ratio

The sex ratio calculated from the 2013 survey was the highest observed since 1998 (0.685, 95% CI = 0.63 – 0.74) (Table 4, 5). We consider this value to be biologically implausible and to reflect the preponderance of samples from schools of non-spawning fish. The mean sex ratio between 1998 and 2011 was 0.52 and ranged between 0.36 and 0.63 (Table 5).
Table 3. Number of sardine in samples by sex and estimates of female weight, $W$ and sex ratio, $R$ (proportion of females by weight) for samples collected in 2013. Values in bottom row are sums (*) and weighted means (#).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Date</th>
<th>Male</th>
<th>Female</th>
<th>Mean Male Weight</th>
<th>Mean Female Weight ($W$)</th>
<th>Sex Ratio by weight ($R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Scotts Cove</td>
<td>04/02/2011</td>
<td>51</td>
<td>97</td>
<td>48.7</td>
<td>58.4</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>Scotts Cove</td>
<td>04/02/2011</td>
<td>70</td>
<td>103</td>
<td>51.0</td>
<td>55.8</td>
<td>0.62</td>
</tr>
<tr>
<td>3</td>
<td>Scotts Cove</td>
<td>04/02/2011</td>
<td>54</td>
<td>114</td>
<td>48.1</td>
<td>54.9</td>
<td>0.71</td>
</tr>
<tr>
<td>4</td>
<td>N. Neptune Is.</td>
<td>07/03/2011</td>
<td>18</td>
<td>15</td>
<td>48.7</td>
<td>49.8</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>N. Neptune Is.</td>
<td>07/03/2011</td>
<td>45</td>
<td>123</td>
<td>51.3</td>
<td>54.6</td>
<td>0.74</td>
</tr>
<tr>
<td>6</td>
<td>N. Neptune Is.</td>
<td>07/03/2011</td>
<td>9</td>
<td>20</td>
<td>53.3</td>
<td>58.9</td>
<td>0.71</td>
</tr>
<tr>
<td>7</td>
<td>Pearson Is.</td>
<td>15/03/2011</td>
<td>26</td>
<td>65</td>
<td>39.9</td>
<td>43.5</td>
<td>0.75</td>
</tr>
<tr>
<td>8</td>
<td>Pearson Is.</td>
<td>15/03/2011</td>
<td>31</td>
<td>92</td>
<td>38.3</td>
<td>43.7</td>
<td>0.77</td>
</tr>
<tr>
<td>9</td>
<td>Pearson Is.</td>
<td>15/03/2011</td>
<td>62</td>
<td>94</td>
<td>40.9</td>
<td>40.7</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>366*</td>
<td>723*</td>
<td>46.5#</td>
<td>51.2#</td>
<td>0.685#</td>
</tr>
</tbody>
</table>
3.5.3 Batch fecundity

Batch fecundity ranged from 7,564 to 38,200 hydrated oocytes for the 54 hydrated female sardines examined in 2013. Based on the relationship (Batch Fecundity = 311.0 x Gonad Free Female Weight + 1,653.9, $R^2 = 0.518$, Fig. 9) and the mean gonad free female weight (49.0 g) for all samples collected in 2013, mean batch fecundity was 16,902 hydrated oocytes per batch (95% CI = 14,983 – 18,616, Table 5).

![Figure 9. Relationship between gonad-free weight and batch fecundity in 2013](dotted line = 95% CI).

3.5.4 Spawning fraction

Of the 723 ovaries examined, 77 had hydrated oocytes and/or day-0 POFs, 21 had day-1 POFs and 54 day-2 POFs (Table 4). The spawning fraction of females in samples ranged from 0.000 to 0.222. The weighted mean spawning fraction for all 2013 data was 0.070 (95% CI = 0.038 – 0.101). For 2002 – 2011, the mean spawning fraction was 0.108 and ranged between 0.044 and 0.170 (Table 5).
### Table 4. Number of female sardine in samples and estimates of spawning fraction (S) for samples collected in 2013. Values in bottom row are sums* and weighted means#.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Date</th>
<th>POF 0</th>
<th>POF 1</th>
<th>POF 2</th>
<th>Total</th>
<th>Spawning Fraction (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Scotts Cove</td>
<td>04/02/2011</td>
<td>14</td>
<td>6</td>
<td>11</td>
<td>97</td>
<td>0.107</td>
</tr>
<tr>
<td>2</td>
<td>Scotts Cove</td>
<td>04/02/2011</td>
<td>8</td>
<td>5</td>
<td>17</td>
<td>103</td>
<td>0.097</td>
</tr>
<tr>
<td>3</td>
<td>Scotts Cove</td>
<td>04/02/2011</td>
<td>3</td>
<td>4</td>
<td>20</td>
<td>114</td>
<td>0.079</td>
</tr>
<tr>
<td>4</td>
<td>North Neptune Island</td>
<td>07/03/2011</td>
<td>9</td>
<td>1</td>
<td></td>
<td>15</td>
<td>0.222</td>
</tr>
<tr>
<td>5</td>
<td>North Neptune Island</td>
<td>07/03/2011</td>
<td>36</td>
<td>1</td>
<td>3</td>
<td>123</td>
<td>0.108</td>
</tr>
<tr>
<td>6</td>
<td>North Neptune Island</td>
<td>07/03/2011</td>
<td>2</td>
<td>3</td>
<td></td>
<td>20</td>
<td>0.083</td>
</tr>
<tr>
<td>7</td>
<td>Pearson Island</td>
<td>15/03/2011</td>
<td>4</td>
<td></td>
<td></td>
<td>65</td>
<td>0.021</td>
</tr>
<tr>
<td>8</td>
<td>Pearson Island</td>
<td>15/03/2011</td>
<td></td>
<td></td>
<td></td>
<td>92</td>
<td>0.000</td>
</tr>
<tr>
<td>9</td>
<td>Pearson Island</td>
<td>15/03/2011</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>94</td>
<td>0.018</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>77*</td>
<td>21*</td>
<td>54*</td>
<td>723*</td>
<td>0.070#</td>
</tr>
</tbody>
</table>

* Sums
# Weighted means
Table 5. Parameters used in the calculations of spawning biomass. Values for 2013 and the mean, minimum and maximum for 2002 to 2011 are presented (for spawning area 2004 is excluded as the survey was not completed in this year).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2013 (95% CI)</th>
<th>Mean 2002-2011 (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg Production (Po, eggs.day⁻¹.m⁻²)</td>
<td>44.3 (30.3 – 67.1)</td>
<td>74.9 (44.0 – 120.9)</td>
</tr>
<tr>
<td>Sex Ratio (R)</td>
<td>0.68 (0.63 – 0.74)</td>
<td>0.52 (0.36 – 0.63)</td>
</tr>
<tr>
<td>Fecundity (F, eggs.female⁻¹)</td>
<td>16,902 (14,983 – 18,616)</td>
<td>18,404 (10,904 – 24,790)</td>
</tr>
<tr>
<td>Spawning Fraction (S)</td>
<td>0.070 (0.037 – 0.101)</td>
<td>0.108 (0.044 – 0.170)</td>
</tr>
<tr>
<td>Female Weight (W, g)</td>
<td>51.2 (46.1 – 55.4)</td>
<td>63.6 (46.6 – 78.7)</td>
</tr>
<tr>
<td>Spawning Area (A, km²)</td>
<td>36,549</td>
<td>43,828 (34,433 – 53,553)</td>
</tr>
</tbody>
</table>

3.6 Re-sampling: Bootstrapping Procedures

The distributions for each variable calculated using ‘bootstrap replacement’ procedures and the percentile method are shown in Table 5.

3.7 Spawning Biomass

The estimate of spawning biomass, calculated using data from 2013 and the mean sex ratio recorded between 1998-2011 of 0.52 (95% CI = 38.0 – 65.9) was 135,484 t (95% CI = 78,854 – 287,533; Table 1, Fig. 10). When alternate models were used to estimate egg production the estimates of spawning biomass were higher (Fig. 10). When the mean value of spawning area for 2002-2013 was used in the calculations the estimate of spawning biomass was 162,645 t.
Figure 10. Estimates of spawning biomass obtained for 2013 using estimate of egg production obtained with four alternate egg models considered in Ward et al. (2009b). Error bars are 95% confidence intervals, red dotted line is the 150,000 and 300,000 t trigger points for the TACC, green dotted line is the 2013 TACC according to the current harvest strategy.

3.8 Sensitivity Analysis

Sensitivity analyses show where values of parameters estimated from the 2013 surveys lie in comparison to the range obtained in surveys conducted since 2002 (Fig. 11). The sex ratio (R) was the highest observed since 2002. Estimates of sex ratio are correlated with spawning fraction (Fig. 12) and may reflect relative encounter rates with male-dominated spawning and female-dominated non-spawning schools. We consider that the estimate of sex ratio for 2013 reflects the over-representation of fish from female-dominated non-spawning schools in samples during a year when spawning fraction was low. As we have done in previous years, when either males or females have dominated samples, we have used the historical mean value of sex ratio to calculate spawning biomass.
Egg production ($P_0$) was particularly low in 2013, being only marginally higher than the lowest value determined since 2002. Spawning fraction ($S$) was also relatively low, being the third lowest value since 2002. The low level of egg production is largely explained by the low level of spawning activity as evidenced by the low spawning fraction. The co-occurrence of low values of each of these two parameters offsets their effects on the estimates of spawning biomass. Although the measures of these two parameters are relatively imprecise, we consider that they are likely to provide unbiased estimates of the two parameters and are suitable for use in calculation of the spawning biomass.

Spawning area ($A$) was the lowest observed since 2002 and probably reflects the high water temperatures recorded on the shelf in 2013. Spawning area is the primary driver of the low estimate of spawning biomass for 2013. The presence of high densities of eggs at stations on the seaward end of seven transects in the central and eastern Great Australian Bight (Fig. 6) provides strong evidence that the survey did not cover the entire area over which sardines spawned in 2013. For this reason, we suggest that the spawning biomass estimate obtained by using the estimate of spawning area measured in 2013 should be considered as the minimum likely value. The potential impact of incomplete sampling of the spawning area on the estimation of the spawning biomass was evaluated by using the mean value of spawning area for 2002-2013 in the calculations (Table 5).
Figure 11. Sensitivity analysis of the effect of varying each parameter on biomass (in each case the other parameters are those of the 2013 survey). Female weight and fecundity are combined to eggs/g as the two are correlated. The red arrow is the value determined in 2013. Black arrows indicate the minimum, maximum and median values observed since 2002.
Figure 12. Relationship between spawning fraction and sex ratio for DEPM surveys between 1998 and 2013.
4. DISCUSSION

4.1 Biophysical Variables and Egg and Larval Distribution Patterns

The lowest SST recorded in the 2013 survey was 19.1°C, which is well above the minimum value obtained in previous surveys and the values historically associated with the upwelling of cold water (i.e. 16-17°C, Ward et al. 2009c). In the absence of upwelled water, SSTs on the shelf were several degrees higher than those observed in previous surveys (Fig. 13). The thermocline was also poorly defined and located at greater depths than in previous years (SARDI, unpublished data), providing further evidence that upwelling was comparatively weak during the 2013 surveys. Surface chlorophyll-a concentrations were low with no readings higher than 0.35 µg.L⁻¹ and the majority of the surveyed area having concentrations of <0.01 µg.L⁻¹. There were no discernable areas with increased concentrations of chlorophyll-a in the eastern GAB where seasonal increases in productivity are often observed (McClatchie et al. 2006). Plankton densities were also low across the entire survey. Collectively, these findings suggest that productivity levels during February and March 2013 were below those usually recorded in shelf waters off South Australia during summer-autumn.

As has been the case in previous years, the highest SSTs recorded during the surveys (>22°C) were from sites located in the two gulfs. However, SSTs in 2013 were not particularly high compared to previous years (Fig. 13) although it should be noted GSV was sampled in February whereas abnormally high temperatures reported elsewhere were recorded during late March after a prolonged period of hot weather (PIRSA 2013).

Like most DEPM surveys, egg samples collected in 2013 (total of 2,159 live eggs) were strongly over-dispersed with a few samples containing very high numbers of eggs and many samples containing no eggs. Overall, egg densities were generally lower than in recent years (e.g. Ward et al. 2009c). The number of positive stations was also relatively low. Stations where eggs were collected were widespread across the survey area. Notably, eggs were present in inshore waters between Coffin Bay and Streaky Bay where eggs are often absent, especially in strong upwelling years when SSTs in this area are low. The egg distribution pattern observed in 2013 is somewhat similar to other years of weak upwelling, such as 2005 and 2011 (Ward et al. 2011b).
The abundance of sardine eggs and larvae in 2013 was low at most stations located on transects in Gulf St Vincent, eastern Investigator Strait and Spencer Gulf, especially north of Wedge Island. There were high egg abundances in three areas: southern Spencer Gulf and western Investigator Strait, where high densities occurred over a relatively limited area; the inner shelf in the eastern Great Australian Bight where high egg densities were patchily distributed; and from the mid-shelf to the shelf break in the eastern and central Great Australian Bight, where eggs were found on the outermost stations of seven transects. This presence of high egg densities on the seaward end of these transects suggests that the distribution of eggs in 2013 may have extended beyond the area covered by the survey.
4.2 Spawning Area

The estimate of spawning area in 2013 (36,549 km², Table 1; 5) is the lowest since 2002 (34,433 km², Ward 2011a) and considerably lower than the average spawning area over the last decade (43,828 km², Table 5), perhaps because the survey did not cover the entire area over which spawning occurred (i.e. the area beyond the outmost stations on transects in the eastern and central Great Australian Bight). Importantly, eggs were sparsely distributed on the shelf where water temperatures were unusually high and large quantities of eggs have been collected historically.

The relatively low egg abundances in Spencer Gulf, Gulf St Vincent and eastern Investigator Strait are more difficult to explain. Abnormally high water temperatures were not recorded in these areas during surveys. However, during the second survey localised mortalities of small benthic fish species were recorded in shallow inshore areas and high water temperatures were identified as the possible cause (PIRSA 2013). Dead sardines were not observed during these mortality events. Low egg densities recorded in these areas may suggest that sardines had moved out of these areas during this period.

4.3 Egg Production

The estimate of egg production \( (P_0) \) obtained using the linear version of the exponential egg mortality model, which is the method that has been recommended for sardine in SA waters by Ward et al. (2011a), was 44.3 eggs. day\(^{-1}.m^{-2} \) (95% CI = 30.3 – 67.1). This is similar to the value of 44.0 eggs. day\(^{-1}.m^{-2} \) that was estimated for 2011 which was the lowest estimate of egg production since the year immediately following the mass mortality events. Application of the other models investigated by Ward et al. (2011a) generally provided higher estimates of egg production, e.g. 58.9 eggs. day\(^{-1}.m^{-2} \) for the exponential model and 75.8 eggs. day\(^{-1}.m^{-2} \) for GLM 3.

4.4 Adult Sampling

During the 2013 survey, nine samples of adult sardines containing 723 females were collected from Scotts Cove, North Neptune and Pearson Island. No fish were collected west of Pearson Island. The limited number of sampling sites in the west reflects the paucity of locations (i.e. protected bays off islands) that are suitable for sampling adults using the existing method (i.e.
gillnet and lights). As a significant portion of the spawning biomass of sardines off SA occurs in areas where there are few locations suitable for the deployment of existing sampling tools, the development of alternative sampling methods remains a high priority for the SASF.

Several of the adult samples collected during 2013 (and 2011) included relatively few males and many non-spawning females, whereas samples obtained in 2009 contained a high proportion of males and actively spawning females (Ward et al. 2011b). Small ephemeral schools of sardine are known to form to undertake spawning (Statoudakis 2006). These schools are dominated by males and include a high proportion of spawning females (like the 2009 samples). Conversely, the remainder of the population in non-spawning schools includes a low proportion of males and many non-spawning females (like samples collected in 2011 and 2013). Encounter rates of non-spawning schools (proportion of females in samples) appears to be related to spawning fraction (Fig. 12). Increased encounter rates with non-spawning schools during years of low spawning activity appear to explain the biologically implausible estimates of sex ratio (R) obtained in 2013 and some other years (e.g. 2011). Conversely, increased encounter rates with spawning schools appear to explain the high proportion of males in samples collected in 2009.

The methods used in this report for calculating spawning fraction were explicitly established to reduce the effects of variations in encounter rates with different school types on estimates of spawning fraction. As noted by Ward et al. (2011a), the use of females with hydrated oocytes and all POF stages (d 0,1,2) in the calculations reduces the potential effects of biases resulting from the differential sampling of fish from spawning and non-spawning schools on estimates of spawning fraction.

**4.5 Spawning Biomass Estimates**

The estimate of spawning biomass for 2013 of 135,484 t (95% CI = 59,400 – 216,801) was calculated using estimates of five parameters obtained from the 2013 surveys and the average sex ratio obtained in all surveys conducted since 1998. The estimate of sex ratio obtained for 2013 was not used in this calculation because it was considered to be reflective of encounter rates with actively spawning schools during a year with relatively low spawning fraction activity (fraction) rather than a sudden change in the sex ratio in the population. A similar approach has been taken in previous years when spawning fraction has been either high or low and males or females, respectively, have dominated samples resulting in a skewed sex ratio.
Estimates of egg production and spawning area were both low compared to recent years (Table 5). The low $P_o$ value reflects the low level of spawning activity during 2013 (as indicated by the low spawning fraction) and we consider that these values are unbiased estimates of these parameters. Notably, the concurrent occurrence of low values of these two parameters offsets their individual effects on the estimates of spawning biomass.

Spawning biomass is strongly correlated with spawning area (Gaughan et al. 2004). As discussed above, it is likely that the 2013 survey did not cover the entire area over which spawning occurred. The potential effect of this limitation on the estimate of spawning biomass was examined by using the average spawning area between 2002 and 2011 in the calculations. We suggest that the estimates of spawning biomass obtained using the 2013 and mean 2002-11 values of spawning area, i.e. 135,484 t and 162,645 t, should be used as the minimum and maximum estimates of mean spawning biomass for 2013, respectively.

The range of mean spawning biomass estimates for 2013 (i.e. 135,484 t – 162,645 t) includes the reference point of 150,000 t identified in the current harvest strategy for the fishery. It is well below the estimates of mean spawning biomass obtained since 2006, which have consistently been above 170,000 t (Fig. 14) and were used as the basis for increasing the TACC above the 30,000 t baseline level identified in the current harvest strategy. The survey on which these estimates of spawning biomass are based was conducted immediately following a period during which the size of fish taken in commercial catches on the main fishing grounds declined to a level that has not been seen since the years immediately following the mass mortality events of the 1990s (e.g. Ward et al. 2012). Hence, the reduction in the estimates of spawning biomass may reflect a real decline in the status of the population. However, it is uncertain whether the spawning biomass has fallen below the reference point of 150,000 t identified in the current harvest strategy. Maintaining the TACC at the current level of 34,000 t in 2014 would result in an exploitation rate of more than 20%, which is above the reference point in the current harvest strategy and beyond the low level of risk to the stock that was used as part of the justification for moving from annual to biennial DEPM surveys.
Figure 14. Spawning biomass estimates for sardine in South Australian water from 1994 to 2013, including the 2013 estimate using the mean spawning area for surveys between 2002 and 2011 (x). Error bars are 95% confidence intervals. The red line indicates the 150,000 t reference point.

4.6 Future Research Directions

It is difficult to separate the extent to which the low estimate of spawning biomass for 2013 reflects: 1) the imprecision of the methodology; 2) failure to cover the entire spawning area; and/or 3) a decline in adult abundance. The quantum of the effect of not covering the entire spawning area was calculated by comparing estimates obtained from the 2013 survey and the average spawning area over the last decade. The effect of imprecision in the estimation of individual parameters of the estimate of spawning biomass is depicted by the results of the sensitivity analysis.

Improving the precision of estimates of spawning biomass used to management the SASF remains a high priority for the SASF. Options for improving the precision of these estimates could involve developing methods for using data collected from a Continuous Underway Fish
Egg Sampler (CUFES) to estimate mean daily egg production. Habitat models could also be developed to identify the probability of spawning occurring in areas not sampled during egg surveys. Other options include the development of alternative methods for sampling adult sardines. The development of these alternative adult sampling methods would ideally be done in collaboration with members of the SASF and involve comparisons of estimates of adult parameters obtained by gillnetting, trawling and purse-seining. Consideration could also be given to using estimates of spawning biomass derived from population models as the key biological indicator for the SASF.
REFERENCES


