Guidelines for using the daily egg production method for stock assessment of sardine, *Sardinops sagax*, off South Australia

T.M. Ward, P. Burch, L.J. McLeay and A.R. Ivey

Report to PIRSA Fisheries

SARDI Publication No. F2009/000249-1
SARDI Report Series No. 355

T.M. Ward, P. Burch, L.J. McLeay and A.R. Ivey (2009). Guidelines for using the daily egg production method for stock assessment of sardine, *Sardinops sagax*, off South Australia. SARDI Publication No. F2009/000249-1 SARDI Research Report Series No. 355

South Australian Research and Development Institute (SARDI)
Aquatic Sciences Division
2 Hamra Avenue
West Beach, South Australia 5024

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

SARDI Publication No. F2009/000249-1

SARDI Research Report Series No. 355

Printed in Adelaide, April 2009

Authors: A/Prof. Timothy M. Ward, Paul Burch, Lachlan J. McLeay and Alex Ivey

Reviewers: Dr Tony Fowler, Dr Mike Steer and Dr Stephen Mayfield

Approved by: Dr Marty Deveney

Signed:
Date: 5 May 2009

Distribution: Primary Industries and Resources South Australia, Fisheries
South Australian Research and Development Institute, Aquatic Sciences
South Australian Sardine Industry Association

Circulation: Public Domain

Table of Contents

List of Tables

List of Figures

Acknowledgments

We thank the master of the *RV Ngerin*, Neil Chigwidden and crew Mr Dave Kerr, Mr Chris Small, and Mr John Collinson, for their efforts during the research surveys. Mr Dave Griffith (National Oceans Atmospheric Agency, USA) provided technical assistance during research surveys. Dr Rick McGarvey provided valuable statistical advice. Dr Tony Fowler, Dr Mike Steer and Dr Stephen Mayfield (SARDI Aquatic Sciences), and Michelle Besley (PIRSA Fisheries) reviewed a draft of the report, which was formally approved for release by Dr Marty Deveney (SARDI Aquatic Sciences).

Executive Summary

This study reanalyses data collected between 1998 and 2007 to establish guidelines using the Daily Egg Production Method (DEPM) for stock assessment of sardine, *Sardinops sagax*, off South Australia. Our analyses confirm that estimates of spawning biomass (*SB*) are imprecise and show that if inappropriate analytical methods are used they may also be biased. Estimates of *SB* are most affected by variation in estimates of mean daily egg production (P_0), total spawning area (*A*) and mean spawning fraction (*S*). The log-linear egg mortality model (with one egg added to each day class of eggs at each positive site) is the best method currently available for estimating P_0 because it fits strongly over-dispersed sardine egg density data better and provides more logically consistent and precautionary estimates of P_0 than the exponential mortality model the Generalized Linear Models (GLM) that we tested. GLMs of untransformed data produced much estimates of P_0 that the log-linear model when egg data were strongly over-dispersed. To estimate *A* reliably, it is critical that the surveyed area is sub-divided into a large number (e.g. 300) of similar sized grids. The Voronoi natural neighbour method should be used to estimate *A*, because it reduces subjectivity in sub-division of the sampling area. Potential biases in *S* resulting from over-representation in samples of females with one stage of post-ovulatory follicles (POF) can be minimised by calculating this parameter as the mean of females with ovaries that contain all three POF stages. Research priorities to improve the precision of estimates of spawning biomass include: developing methods for using a continuous underway fish egg sampler to enhance estimation of P_0 and *A*; measuring the degeneration rates of POFs in water bodies with different physical and biological characteristics; and comparing the vulnerability of females with different POF stages to capture in various sampling gears.

Introduction

The Daily Egg Production Method (DEPM) was originally developed for direct stock assessment of the northern anchovy, *Engraulis mordax*, off the west coast of North America (Parker 1980). The method 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:

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

The DEPM can be applied to fishes that spawn multiple batches of pelagic eggs over an extended spawning season (Parker 1980). Data used to estimate DEPM parameters are typically obtained during fishery-independent surveys. The key assumptions of the method are that: 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).

The DEPM has been used for stock assessment for at least fifteen species of small pelagic fishes, mostly clupeoids (e.g. Stratoudakis et al. 2006). Although the method 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). The imprecision that characterises the DEPM 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; Gaughan et al. 2004; Stratoudakis et al. 2006). However, many studies have also been impeded by difficulties associated with obtaining representative samples of spawning adults for estimation of adult reproductive parameters, especially S (see Stratoudakis et al. 2006). There are relatively few published examples where the DEPM has been applied for extended periods and robust estimates of all parameters have been obtained consistently.

At least two reviews have concluded that the DEPM may be better tailored to anchovies (*Engraulis* spp.) than to 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 of the season, daily fecundity can be estimated more precisely for anchovy than sardine (e.g. Alheit 1993; Stratoudakis et al. 2006). Despite the apparent limitations of the method for stock assessment of sardine, the DEPM is currently considered the best technique available for this species off the west coast of North America (e.g. Lo et al. 1996, 2005) and the western and southern coasts of Australia (Fletcher et al. 1996; Ward et al. 2001b; Gaughan et al. 2004). However, there are important differences between locations in the manner in which egg and adult samples are collected, data are

analysed and estimates of spawning biomass used to support fisheries management. For example, 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.* 2001b). Furthermore, estimates of SB obtained from DEPM surveys are used directly for fisheries management in South Australia, but are incorporated into age-structured stock assessment models in California and Western Australia.

Shelf waters off South Australia (Fig. 1) form part of the world's only northern boundary current system (Middleton and Cirano 2002; Kaempf et al. 2004; McClatchie et al. 2006) and support the highest levels of primary, secondary and fish production in Australian waters (Ward et al. 2001a, b). Australia's largest single-species fishery (by weight), the South Australian Sardine Fishery (SASF), was established in 1991 to provide fodder for the ranching of wild-caught southern blue fin tuna (*Thunnus maccoyi*). A Total Allowable Catch (TAC) is set annually and each of the 14 licence holders is allocated an equal Individual Transferable Quota. There are also some input controls, such as a maximum net size. The total catch in 1991 was less than 10 t and this increased to ~3,500 t in 1995. Estimates of spawning biomass obtained using the DEPM have been used to set the TAC since 1996. The fishery has grown quickly despite mass mortality events in 1995 and 1998 that each killed over 70% of the adult population (Ward et al. 2001a; b). Annual catches since 2004 have ranged between approximately 25,000 and 42,500 t. The TAC has been set at 30,000 t

since 2006. Under the current management arrangements the TAC will remain at 30,000 t while estimates of spawning biomass remain between 150,000 and 300,000 t.

This paper re-analyses data collected between 1998 and 2007 to establish guidelines for using the DEPM to estimate the spawning biomass of sardine (*Sardinops sagax)* in waters off South Australia. To do this, estimates of each DEPM parameter are calculated using the full range of data available (i.e. samples obtained from research and commercial vessels) and the main analytical methods described in the literature. The most appropriate sampling methods and statistical approaches are identified on the basis of model diagnostics and the logical consistency of inter-annual trends. Estimates of each parameter obtained using the most appropriate sampling method and statistical approach are used to calculate estimates of *SB* between 1998 and 2007. The relative importance of each parameter as a determinant of *SB* is estimated by dividing the range of estimates obtained over the ten years by the overall mean value. The scales of potential biases in estimates of *SB* resulting from the use of sub-optimal methods to estimate each key parameter are also calculated. Research priorities to enhance future stock assessment of sardine using the DEPM are identified.

Methods

Total Daily Egg Production

Plankton Sampling

Plankton samples for application of the DEPM have been collected each year using paired Californian Vertical Egg Tow (CalVET) nets (0.225 m diameter in 1998-2003, 0.300 m diameter in 2004-2007, 300 μm mesh). Sampling design varied among years (Table 1, Fig.2). CalVET nets were deployed to within 10 m of the seabed in waters less than 80 m deep and to 70 m in depths greater than 80 m, and retrieved at approximately 1.0 ms^{-1}. General Oceanics™ flow-meters were used to estimate the distance travelled by each net. Samples from the two cod-ends were combined and stored in 5% buffered formaldehyde and seawater solution.

Egg density

Sardine eggs obtained in each plankton sample were assigned to stages (1-12) according to the criteria identified in White and Fletcher (1996) and counted. The number of eggs of each stage under one square metre of water (P_t) was estimated at each site according to equation 2:

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

where C is the number of eggs of each age in each sample, V is the volume filtered (m^3), and D is the depth (m) to which the net was deployed (Smith and Richardson 1977). The densities of day-1 and day-2 eggs were weighted according to the relative size of the area from which each sample was taken (see section on spawning area below).

Spawning Time and Egg Ages

A reliable model relating egg development rate to temperature has not been developed for sardine in Australian waters. Peak spawning time and the modal age of each egg stage were estimated for all samples combined and for three temperature ranges (<19°C, 19-20°C, >20°C) using kernel density smoothing to estimate the relative abundance of each egg stage by sampling time.

The time of peak abundance for each egg stage between Stages 2 and 11 (White and Fletcher (1996) was estimated by combining samples for all years and using a Kernel density estimation function with a Gaussian (normal) kernel and a bandwidth of three hours (Simonoff 1998). The ages of eggs (in days) in each sample were estimated in three ways. Firstly, the effects of temperature on development rates were ignored and the age of all day-1 eggs (assumed to be Stages 1-7) was estimated by subtracting the estimated the assumed spawning time (0200 hours) from the collection time. The age of day-2 eggs (Stages 8-12) was also estimating by subtracting the spawning time from the collection time and adding 24 hours to each age estimate. Secondly, the same approach was applied with the additional assumption that in waters in <19^{o}C, 19-20^{o}C and >20^{o}C, day-1 eggs were Stages 1-6, 1-7 and 1-8, respectively. Thirdly, based on this assumption each sample was divided into Day-1 and Day-2 eggs, and all eggs from each day were aggregated into the stage with the maximum number of eggs (in the case of a tie to the earlier stage). Each egg stage was then assigned the age of the modal time obtained in each temperature range in the kernel density plot. The effects on estimates of egg production of using different methods to estimate egg age were examined by fitting the log-linear model (see below) to weighted egg density with the estimates of egg age.

Spawning area (A)

For each year, two methods were used to establish a contiguous series of grid areas that each included a single sampling site (Fig. 1). Firstly, in MAPINFO Version 8 the sampling area was manually divided into a series of contiguous grids located around each sampling site (Fig. 1) using the method described by Lasker (1985). Secondly, the Voronoi natural neighbour (VNN) method (Watson 1981) was implemented in MAPINFO Version 8 to generate a polygon around each sampling site with the boundary as the midpoint equidistant between the survey sites (Fig. 1). For each year *A* was estimated by summing separately the area of all grids generated using the Lasker (1985) and the VNN methods, based on the presence of live eggs.

Mean daily egg production (P_0)

P_0 and mean daily egg mortality (Z) were estimated using the exponential model (Lasker 1985), a log-linear model (Lasker 1985) and several generalized linear models (ICES 2004; Stratoudakis et al. 2006; Wood 2006). The log-linear model was fitted to data-sets from which 1) all zero values were excluded and 2) to which one egg was added to each day class of eggs (Day-1, Day-2) at each site where at least one egg was collected. In cases (models and years) when Z could not be estimated reliably, P_0 was estimated by assigning a value of Z equal to the mean value obtained in all other years.

Non-linear least squares regression with starting values of P_0 and Z were 100 and 0.5, respectively, was used to solve the exponential egg mortality model of Lasker (1985):

$$P_t = P_0 e^{-zt}, \qquad \text{Equation 3}$$

where P_t is density of eggs of age *t* and *Z* is the instantaneous rate of daily egg mortality.

Using the log-linear model, biased mean daily egg production (P_b) was calculated by fitting a linear regression to ln-transformed estimates of egg density by age at each site (Picquelle and Stauffer 1985):

$$\ln P_b = \ln(P_i) - Zt, \qquad \text{Equation 4}$$

where P_i is the density of eggs of age *t* at site *i* and *Z* is the instantaneous rate of daily egg mortality. As estimates of P_b obtained using the log-linear model have a negative bias, a bias correction factor was applied following the equation of Picquelle and Stauffer (1985):

$$P_0 = e^{(\ln P_b + \sigma^2/2)} \qquad \text{Equation 5}$$

where σ^2 is the variance of the estimate of P_b.

P_0 was estimated using four GLMs suggested in the literature (McCullagh and Nelder 1983; ICES 2004; Stratoudakis et al. 2006; Wood 2006). For observed egg densities (Pi), the generalized linear models were of the form:

$$g(u) = P_0 - zt, \qquad \text{Equation 6}$$

where u is the link function from an exponential family distribution such that $u_i \equiv E(P_i)$. GLM 1 assumed a Gaussian distribution with a log link function. If the data followed a log-normal distribution, this model would be expected to give similar results to the log-linear model. GLM 2 assumed a Quasi distribution with a log link function and variance proportional to the mean as recommended for overdispersed egg density data (Wood 2006). GLM 3 assumed a Quasi distribution with a log link function and variance proportional to the mean squared, because the residual plots from GLM 2 still showed signs of over-dispersion. GLM 4 was applied to ln-transformed egg density data assuming a Quasi distribution with identity link function and variance proportional to the mean. As estimates of P_0 obtained using GLM 4 were negatively biased, we applied the same bias correction factor that we applied for the log-linear model (Eqn. 5, variance assumed to equal residual deviance).

To compare the fit of the seven models to the observed data over the ten year period, especially to determine how the models coped with the over-dispersion of data, particularly the small number of samples with very high egg densities, we plotted residuals and calculated standard errors, coefficients of variation and 95% confidence intervals using the bootstraps methods described below.

Confidence Intervals

As the egg data were over-dispersed, confidence intervals were calculated by bootstrapping (Efron and Tibshirani 1993). A total of 100,000 bootstraps were generated from each data pair with confidence intervals calculated using the percentile method.

Mean daily fecundity

Adult sampling

Between 1998 and 2007, samples of mature *Sardinops sagax* were collected using a multi-panelled gillnet described in Ward et al. (2001b). Each afternoon, areas where sardine schools aggregate were searched using a dual frequency echo sounder (*Furuno* - 60 and 180 KHz). The *RV* Ngerin was anchored in an area where several schools were observed. The gillnet comprised of three panels, each with a different multi-filament nylon mesh size (Double Diamond: 210/4 ply meshes – 25, 28 and 32mm). Surface and sub-surface lights (500 W) were illuminated after the net was set. Soak time varied from 15 minutes to 3 hours depending on the number of sardine caught. After the net was retrieved, sardine were removed and dissected immediately. Males and immature females were counted and frozen. Mature females were fixed in 5% buffered formaldehyde solution. Frozen samples of both sexes were also obtained from commercial catches taken by purse-seine vessels in the SASF.

Mean female weight (W)

Mature females from each research sample were removed from formalin and weighed (± 0.01 g). *W* was calculated from the average of sample means weighted by proportional sample size:

$$W = \left[\overline{W_i * \frac{n_i}{N}} \right], \qquad \text{Equation 7}$$

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

commercial samples were calculated in the same way using mature females captured in the months of February and March to be consistent with the timing of research samples.

Sex ratio (R)

For research samples, *R* was calculated from the average of sample means weighted by proportional sample size:

$$R = \overline{\left[\overline{R_i} * \frac{n_i}{N} \right]}, \qquad \text{Equation 8}$$

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

$$\overline{R_i} = \frac{F}{(F+M)}, \qquad \text{Equation 9}$$

where F and M are the respective total weights of mature females and males in each sample i. Sex ratios for commercial samples were calculated in the same way using all mature fish captured in the months of February and March to be consistent with the timing of research samples.

Batch fecundity (F)

F was estimated from ovaries containing hydrated oocytes using the gravimetric method (Hunter and Macewicz 1985). Both ovaries were weighed and the number of hydrated oocytes in three ovarian sub-sections (proximal, central and distal) were

counted and weighed. 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. To determine the relationship between female weight (ovaries removed) and batch fecundity, three models were tested: a linear regression, a Gamma GLM and a Negative Binomial GLM, both with identity link functions (ICES 2004). The optimal model over all ten years was selected by comparing the pseudo R^2 statistic (1 – Residual Deviance/Null Deviance, Swartzman et al. 1992). The optimal model was used to estimate *F* for each year from the gonad-free female weights from individual samples collected in that year.

Samples obtained from commercial vessels did not include females with hydrated oocytes. *F* for commercial samples was calculated by applying the model obtained from fishery independent samples to the estimates of mean gonad-free weight of mature females collected in samples obtained in February and March for each year that data were available.

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). *S* of each sample was estimated in four ways, the proportion of day-0 females (i.e. with hydrated oocytes plus POFs assumed to be 0-24 hrs old), the proportion of day-1 females (i.e. with POFs assumed to be 24-48 hrs old), the proportion day-2 females (i.e. with POFs assumed to be 48+ hrs old), and the mean number of day-0, day-1 and day-2 females

divided by the total number of females in the sample. The mean spawning fraction of the population was calculated from the average of sample means weighted by proportional sample size.

$$S = \overline{\left[\overline{S}_i * \frac{n_i}{N} \right]}, \qquad \text{Equation 10}$$

where n_i 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:

$$\overline{S}_i = \frac{[\sum d_j]}{m.n_i}, \qquad \text{Equation 11}$$

where d_j is the number of mature female sardine with POFs of either day-0, day-1 or day-2 found in each sample, m is 1 where the individual number of day-0, day-1 or day-2 females is used and 3 when the sum of day-0, day-1 and day-2 is used and n_i is the total number of female sardine within a sample.Spawning fraction could not be estimated from frozen samples obtained from commercial vessels.

Confidence Intervals

To allow for the covariance of adult parameters within individual samples, confidence intervals for all four adult parameters were calculated using a two stage bootstrap (Efron and Tibshirani 1993) with 100,000 bootstrap iterations. For each bootstrap iteration, the adult samples were resampled with replacement and the fish in each new sample were then resampled with replacement. The adult parameters *W*, *S* and *R* were

calculated from the bootstrapped values using the method described above. 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 $W / R.F.S$ using the percentile method.

Relative influence of each parameter on estimates of spawning biomass

Variations in each parameter have proportional effects on *SB* (e.g. Ward et al. 2009). To identify the parameter with the greatest potential influence on estimates of *SB* we calculated the relative variation in each parameter as the range of estimates obtained over the entire study period as a percentage of the mean value for that parameter. Parameters with high levels of relative variation have more potential influence on estimates of spawning biomass than parameters with lower levels of variation. To determine the potential levels of bias resulting from use of sub-optimal data or methods we estimate the range of proportional variation in estimates of *SB* resulting from these approaches.

Results

Total Daily Egg Production

Egg abundance and distribution

In 1998, 2,738 sardine eggs were collected from 110/164 (67.1%) sites but in 1999, after the second mass mortality event, only 397 eggs were collected from 50/213 (23.5%) of sites (Table 1, Fig. 2). The number of sites sampled (290-328), number of eggs collected (1,362-1,718) and percentage of positive sites (35.0-38.3%) remained relatively stable between 2000 and 2003. In 2004, rough weather prevented the collection of samples from four transects (Fig. 2), yet 3,186 eggs were collected from 115/284 sites (40.5%). In 2005, only 1,808 eggs were collected from 124/334 sites (37.1%), whereas in both 2006 and 2007 (like 2004), over 3,000 eggs were collected from 341 sites.

Mean egg density at positive sites (i.e. those with eggs) was 217.9 ($\pm$ 44.2 SE) eggs m^{-2} in 1998, but fell to 71.7 ($\pm$ 16.4 SE) eggs m^{-2} in 1999, after the second mass mortality event. In the seasons between 2001 and 2003 inclusive, egg density remained between 117.3 ($\pm$ 18.2 SE) and 131.3 ($\pm$ 16.9 SE) eggs m^{-2}, but increased to 200.7 ($\pm$ 48.8 SE) in 2004 before falling to 103.8 ($\pm$ 34.7 SE) eggs m^{-2} in 2005. Mean egg density at positive sites was 142.1 ($\pm$ 43.4 SE) and 156.0 ($\pm$ 27.1 SE) eggs m^{-2} in 2006 and 2007, respectively.

Spawning Time

The progression of the modal abundance of eggs Stages 2-7 and, less clearly, Stages 7-12 is shown in Fig. 3. Stage 2, 3 and 4 eggs were most commonly collected at 5.30-6.00 am, 8.00 am and 12.00 pm, respectively. Assuming that Stage 2 eggs are 3-4

hours old, the peak spawning time would be approximately 2.00 am. These plots suggest that most Stage 1-7 eggs are less than 24 hours old and most Stage 8-12 eggs are 24-48 hours old. The modal age of each egg stage for each of three temperature ranges shown in Table 2 suggests that in waters <19.0^{o}C, 19.0-20.0^{o}C and >20.0^{o}C, respectively, Stages 1-6, 1-7 and 1-8 were less than 24 hours old and that Stage 7-12, 8-12 and 9-12 eggs were 24-48 hours old, respectively (Table 2).

Estimates of P_0 obtained (from the log-linear model) using the three different methods to estimate egg age were generally similar. The differences in the estimates from the two models that include temperature were between 3 and 12% different from one another. The estimates of P_0 from the model where the effects of temperature on the rates of egg development were ignored were between 1 and 30% different to those from the other two models. In 1998 and 1999, the estimates of P_0 obtained from the model that ignored temperature were 20 to 30% higher than those from the other two models, suggesting that this approach may not be justified. As estimates of mean egg age of some egg stages in some temperature ranges were based on small sample sizes, the best approach for estimating P_0 may be to subtract collection time from spawning time, but recognising that at <19.0^{o}C Stages 1-6 are day-1 eggs, at 19.0-20.0^{o}C Stages 1-7 are day-1 eggs and at >20.0^{o}C Stages 1-8 are day-1 eggs.

Spawning Area (A)

The area surveyed varied between years, covering 46,500 and 63,200 km^2 in 1998 and 1999, respectively, but increasing to over 100,000 km^2 from 2000 onwards. The only year after 2000 when the survey area was less than 100,000 km^2 was 2004, when the

four transects in the far west (Fig. 2) were not sampled due to poor weather and the total survey area was 95,240 km^2.

Similar estimates of *A* were obtained using the original Lasker (1985) and VNN methods (Fig. 4). *A* fell by 52.8% from 31,510 km^2 in 1998 to 14,877 km^2 in 1999, following the 1998 mass mortality event, despite the 25% increase in the area surveyed. Estimates of *A* were between 34,000 and 38,000 km^2 from 2000 to 2004, but increased to 40,817 in 2005 and were 49,220 and 49,629 km^2 in 2006 and 2007, respectively. The large range of estimates of *A* as a percentage of the mean value (94.9%) is the results of the combined effects of the variations in survey area and the differences in spawning area in 1999, immediately after the 1998 mass mortality event, and in 2007 after the population reached the highest level recorded (Table 3). This large range shows the importance of *A* as a determinant of *SB*.

Daily egg production (P_0), egg mortality (Z) and model variance

Due to the extremely over-dispersed nature of the data (many zeros, some moderate values and a few very high values) the fits for all seven models tested were poor and confidence intervals were broad (Table 4, Fig.5). However, comparison of Q-Q plots and model CVs provide clear evidence that the log-linear model and GLM 4 of log-transformed data fit better than the exponential model and GLMs 1–3. In terms of coefficient of variation, GLM 4 performed best in every year followed by the log-linear model. There was no trend over the ten years in the CVs of the remaining models. A similar trend is evident in Q-Q plots of the residuals, with the exponential model and GLMs 1 and 2 performing extremely poorly at the tails of the distribution (Fig. 5). The Q-Q plots of the residuals of the log-linear model and GLM 4 show

some evidence of poor fit at the tails of the distributions, but are far superior to the other models. The residuals of GLM 3 are better than GLMs 1 and 2 and the exponential model, but are still overly affected by extreme values in egg density.

Estimates of P_0 obtained using the seven methods vary considerably among and within years and provide further insights into the suitability of the various models. Estimates obtained by fitting the exponential model and using GLM 1 with a Gaussian family and a log link were almost identical and varied more among years than any other models. For example, estimates of P_0 in 2004 (>229 eggs.m^{-2}) obtained using these models were more than three times higher than the estimates for 2003 and 2005 (65-75 eggs.m^{-2}). In addition, these methods produced estimates of P_0 that were almost twice as high as the estimate obtained using the log-linear model in 2004 but approximately equal to the estimate obtained using that method in 2003. These results, in conjunction with Q-Q plots, show that the exponential model and GLM 1 produce highly inflated estimates of P_0 when datasets include a few samples with very high densities of early stage eggs.

The estimates of P_0 obtained using the GLM 2 (Quasi distribution with a log link function and variance proportional to the mean) and GLM 3 (Quasi distribution with a log link function and variance proportional to the mean squared) were generally similar to each other, but highly variable between years. For example, the estimates of P_0 for 2004 obtained using these methods (200.28 and 186.40 eggs.m^{-2}, respectively) were more than three times estimates obtained in 2003 (~65 eggs.m^{-2}) and were more than two times greater in 2005 (74.69 and 78.60 eggs.m^{-2}, respectively). Estimates of

P_0 obtained using the GLMs 2 and 3 in 2004 were more than 50% higher than that obtained using the log-linear model (i.e. 121.91 eggs.m^{-2}).

In contrast to the exponential model and GLMs 1-3, GLM 4 (ln-transformed egg density with a Quasi distribution, identity link and variance proportional to mean) provides low and stable estimates of P_0. Estimates of egg production obtained using GLM 4 varied between ~ 27 eggs.m^{-2} in 1999 and ~70 eggs.m^{-2} in 2004, with estimates typically 50-70% of the estimates obtained using the log-linear model. The difference between estimates of P_0 obtained using GLM 4 and the log-linear model reflect the lower residual deviance of the GLM compared to the variance for log-linear model, which reduces the size of the bias correction factor applied to the GLM.

Estimates of P_0 obtained using the log-linear model were more stable between years than estimates obtained using the exponential model and GLMs 1-3, but less stable (and consistently higher) than the estimates obtained using GLM 4. The trend in estimates of P_0 obtained using the log-linear model can be logically explained. The large decrease in P_0 between 1998 and 1999, which was identified using all methods, reflects the effects of the mass mortality event that occurred between the two surveys (Ward et al. 2001b), and the increase in P_0 between 1999 and 2004 reflects the recovery of the stock. The large reduction in P_0 for 2005, which was observed for all methods, appears to reflect the timing of the second survey which was probably conducted after the peak spawning season. Estimates of P_0 for 2006 and 2007 obtained using the log-linear model are similar to those obtained in 2003 and 2004. These results suggest that log-linear model produces estimates of egg production that are less affected by a small number of samples with high densities of early stage eggs

than the exponential model and GLMs 1-3. The large range of estimates of P_0 as a percentage of the mean (85.6%) reflects the high potential for this parameter to drive variations in *SB* (Table 3).

Total Daily Fecundity

Sex ratio (R)

Estimates of *R* from fishery-independent samples ranged between 0.44 in 2003 and 0.64 in 2006, whereas *R* from commercial catch samples ranged between 0.40 in 2000 and 0.66 in 2005. The overall (all years) mean *R* from fishery-independent and commercial samples were 0.53 and 0.55, respectively. Estimates of *R* from commercial catch samples lay within the 95% CIs of estimates from fishery-independent samples in four out of the eight years for which commercial catch data were available (Fig. 7). The relatively small range of estimates of *R*, as a percentage of the mean (37.9%), reflects the stability of this parameter between years and the relatively low potential to influence estimates of *SB* (Table 3).

Mean female weight (W)

Estimates of *W* from fishery-independent samples, which were mainly taken from shelf waters, were typically larger (range 46.3 g in 1998 to 78.7 g in 2004) than estimates from commercial catch samples, which were mainly taken from Spencer Gulf (range 37.3 g in 2000 to 52.9 g in 2003). The overall (all years) means of *W* from fishery-independent and commercial samples were 62.6 and 46.0 g, respectively. Estimates of *W* from commercial catch samples lay within the 95% CI of estimates from fishery-independent samples in 2003 only (Fig. 7). The relative variation in estimates of *W*, as a percentage of the mean (56.4%), is moderate compared to other

parameters and is offset by the linkage of this parameter to *F*. *F/W* (eggs/g) only varies by 30.8% of the mean among years (Table 3).

Batch Fecundity (F)

The number of females with hydrated oocytes collected fishery-independent samples ranged between 6 in 2003 to 250 in 2000 (Table 6). Due to the small number of females with hydrated oocytes collected in 2003 and 2004 estimates of batch fecundity for these years were calculated by applying the relationship between female ovary-free weight and batch fecundity derived from data obtained in all years.

The linear regression model fitted the observed data better than either the Gamma or Negative Binomial GLMs in 6 out of the 10 years in terms of pseudo R^2 and residual plots (Table 5). Between 1998 and 2007, estimates of *F* from fishery-independent samples obtained using the linear regression model ranged between 13,600 and 23,729 hydrated oocytes (Fig. 7). Variation in *F* between years mainly reflected variation in gonad-free female weight (and *W*). Estimates of *F* obtained from fishery-dependent samples were below the 95% CIs of estimates from fishery-independent samples in five of the eight years for which both estimates were available. Like *W*, the potential effects of *F* on estimates of *SB* are relatively low, because of the moderate range of estimates of *F* as a percentage of the mean (57.8%). This potential effect is also offset by the linkage to *W*, with *F/W* (eggs/g) only varying by 30.8% of the mean among years (Table 3).

Spawning Fraction (S)

The proportion of females with ovaries containing day-0 (including hydrated oocytes), day-1 and day-2 POFs varied between years. For example, the proportion of females

with day-2 POFs was much lower than the proportion of females with day-0 or day-1 POFs in 2002, 2005, 2006 and 2007, suggesting that females with day-2 POFs may have been under-represented in samples. Estimates of *S* based on the presence of one type of POF vary more significantly between years than those obtained from all three types of POF (Fig. 8). For this reason we used the mean proportion of females with ovaries containing all stages of POFs (i.e. day-0, day-1 and day-2) to estimate *S*.

Estimates of *S* varied between 0.07 in 2002 and 0.18 in both 1999 and 2001 (Fig. 8). Estimates of *S* for 2003 and 2004 were based on less than 500 females. The overall mean annual *S* for all years was 0.14. The large range of estimates of *S* as a percentage of the mean (83.0%) reflects the importance of this parameter in determining *SB* (Table 3).

Relative influence of each paramter on estimates of spawning biomass (*SB*)

Estimates of *SB* calculated from estimates of *A* obtained using the Voroni natural neighbour method, estimates of P_0 calculated using the log-linear model (with one egg added to each day class of eggs at each positive site), estimates of adult parameters obtained from fishery-independent samples and estimates of *S* based on females with all stages of POFs combined are shown in Fig. 9. The estimate of *SB* for 1998 was 169,573 t, but this fell to 22,906 t in 1999, following the mass mortality event in 1998 - despite the >25% increase in area sampled. In 2000 and 2001, the estimates of *SB* remained below 120,000 t. Between 2002 and 2005 (inclusive) estimates of *SB* ranged between 152,089 and 180,724 t. Estimates of *SB* increased to 202,624 t in 2006 and 263,049 t in 2007.

The large range of estimates of P_0, S and A as a percentage of the mean emphasizes the importance of these parameters in estimating SB (Table 3). Estimates of P_0, and hence SB, obtained using the exponential model and GLMs 1-3 were consistently higher than those obtained using the log-linear model. The most extreme variations occurred in 2004, when the estimates of SB obtained using the exponential model and GLM 1 (Fig. 5) were both 90% higher (~321,200 t) than the estimate obtained using the log-linear model (169,209 t). The estimate of SB for 2004 obtained using the exponential model and GLM 1 was also 91% and 68% higher than the estimates obtained for 2003 and 2005, respectively. In 2006, GLMs 2 and 3 (Fig. 5) produced SB estimates of 354,834 t (75% higher) and 401,963 t (98% higher), respectively than those obtained using the log-linear model. In contrast, each year the estimate of SB obtained using GLM 4 was 28-46% lower than the estimate obtained using the log-linear model, which is approximately equal to the bias correction factor used to calculate Po using the log-linear model.

Estimates of SB obtained using estimates of S from females with day-0, day-1 and day-2 POFs (combined) typically fell between estimates of S obtained from females with only one day class of POFs. For example, in 2005 the estimate of SB obtained using the estimate of S obtained using day-0 females only was 322,955 t, which is 2.1 times higher than the estimate obtained from females with day-0, day-1 and day-2 POFs combined (153,788 t). In contrast, the estimate obtained using females with day-1 POFs (92,272 t) and day-2 POFs (46,136 t) were ~40% and 70% lower, respectively, than the estimate obtained from females with day-0, day-1 and day-2 POFs combined.

The effect of varying the method used to estimate *A* had minimal effect on estimates of *SB*, because estimates obtained using manual and VNN methods were virtually identical. The major factor other than egg distribution that is likely to affect estimates of *A* is the size of the area surveyed. However, it is also important to ensure that *A* is estimated from a large number of similar-sized grids to ensure that estimates of *A* are not unduly biased by the presence/absence of eggs in one or two large grids.

Table 1. Summary of information on the timing of surveys, number of samples collected and distribution and abundance of *Sardinops sagax* eggs in ichthyoplankton surveys conducted between 1998 and 2007.

Year	Survey month	No. transects	No. stations	No. Eggs	No. (%) Positive stations	Mean egg density in spawning area (eggs m^{-2} ±SE)
1998	2, 3	19	164	2738	110 (67.1)	217.9 (44.2)
1999	2, 3	26	213	397	50 (23.5)	71.7 (16.4)
2000	2, 3	32	290	1362	111 (38.3)	79.4 (14.2)
2001	2, 3	31	291	1396	107 (36.8)	117.3 (18.2)
2002	2, 3	33	328	1475	115 (35.1)	129.8 (27.2)
2003	2, 3, 4	32	320	1718	112 (35.0)	131.3 (16.9)
2004	2, 3	28	284	3186	115 (40.5)	200.7 (48.8)
2005	2, 3	31	334	1808	124 (37.1)	103.8 (34.7)
2006	2, 3	34	341	3083	135 (39.6)	142.1 (43.4)
2007	2, 3	34	341	3909	151 (44.3)	156.0 (27.1)

Table 2. Estimates of egg age by stage for three categories of sea surface temperature, cool (<19 deg. C), intermediate (19-20 deg. C) and warm (>20 deg. C). Kernel density smoothing models with a Gaussian kernel and a bandwidth of 3 hours were fitted to time of sampling for each stage and temperature category. Age for day 1 eggs was estimated as the modal time of sampling subtracting the 2am peak spawning time. Age for day 2 eggs was estimated by adding 22 hours to modal time of sampling.

Stage	Cool	Intermediate	Warm	N. cool	N. Intermediate	N. Warm
2	4.6	3.4	3.5	76	86	41
3	7.8	6.2	5.9	87	74	50
4	10.1	11.0	7.9	50	86	42
5	16.5	16.1	13.8	74	120	64
6	22.8	16.8	14.0	28	30	17
7	23.6	19.3	20.3	82	83	49
8	31.0	26.0	21.1	103	86	70
9	32.0	29.2	24.1	91	54	35
10	32.4	30.4	25.3	63	75	43
11	37.4	36.0	32.5	75	133	67
12	40.7	35.9	38.5	60	98	59

Table 3. The mean, minimum and maximum estimated for each DEPM parameter for Sardinops sagax in waters off South Australia between 1998 and 2007. The range is presented as a percentage of the mean to indicate the relative variability of parameters among years. Eggs/g of female is calculated to show that the effects of variation in W and F on estimates of spawning biomass are buffered by the relationship of the two parameters.

Parameter	P_0	A	W	R	F	S	Eggs/g
Mean of all years	90.30	36637	59.42	0.53	17525	0.14	295.22
Minimum observed	50.99	14877	45.18	0.44	13600	0.07	249.40
Maximum observed	128.29	49629	78.72	0.64	23729	0.18	340.35
Range as % of mean	85.6	94.9	56.4	37.9	57.8	83.0	30.8

Table 4. Diagnostics obtained from six models used to estimate P_0 from data where all stations with eggs present were included and one egg was added to the total for each day: exponential egg mortality model of Lasker (1985); log-linear version of egg mortality model (Picquelle and Stauffer 1985); GLM 1 with a Gaussian distribution, a log link function (McCullagh and Nelder 1983); GLM 2 with a Quasi distribution, a log link function and variance proportional to the mean (Wood 2006); GLM 3 with a Quasi distribution, a log link function and variance proportional to the mean squared and GLM 4 fitted to ln-transformed egg density with a Quasi distribution, identity link function and variance proportional to the mean. In addition, diagnostics from the log-linear model fitted to data where all zeroes have been excluded are included for comparison. Estimates of egg production (P_0), egg mortality (Z), 95% confidence intervals (CI), standard errors (SE) and coefficients of variation (CV). Models where egg mortality was negative (in grey) were refitted using the mean Z from other years. The confidence intervals, standard errors and coefficient of variations were calculated from 100,000 bootstrapped resamples for the data.

Year	Diagnostic	Exponential	Log Linear	Log Linear (no zeroes)	GLM1	GLM2	GLM3	GLM4
1998	P_0	136.21	111.78	120.72	136.21	139.41	146.73	64.48
	Z	0.55	0.43	0.28	0.55	0.58	0.64	0.44
n=212	95% CI	81-219	74-168	73-193	81-219	83-221	85-231	45-92
	SE	35.5	24.0	30.8	35.5	35.3	37.6	12.1
	CV	0.26	0.21	0.25	0.26	0.25	0.25	0.19
1999	P_0	(29.75) 51.58	38.12	(38.65) 50.10	(29.75) 37.20	(29.62) 37.24	(29.61) 37.31	27.30
	Z	(-0.21) 0.53	0.12	(>-0.01) 0.29	(-0.21) 0.53	(-0.22) 0.58	(-0.22) 0.65	0.12
n = 92	95% CI	15-46	25-57	24-60	14-46	17-46	18-47	18-41
	SE	100.2	8.2	9.3	7.8	7.3	7.4	5.7
	CV	3.30	0.21	0.24	0.26	0.24	0.24	0.21
2000	P_0	62.88	65.03	57.63	62.87	67.31	75.45	45.82
	Z	0.30	0.50	0.07	0.30	0.38	0.51	0.53
n = 190	95% CI	44-89	46-92	38-88	44-89	45-100	46-122	34-63
	SE	11.4	11.6	13.1	11.4	14.1	19.5	7.7
	CV	0.18	0.18	0.22	0.18	0.21	0.25	0.16
2001	P_0	93.09	74.53	91.57	93.09	96.43	105.45	49.87
	Z	0.58	0.44	0.44	0.58	0.63	0.74	0.46
n = 184	95% CI	59-152	50-109	56-145	59-153	61-143	65-159	35-71
	SE	90.1	15.1	22.7	>1000	21.0	23.9	9.4
	CV	0.93	0.20	0.24	>100	0.22	0.22	0.19
2002	P_0	87.87	59.75	97.35	87.86	91.92	99.45	36.77
	Z	0.21	0.13	0.27	0.21	0.27	0.35	0.13
n = 176	95% CI	51-140	39-93	54-177	51-140	50-155	47-183	26-53
	SE	22.9	14.1	31.8	22.9	27.0	35.0	7.0

	CV	0.26	0.23	0.32	0.26	0.29	0.34	0.19
2003	P_O	65.74	66.31	97.11 (72.57)	65.74	65.73	65.72	42.24
	Z	0.06	0.05	0.29 (-0.02)	0.06	0.06	0.06	0.05
n = 190	95% CI	45-93	47-92	49-105	45-93	45-93	45-93	31-58
	SE	12.4	11.7	14.5	12.4	12.3	12.3	7.0
	CV	0.19	0.17	0.20	0.19	0.19	0.19	0.16
2004	P_O	229.53	120.91	128.29	229.54	200.28	186.40	69.56
	Z	1.25	0.56	0.33	1.25	0.99	0.90	0.58
n = 206	95% CI	76-712	78-187	76-213	76-712	78-447	80-341	47-103
	SE	175.1	28.0	35.3	175.1	98.7	68.7	14.4
	CV	0.67	0.23	0.27	0.67	0.47	0.37	0.20
2005	P_O	70.05	55.66	56.29 (43.48)	70.05	74.69	78.60	39.71
	Z	0.49	0.52	0.29 (-0.03)	0.49	0.58	0.64	0.54
n = 214	95% CI	35-132	40-79	28-69	35-132	35-154	36-180	30-54
	SE	26.1	9.8	10.4	26.1	32.2	39.1	6.2
	CV	0.37	0.17	0.23	0.37	0.42	0.47	0.15
2006	P_O	131.04	86.21	97.90	131.05	150.96	171.01	53.05
	Z	0.77	0.68	0.46	0.77	1.01	1.18	0.69
n = 262	95% CI	67-231	59-126	62-154	67-231	71-295	74-389	38-74
	SE	43.2	17.1	23.8	43.2	59.9	85.0	9.0
	CV	0.33	0.20	0.24	0.33	0.39	0.48	0.17
2007	P_O	131.89	102.98	105.16	131.89	142.07	163.50	55.94
	Z	0.58	0.60	0.18	0.58	0.69	0.86	0.62
n = 274	95% CI	77-239	69-152	65-167	77-239	80-250	86-285	40-79
	SE	42.6	21.3	26.2	42.6	44.6	51.8	9.9
	CV	0.31	0.20	0.24	0.31	0.31	0.31	0.17

Table 5. Numbers of samples, individuals of each sex used to estimate mean sex ratio, number of females used to estimate spawning fraction and batch fecundity for *Sardinops sagax* collected in February and March in fishery-independent samples and commercial catches in South Australian waters between 1998 and 2007.

	Fishery-independent samples						Commercial catch	
Year	No. adult samples	No. individuals	No. ♀ Sex Ratio	No. ♂ Sex Ratio	No. ♀ Spawning Fraction	No ♀ Batch Fecundity	No. Females	No. Males
1998	11	933	451	482	445	96		
1999	13	1,562	690	872	690	179	93	147
2000	15	2,199	1,012	1,187	1012	250	78	125
2001	10	1,394	745	649	741	73	203	146
2002	22	2,823	1,576	1,247	1576	53	311	267
2003	5	971	416	555	414	6	284	263
2004	10	867	405	462	403	8	311	263
2005	28	4,688	2,157	2,531	2153	190	520	292
2006	19	2,433	1,330	1,103	874	29	95	114
2007	20	2,244	1,084	1,160	1084	69		

Table 6. Estimates of batch fecundity with R^2 values (pseudo R^2 used for GLMs) obtained from three models fitted to estimate the relationship between fecundity and mean gonad free female weight: Linear was a linear regression, Gamma was a GLM with a gamma family and an identity link and Negative Binomial was a GLM with a negative binomial family and an identity link. The number of fish sent for histology analysis and mean gonad free weight of all female fish by year is provided for comparison. In 2003 and 2004 only 6 and 8 hydrated fish were collected, in those years the batch fecundity relationship obtained from all years was applied to the mean gonad free female weight from that year.

Year (N)		Gonad-free weight (g)	Batch fecundity (R^2)		
			Linear	Gamma	Negative Binomial
1998	(55)	42.8	13600 (0.34)	13323 (0.41)	13324 (0.41)
1999	(144)	49.6	15176 (0.29)	15367 (0.30)	15367 (0.30)
2000	(224)	45.8	13965 (0.55)	13950 (0.44)	13950 (0.44)
2001	(73)	48.7	17372 (0.47)	17349 (0.45)	17349 (0.45)
2002	(53)	60.5	18294 (0.64)	18151 (0.57)	18152 (0.57)
2003	(6)	51.3	10904 (0.66)	10665 (0.71)	10665 (0.71)
2003 all	(838)	51.3	15996 (0.54)	15975 (0.50)	15975 (0.50)
2004	(8)	75.0	24790 (0.18)	24862 (0.16)	24862 (0.16)
2004 all	(838)	75.0	23729 (0.54)	23996 (0.50)	23996 (0.50)
2005	(178)	70.3	22266 (0.18)	22316 (0.17)	22316 (0.17)
2006	(25)	59.7	16166 (0.37)	16078 (0.26)	16078 (0.26)
2007	(67)	67.6	20581 (0.49)	20649 (0.51)	20649 (0.51)

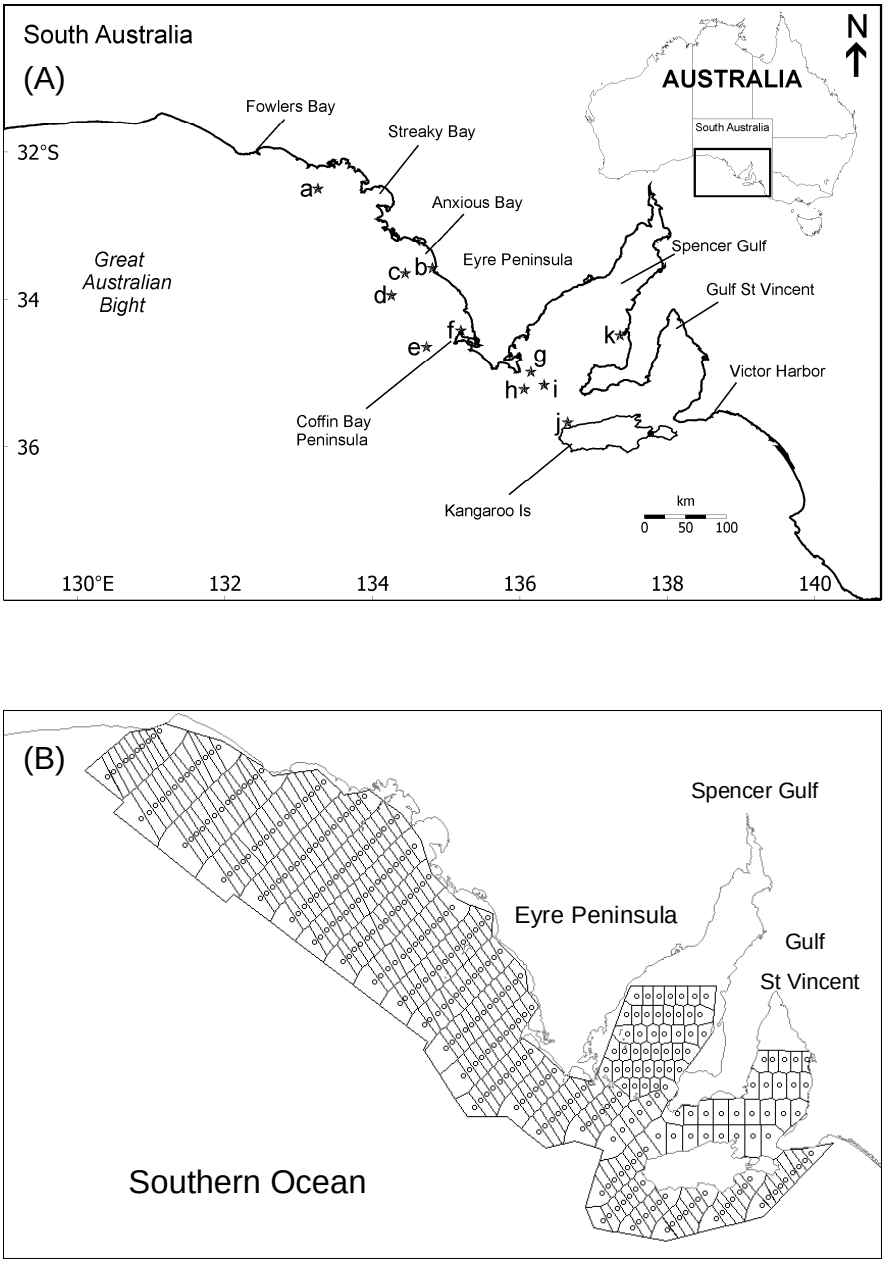


Figure 1. (A) Map showing locations mentioned in the text and sites where adult samples were collected in surveys of *Sardinops sagax* in South Australian waters between 1998 and 2007. (a) St Francis Island, (b) Waldegrave Island, (c) Flinders Island, (d) Pearson Island, (e) Greenly Island, (f) Coffin Bay, (g) Thistle Island, (h) Neptune Island, (i) Wedge Island, (j) Scotts Cove and (k) Wardang Island. (B) Grids generated for 2007 using the Voroni natural neighbour method.

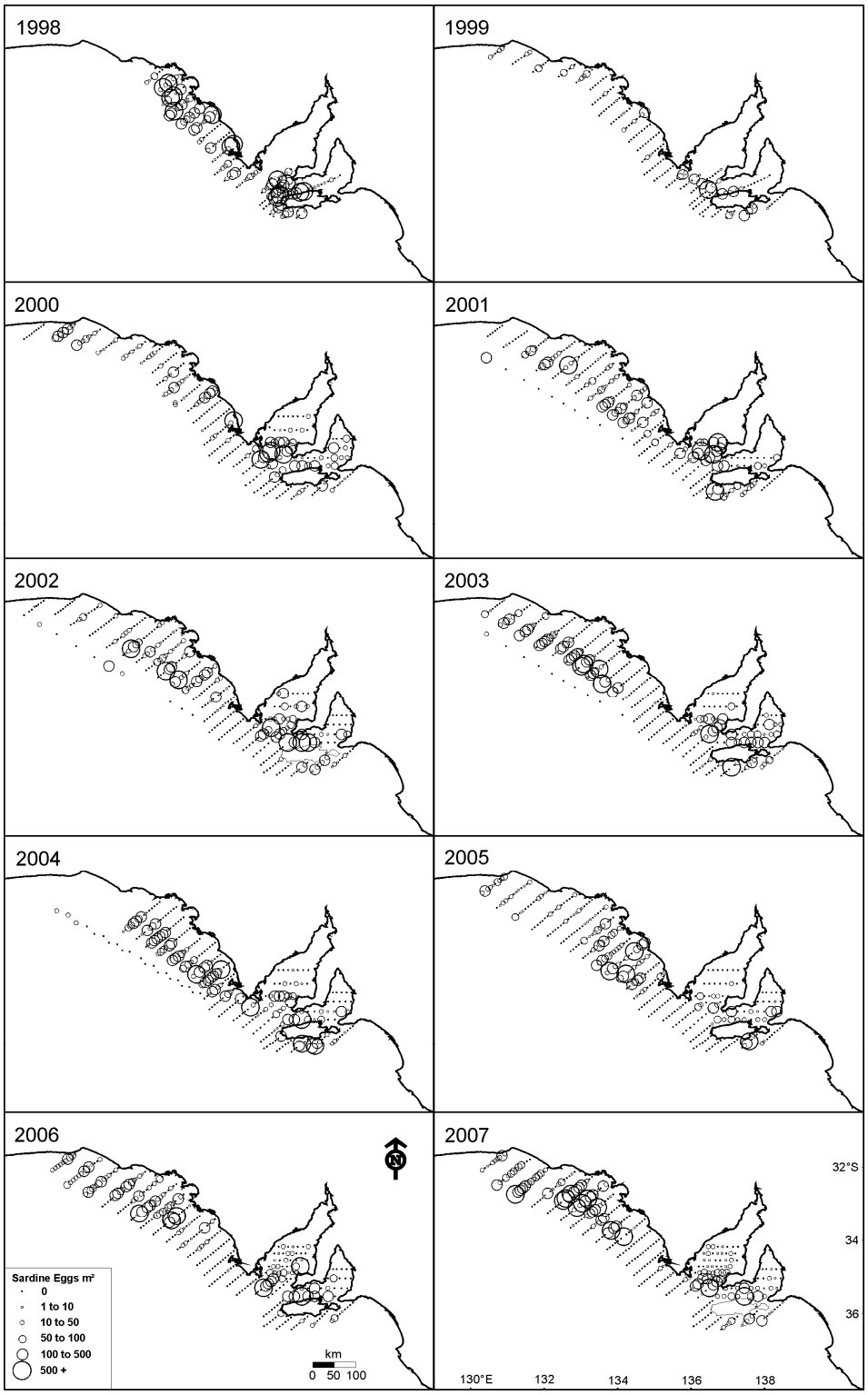


Figure 2. Location of stations where ichthyoplankton samples were collected and densities of *Sardinops sagax* (eggs.m^{-2}) collected in surveys of *Sardinops sagax* in South Australian waters between 1998 and 2007.

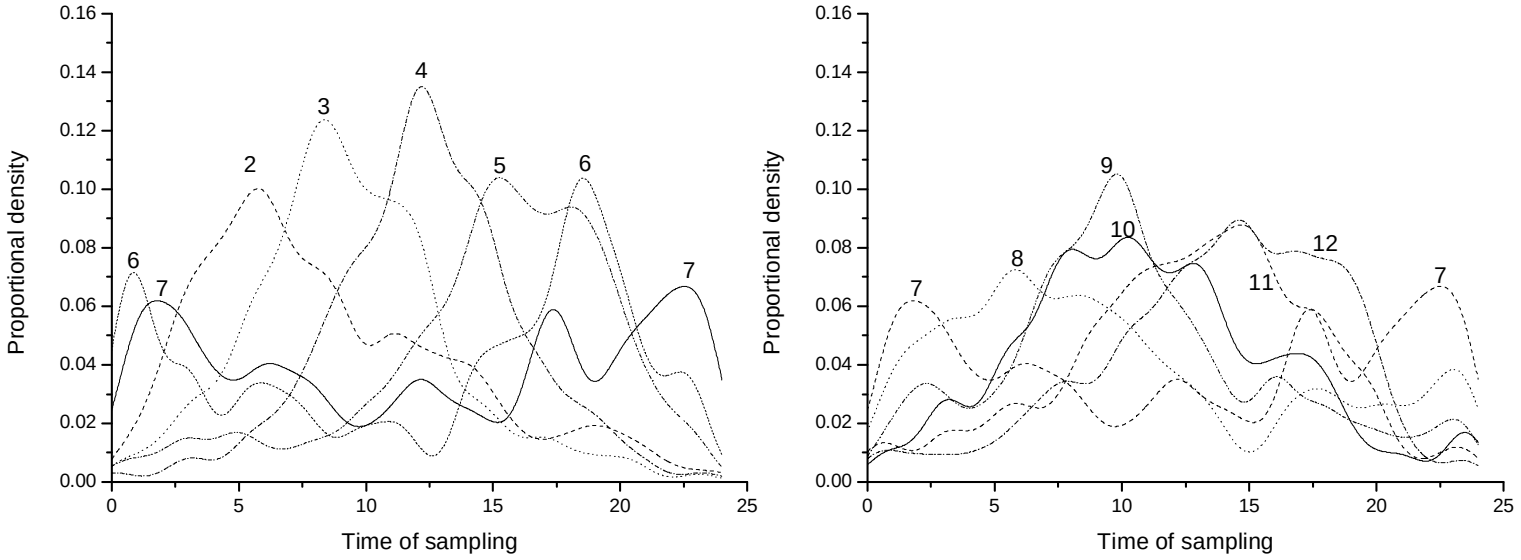


Figure 3. Plots of proportion of eggs of each stage by sampling time from surveys of *Sardinops sagax* in South Australian waters between 1998 and 2007. Kernel density smoothing techniques with a Gaussian (normal) kernel function and a bandwidth of three hours was used to produce these plots.

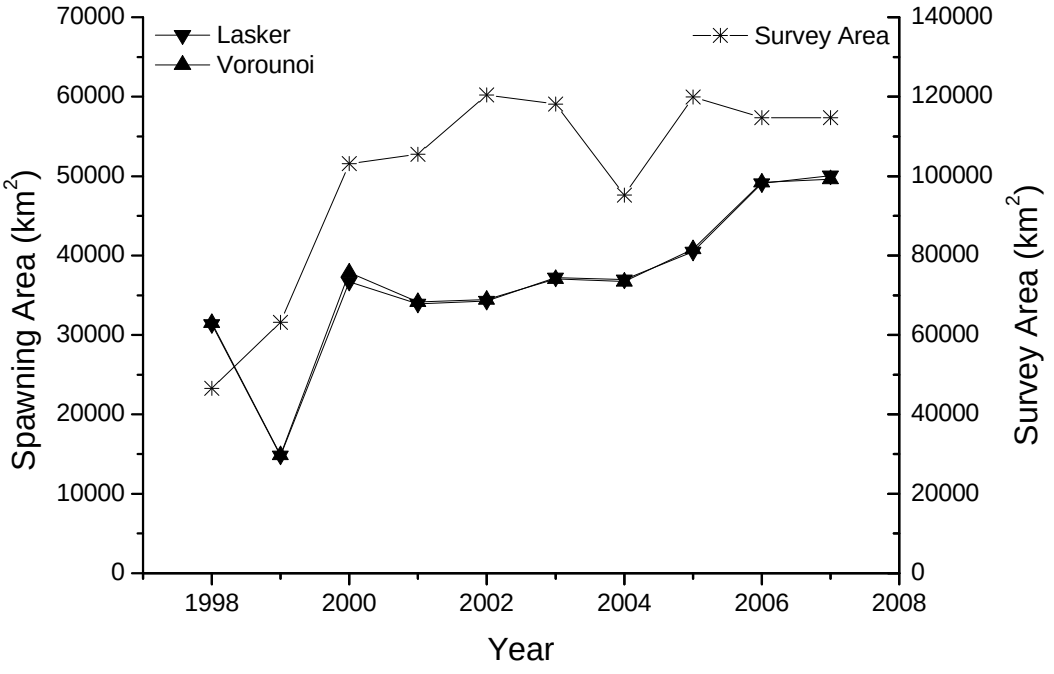


Figure 4. Estimates of survey area (✳) and spawning area obtained using Lasker (1985) (▼) and Voroni natural neighbour (▲) method.

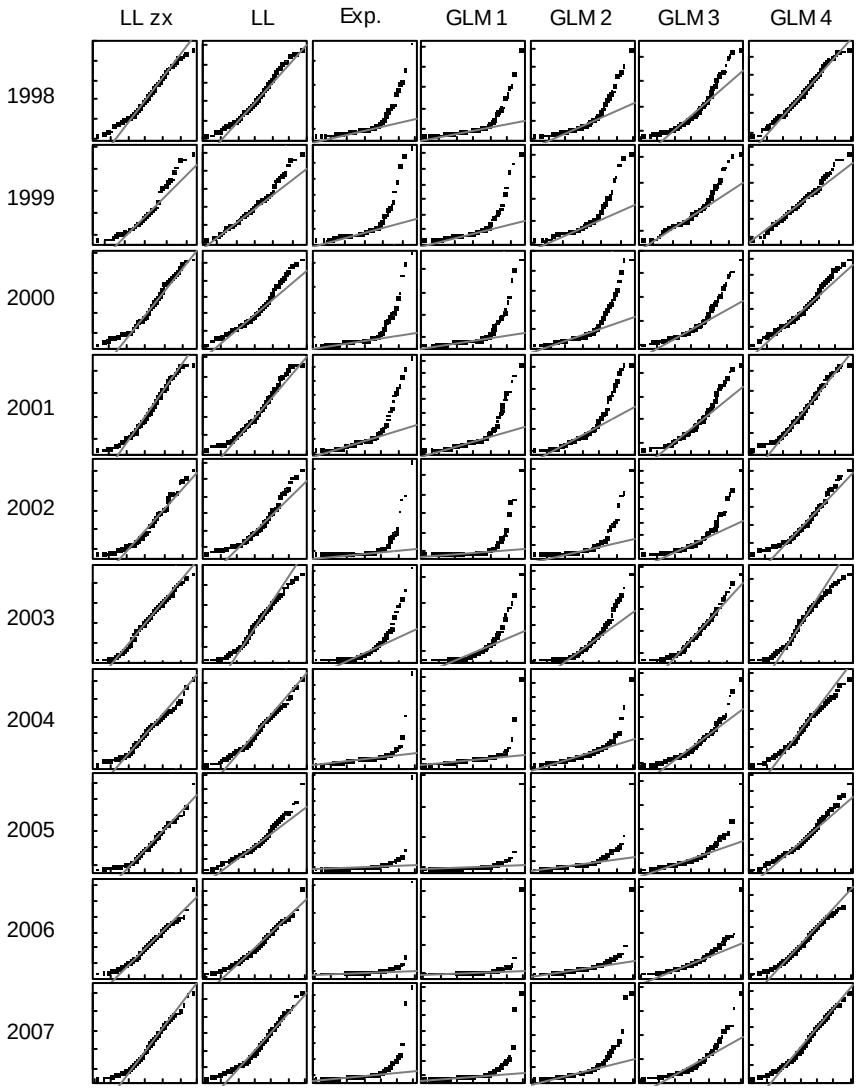


Figure 5. Q-Q plots for seven models fitted to egg density data to estimate P_0 from 1998 to 2007. LLzx = log-linear model with zero values excluded. All other models are fitted to datasets with one egg added to each day class of eggs at each positive station. LL = log-linear, Exp = exponential, Generalized Linear Model (GLM) 1 = Gaussian GLM with a log link, GLM 2 = Quasi GLM with a log link and variance proportional to the mean, GLM 3 = Quasi GLM with a log link and variance proportional to the mean squared and GLM 4 = Quasi GLM with an identity link and variance proportional to the mean fitted to the log of the egg density. The theoretical quantiles (x-axis) are plotted against, the standardized residuals (log-linear and exponential models) and the standardized deviance residuals (GLMs). The grey line passes through the 1st and 3rd quantiles.

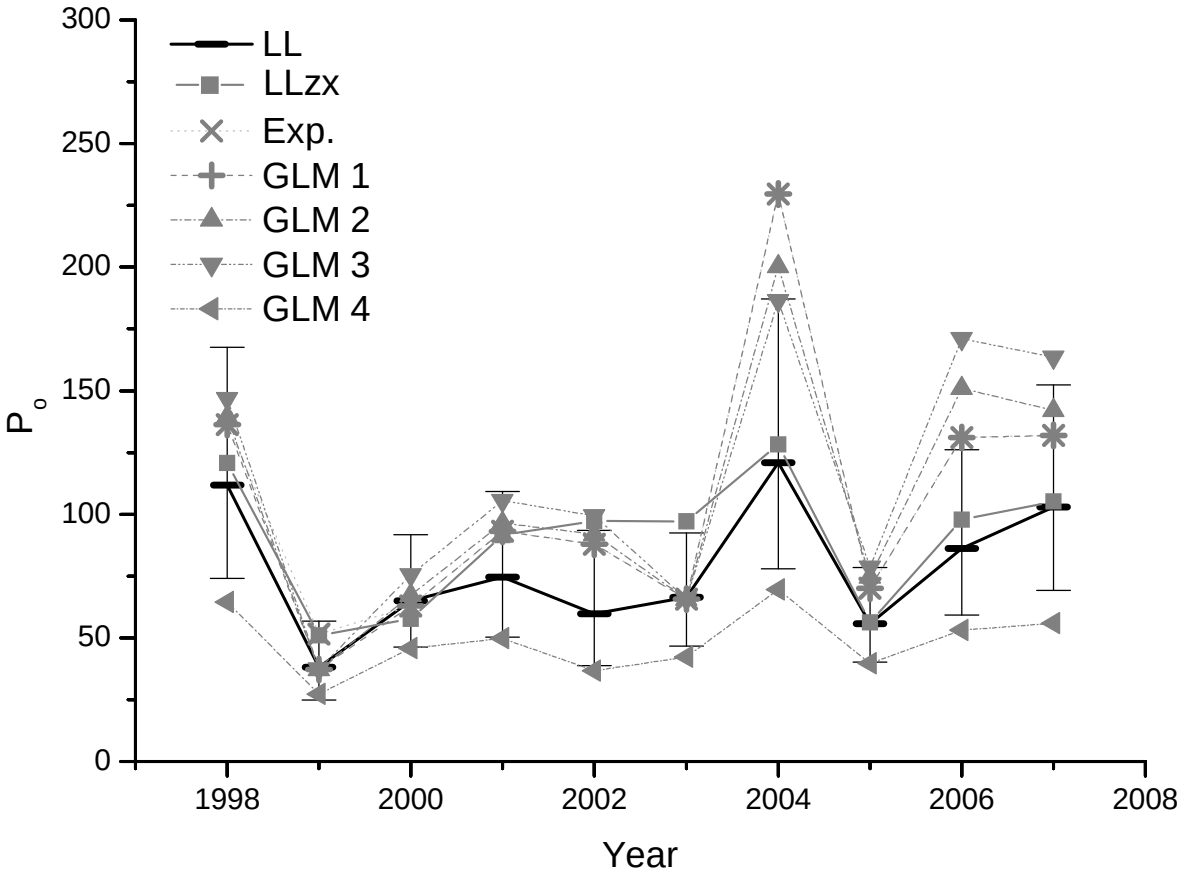


Figure 6. Estimates of mean daily egg production (P_0) obtained using seven models. LLzx = log-linear model with zero values excluded. All other models are fitted to datasets with one egg added to each day class of eggs at each positive station. LL = log-linear, Exp = exponential (Lasker 1985), Generalized Linear Model (GLM) 1 = Gaussian GLM with a log link, GLM 2 = Quasi GLM with a log link and variance proportional to the mean, GLM 3 = Quasi GLM with a log link and variance proportional to the mean squared and GLM 4 = Quasi GLM with an identity link and variance proportional to the mean fitted to ln egg density. Error bars are for the log-linear model only and are 95% confidence intervals calculated from 100,000 bootstraps using the percentile method. Confidence intervals for other models are shown in Table 4, along with other model diagnostics. In years when Z could not be estimated reliably, P_0 was estimated using a value of Z equal to the mean value of all other years.

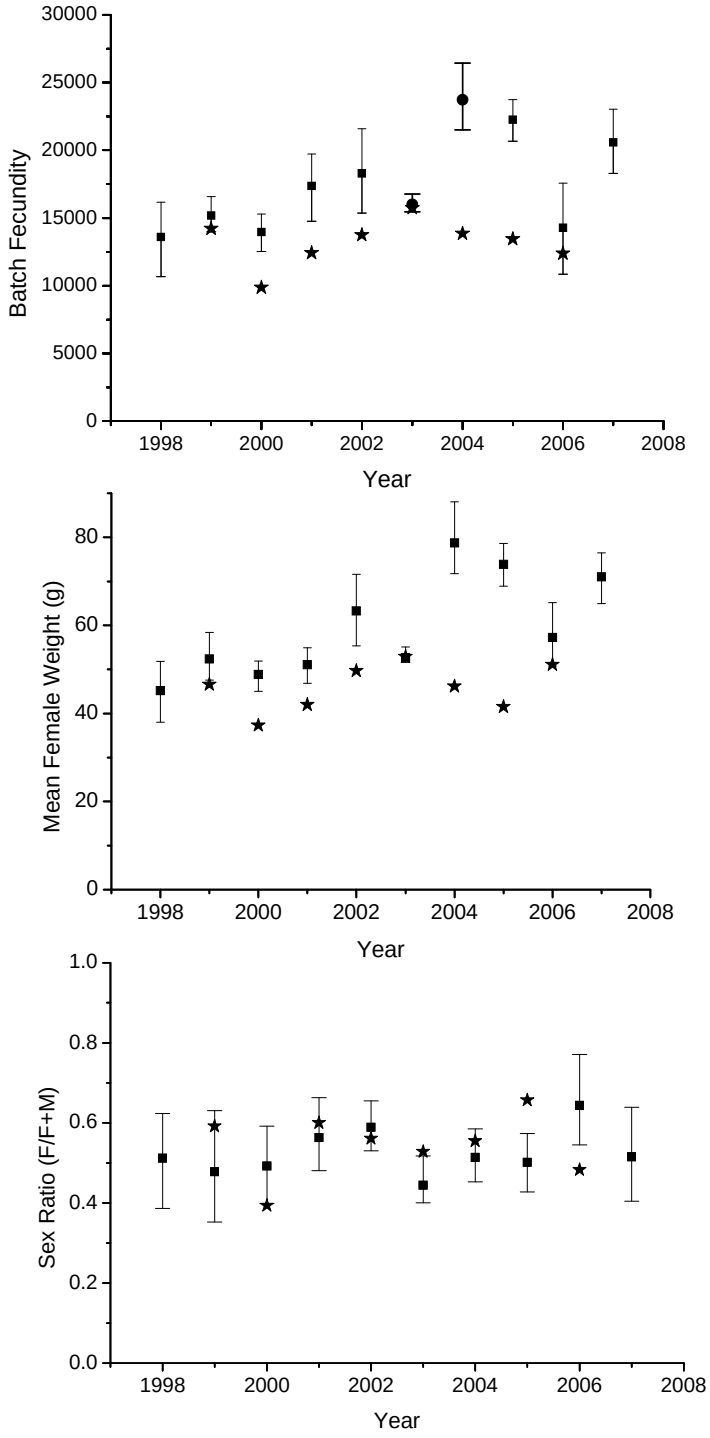


Figure 7. Mean sex ratio, mean female weight and mean batch fecundity for sardine *Sardinops sagax* collected for daily egg production studies off South Australia between 1998 and 2007. Mean female weight (g) and mean sex ratio by weight are from both fishery-independent (■) and commercial catch samples (★). Batch fecundity was estimated using linear regression from mean female weight (ovary removed) of females collected each year between 1998 and 2007 except for 2003 and 2004 (■). In 2003 and 2004 the number of hydrated females sampled was 6 and 8,

respectively. Estimates for these years based on the relationship of female weight (ovary removed) and batch fecundity for all years data (●). Error bars for all estimates are 95% confidence intervals derived from 100,000 bootstrapped estimates.

.

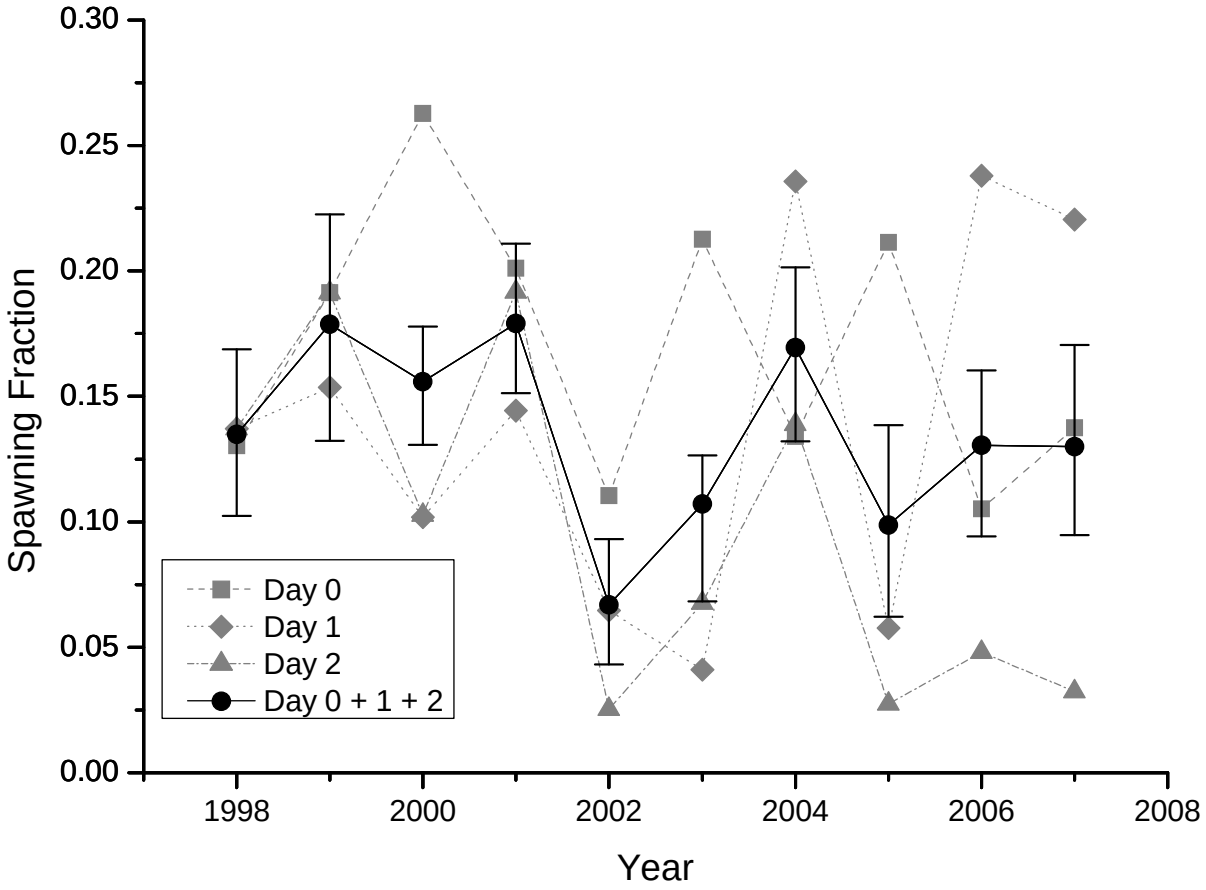
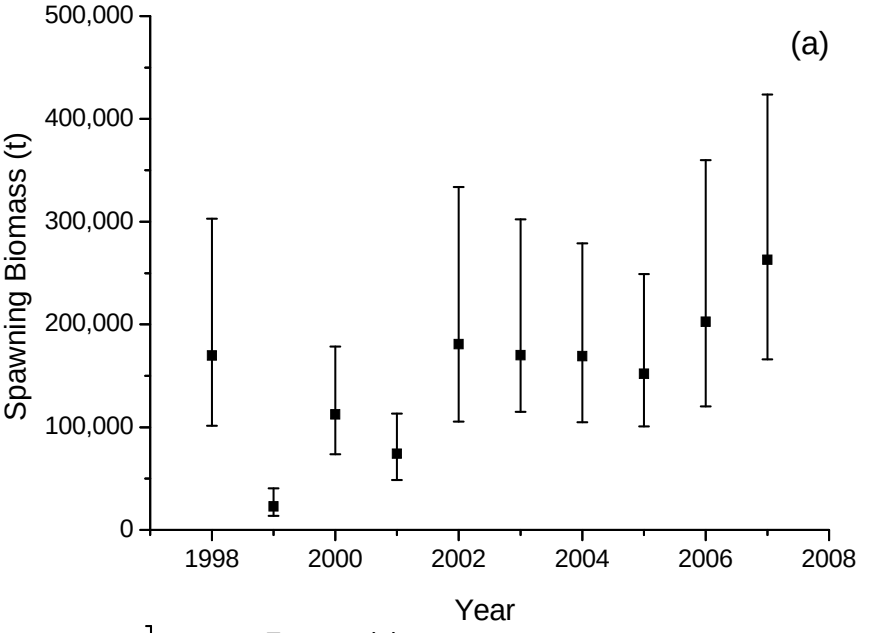
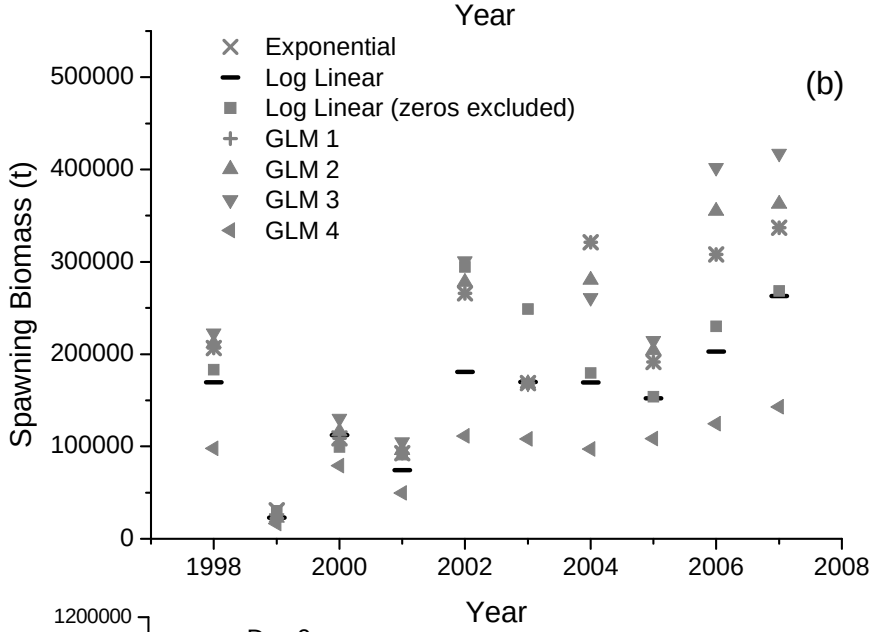


Figure 8. Estimates of spawning fraction obtained using the proportion of females in samples with ovaries containing day-0 (including hydrated oocytes), day-1 and day-2 POFs (post ovulatory follicles) and all POF stages (i.e. day-0, day-1 and day-2) combined. The 95% confidence intervals are derived from 100,000 bootstrapped estimates and are for the mean proportion of all POF stages combined.


(a)
Spawning Biomass (t)
500,000
400,000
300,000
200,000
100,000
0
1998
2000
2002
2004
2006
2008
Year


(b)
Exponential
Log Linear
Log Linear (zeros excluded)
GLM 1
GLM 2
GLM 3
GLM 4
Spawning Biomass (t)
500000
400000
300000
200000
100000
0
1998
2000
2002
2004
2006
2008
Year

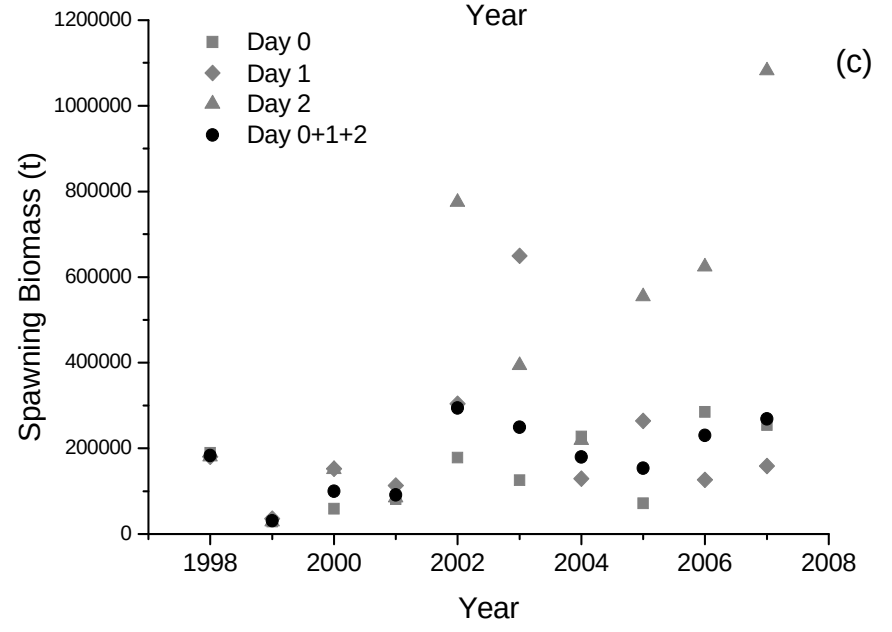

(c)
Day 0
Day 1
Day 2
Day 0+1+2
Spawning Biomass (t)
1200000
1000000
800000
600000
400000
200000
0
1998
2000
2002
2004
2006
2008
Year

Figure 9. (a) Estimates of spawning biomass of *S. sagax* in South Australian waters between 1998 and 2007 based on estimates of mean daily egg production obtained using log-linear model (mean annual estimates of Z in 1999 and 2003) and datasets with one egg added to each day class of eggs at each positive station, spawning area estimated using Voroni natural neighbour method (all live eggs), mean female weight and mean sex ratio from fishery independent samples, batch fecundity using linear regression model (mean relationship for all years used to estimate values for 2003 and 2004) and spawning fraction based on day-0 POFs (plus hydrated females), day-1 POFs and day-2 POFs (combined). Error bars are 95% confidence intervals. (b) Estimates of spawning biomass based on estimates of mean daily egg production (P_0) obtained using seven models. LLzx = log-linear model with zero values excluded. All other models are fitted to datasets with one egg added to each day class of eggs at each positive station. LL = log-linear, Exp = exponential (Lasker 1985), Generalized Linear Model (GLM) 1 = Gaussian GLM with a log link, GLM 2 = Quasi GLM with a log link and variance proportional to the mean, GLM 3 = Quasi GLM with a log link and variance proportional to the mean squared and GLM 4 = Quasi GLM with an identity link and variance proportional to the mean fitted to ln egg density. (c) Estimates of spawning biomass based on estimates of mean spawning fraction obtained using the proportion of females in samples with ovaries containing day-0 (including hydrated oocytes), day-1 and day-2 POFs (post ovulatory follicles) and all POF stages (i.e. day-0, day-1 and day-2) combined.

Discussion

Total Daily Egg Production

Estimating P_0

The most important finding of this study is that the log-linear model (with one egg added to each day class of eggs at each positive site) is the best option currently available for estimating P_0 in DEPM studies of sardine because it fits strongly over-dispersed egg density data better (e.g. lower CVs) and provides logically consistent and more precautionary estimates of P_0 than the traditional exponential model (Lo et al. 1996, 2005) and GLMs of untransformed data (ICES 2004; Stratoudakis et al. 2006; Wood 2006). Using the log-linear model to deal with the non-normal distribution of residuals and non-homogenous variances of egg density data is not a new idea. This approach was suggested by Piquelle and Stauffer (1985) in the original report by Lasker (1985), but has been rarely adopted (e.g. Ward et al. 2001a). However, the results of the present study suggest that the exponential model and GLMs of untransformed data may introduce a significant positive bias into estimation of P_0 when datasets include a few samples with very large numbers of early stage eggs. For example, estimates of *SB* for sardine obtained using the exponential model and GLM1 in 2004 (~321,200 t), when a few samples contained very high densities of early stage eggs, were more than 80% higher than that obtained using the log-linear model (169,209 t). The *SB* estimate for 2004 obtained using the exponential model and GLM1 was also 91% and 68% higher than the estimates obtained for 2003 and 2005, respectively, using these methods. From a precautionary fisheries management perspective it is clear that the log-linear model is the best option currently available for estimating P_0.

Although the log-linear model is the currently the best option for estimating P_0, the diagnostics show that estimates have low precision (CV = 0.17- 0.23). The problem of estimating P_0 from strongly over-dispersed data may not be overcome by developing more sophisticated statistical techniques. Furthermore, logistical constraints virtually ensure that the problem of estimating P_0 from strongly over-dispersed data will not be overcome by simply collecting more CalVET net samples. Collecting additional data using complementary methods, such as a CUFES, may be a more suitable approach. It has been widely acknowledged that using a CUFES has the potential to increase the precision of estimates of egg production obtained using the DEPM (van der Lingen *et al.* 1998; Lo *et al.* 2001; Stratoudakis *et al.* 2006). However, in most studies conducted to date, a CUFES has mainly been used to reduce sampling intensity or to stratify the spawning area. A recent study suggests that CUFES data could be integrated with data from CalVET nets to provide more precise estimates of P_0 (Ward and Ivey, in revew 2009) Specifically, data collected at each CalVET net site could potentially be used to address the effects on estimates of P_0 of samples that include high densities of early stage eggs. CUFES data obtained between each CalVET net site could potentially be used to enhance estimation of both P_0 and A.

Estimating egg age

Temperature egg development keys are widely used to inform application of the DEPM, but such a key has not been developed for sardine off South Australia. The kernel egg density plots provide estimates of modal age for each egg stage that are analogous to information obtained from temperature egg development keys. The main disadvantage of these types of plots is that sufficient data are only

available after many samples have been collected. The main advantage is that data are collected *in situ* and are not susceptible to the potential 'tank' effects on rates of egg development that can affect laboratory studies.

The plots of proportional density of various egg stages versus time of sampling provide evidence that in South Australian waters most sardine eggs reach Stage 7 after 24 hours. However, at cooler water temperatures (<19.0^{o}C) most eggs only reach Stage 6 after 24 hours, whereas at warmer temperatures (>20.0^{o}C) most eggs reach Stage 8. Assuming that all eggs reached Stage 7 after 24 hours (i.e. ignoring effects of temperature on developmental rates) produced estimates of P_0 that were up to 30% different from the two temperature adjusted estimates. Despite our large dataset, some estimates of the modal age of some egg stages in some temperature ranges were based on relatively small sample sizes and may be biased. This problem can also affect estimates of age from temperature egg development keys (e.g. White and Fletcher 1996). The best approach to estimating egg age in most situations may be to recognise that in different temperature ranges, eggs reach different stages after 24 hours, and use this information to define stages that constitute day-1 and day-2 eggs in samples and then estimate egg age by subtracting spawning time from collection time.

Estimating spawning area

The large range of estimates of *A* as a percentage of the mean (94.9%) emphasizes the importance of estimating this parameter reliably to optimise estimates of *SB*. Our results show that in situations where sites are evenly distributed along equidistant transects, estimates of *A* obtained using manually drawn grids and

those generated using the VNN method are similar. The VVN method should probably be used in most situations because it removes subjectivity, especially when the sampling design is complex (i.e. not simple equidistant transects and sites). However, in some situations where the survey design is very complex and the number of sites sampled is small compared to the size of the area surveyed (see example for East Coast of Australia in Ward *et al.* 2009), the VNN method cannot always be used effectively. A critical element to estimating *A* reliably is to establish a large number of grids of approximately the same size, to minimise bias resulting from the presence or absence of eggs in one or two large grids.

It has been widely acknowledged that to estimate *SB* reliably, the entire area over which spawning occurs should be sampled. However, the presence of eggs on the last site on the far western transect in some years (e.g. 2003, Figure 2), shows that ensuring the entire *A* is sampled can be difficult to achieve in practice. It is particularly difficult when there are no natural barriers to the distribution of adults or eggs, as is the case off South Australia. In the present study, it is highly likely that some spawning occurred outside the area surveyed in at least some years. While sampling the entire spawning area is clearly desirable, our results show that large fluctuations in *SB* can be identified even if the entire *A* is not sampled. For example, the sampling area in 1999 was >25% larger than the area sampled in 1998, but the estimate of *A* was more than 50% lower in 1999 than in 1998. In situations where the spawning area of sardine is not discreet, as is the case in southern Australia, the most practical option may be to consistently survey the largest area that is logistically feasible to sample.

As is the case in almost all other DEPM studies, our approach assumed that *A* was estimated without error. This assumption is incorrect and requires further consideration because of the strong correlation of estimates of *A* and *SB* (e.g. Gaughan *et al.* 2004). There are several potential sources of error associated with estimating *A* from ichthyoplankton data. Most notably, data from a small area (0.05 m^2) is used to characterise egg presence/absence over a much larger area. Like Pepin *et al.* (2005), we recognise the potential for using a CUFES to enhance estimation of *A* in future DEPM studies of sardine.

Mean Daily Fecundity

Prior to this study (Ward *et al*. 1998, 2001b), some DEPM samples of adult sardines were collected from South Australian waters by purse-seining and mid-water trawling. However, samples obtained by purse-seining included few spawning fish and few samples were collected in mid-water trawls, perhaps due to the slow towing speed (~ 4 knots) of the RV *Ngerin* (Ward *et al*. 1998, *et al.* 2001b). Samples collected from commercial purse-seine vessels during the present study were frozen, which prevented histological determination of spawning fraction. However, absence of females whose ovaries contain hydrated oocytes suggests these samples also contained few actively spawning fish. It is unclear whether this problem reflects the selectivity of the gear or targeting practices of fishers. Samples obtained from purse-seine vessels in southern Western Australia do contain actively spawning fish, including females with ovaries that contain hydrated oocytes (Fletcher et al. 1996; Gaughan et al. 2004).

In the present study, samples of adult fish were collected in all years using fishery-independent methods (gillnets and lights). Annual collection of adults has not always been achieved in other long-term applications of the DEPM to sardine (e.g. Lo *et al.* 2005). Estimates of adult parameters, especially *S* and *F*, obtained from gill net samples were consistent with estimates obtained in other studies using other methods (e.g. (Fletcher et al. 1996; Gaughan et al. 2004). This finding suggests that gill nets may provide samples that are representative of levels of spawning activity in sardine populations. However, the extent to which samples obtained by purse-seining, mid-water trawling or gill-netting are representative of the adult sardine populations is not well known. Experimental comparison of the size and spawning activity of fish in samples obtained using all three methods is warranted.

Relatively few adult samples (<12 samples, <1000 fish) were collected in 1998, 2003 and 2004. In both 2003 and 2004, less than 10 females with hydrated oocytes were collected for estimation of the relationship between gonad-free female weight and batch fecundity. This lack of data, in part, reflects the main weakness of using gillnets and lights to collect samples. The method works best when the vessel is at anchor in a relatively protected location, which means, adult samples can only be collected from a small number of sites, mostly islands (Fig. 1). Using several methods to collect adult samples would be beneficial, especially if any biases associated with each method could be determined reliably.

The relatively small ranges of estimates of *R*, *W* and *F* among years suggest that these parameters may be less critical determinants of *SB* than P_0, *A*, and *S* (Table

3). Estimates of *R* obtained from fishery-dependent purse-seining and fishery-independent gill-netting were also similar. In contrast, estimates of *W* (and *F*) obtained from samples taken from the fishery were generally lower than those obtained from research samples, even though both methods have potential to catch fish across the entire adult size range. This may be because small, young fish are abundant in Spencer Gulf where most commercial fishing is undertaken, and larger older fish are common in shelf waters offshore where most research samples were collected (Rogers and Ward 2007). Although the ranges of *W* and *F* among years were ~50% of the mean, because of the strong correlation between these parameters, the range with *F/W* was only 30.8% of the mean (Table 3). This correlation means that increases in *SB* from increases in *W* are offset by reductions in *SB* resulting from the corresponding increase in *F*.

In contrast to *R*, *W* and *F*, the range of estimates of *S* as a percentage of the mean was large (83.0%), which emphasizes the importance of this parameter in determining *SB* (Table 3). There was also a large variation in estimates of *S* obtained from females with day-0 (including hydrated oocytes), day-1 and day-2 POFs, which suggests that each category may provide a biased estimate of this parameter and that these biases may vary spatially and temporally. In both 2000 and 2005, when large numbers of adult samples were collected, the proportion of females with day-0 POFs was much higher than the proportion of females with either day-1 or day-2 POFs. Assuming that the proportion of fish spawning each night is constant, this finding suggests that day-0 females were over-represented in samples and that day-1 and day-2 females were under-represented. This could have reflected either higher vulnerability of day-0 females to gillnetting or the

misidentification of some day-1 and day-2 POFs to an early stage (i.e. day-0 and day-1 POFs, respectively). This finding is important because using the estimate of *S* obtained from females with day-0 POFs can cause *SB* to be over-estimated by a factor of greater than two (e.g. in 2005). The most logical way to reduce these biases is to use data from all three POF stages (combined) to estimate *S*. However, these findings clearly show that there is a need for detailed studies of the relative vulnerability of females with different stage POFs to capture in gill-nets (and other adult sampling methods) and to quantify the rates of degeneration of sardine POFs in South Australian waters.

Summary and conclusions

This study suggests that DEPM is a suitable tool for stock assessment of sardine, but shows, as other authors have noted, that estimates of *SB* are imprecise. We have also shown that estimates of *SB* can be biased if inappropriate models are used to estimate P_0. Similarly, using females with only day-0, day-1 or day-2 POFs to estimate *S* has the potential to introduce biases into estimates of *SB*. As shown by Gaughan et al. (2004) and others, the parameter that has the greatest potential to influence estimates of *SB* is *A*. A key factor that affect *A* is the size of the area surveyed. Most reviews have identified the need to survey the entire spawning area, but this study shows that this can be difficult to achieve in practice. In situations, such as southern Australia, where it can be logistically difficult to survey the entire area over which sardines are likely to spawn, the best option may be to ensure that the area surveyed is as large as possible and is consistent among years.

The findings of this study suggest that future applications of the DEPM for stock assessment of sardine should: 1) use the log-linear egg mortality model (with one egg added to each day class of eggs at each positive site) to estimate P_0; 2) ensure that the area surveyed is large and consistent among years and is divided into a large number of similar-sized grids; 3) use the VNN method to estimate the size of each grid; 4) ideally utilise more than one adult sampling method; 5) calculate S using data from females with all stages of POFs (combined).

Research priorities for improving future applications of the DEPM for sardine include: 1) determining the best way to utilise data from a CUFES to increase the precision of estimates of P_0 and A; 2) conducting experiments to determine the rates of vulnerability of females with different stages of POFs to capture in various sampling gear (including gillnets, purse-seine nets and mid-water trawls); and 3) determining the effects of temperature on the rates of degeneration of sardine POFs in Australasian waters. The last two priorities could be addressed by spatially replicated studies involving around-the-clock sampling of adult sardines over several days using several methods. Such studies would ideally be conducted in conjunction with around-the-clock plankton sampling designed to elucidate rates and patterns of egg development and mortality in different water bodies with different oceanographic characteristics.

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(2): 750-767.

Borchers, D. L., Buckland, S. T., Priede, I. G. and Ahmadi, S. (1997). "Improving the precision of the daily egg production method using generalized additive models." Canadian Journal of Fisheries and Aquatic Sciences 54(12): 2727-2742.

Dimmlich, W. F. and Ward, T. M. (2006). "Ontogenetic shifts in the distribution and reproductive patterns of Australian anchovy (*Engraulis australis*) by otolith microstructure analysis." Marine and Freshwater Research 57: 373-381.

Efron, B. and Tibshirani, R. J. (1993). An Introduction to the Bootstrap. New York, Chapman and Hall, Inc.

Fletcher, W. J., Lo, N. C. H., Hayes, E. A., Tregonning, R. J. and Blight, S. J. (1996). "Use of the daily egg production method to estimate the stock size of western Australian sardines (*Sardinops sagax*)." Marine and Freshwater Research 47(6): 819-825.

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(4): 617-633.

Gaughan, D. J., Mitchell, R. W. and Blight, S. J. (2000). "Impact of mortality, possibly due to herpesvirus, on pilchard *Sardinops sagax* stocks along the south coast of Western Australia in 1998-99." Marine and Freshwater Research 51(6): 601-612.

Griffin, D. A., Thompson, P. A., Bax, N. J., Bradford, R. W. and Hallegraeff, G. M. (1997). "The 1995 mass mortality of pilchard: No role found for physical or biological oceanographic factors in Australia." Marine and Freshwater Research 48(1): 27-42.

Hunter, J. R. and Goldberg, S. R. (1980). "Spawning incidience and batch fecundity in northern anchovy, *Engraulis mordax*." Fisheries Bulletin 77: 641-52.

Hunter, J. R. and Lo, N. C. H. (1997). "The daily egg production method of biomass estimation: Some problems and potential improvements." Ozeanografika 2: 41-69.

Hunter, J. R. and Macewicz, B. J. (1985). Measurement of spawning frequency in multiple spawning fishes. National Marine Fisheries Services. National Oceanic and Atmospheric Administration Technical Report. 79-94.

Hyatt, A. D., Hine, P. M., Jones, J. B., Whittington, R. J., Kearns, C., Wise, T. G., Crane, M. S. and Williams, L. M. E. (1997). "Epizootic mortality in the pilchard *Sardinops sagax neopilchardus* in Australia and New Zealand in 1995. 2. Identification of the herpesvirus in the gill epithelium." Diseases of Aquatic Organisms 28: 17-29.

ICES (2004). "The DEPM estimation of spawning-stock biomass for sardine and anchovy." ICES Cooperative Research Report 268: 91 pp.

Jones, J. B., Hyatt, A. D., Hine, P. M., Whittington, R. J., Griffin, D. A. and Bax, N. J. (1997). "Australasian pilchard mortalities." World Journal of Microbiology & Biotechnology 13(4): 383-392.

Kaempf, J., Doubell, M., Griffin, D., Matthews, R. and Ward, T. M. (2004). "Evidence of a large seasonal coastal upwelling system along the southern shelf of Australia " Geophysical Research Letters 31: 1-4.

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. National Oceanic and Atmospheric Administration Technical Report. 36. 99 pp.

Lo, N. C. H., Green Ruiz, Y. A., Cervantes, M. J., Moser, H. G. and Lynn, R. J. (1996). Egg production and spawning biomass of Pacific sardine (*Sardinops sagax*) in 1994, determined by the daily egg production method. California Cooperative Oceanic Fisheries Investigations Report. 160-174.

Lo, N. C. H., Hunter, J. R. and Charter, R. (2001). "Use of a continuous egg sampler for ichthyoplankton surveys: application to the estimation of daily egg production of Pacific sardine (*Sardinops sagax*) off California." Fishery Bulletin 99(4): 554-571.

Lo, N. C. H., Macewicz, B. J. and Griffith, D. A. (2005). "Spawning biomass of Pacific sardine (*Sardinops sagax*), from 1994-2004 off California."

Reports of California Cooperative Oceanic Fisheries Investigations 46: 93-112.

McClatchie, S., Middleton, J. F. and Ward, T. M. (2006). "Water mass analysis and alongshore variation in upwelling intensity in the eastern Great Australian Bight." Journal of Geophysical Research-Oceans 111(C8).

McCullagh, P. and Nelder, J. A. (1983). Generalized Linear Models. USA, Chapman and Hall.

McGarvey, R. and Kinloch, M. A. (2001). "An analysis of the sensitivity of stock biomass estimates derived from the daily egg production method (DEPM) to uncertainty in egg mortality rates." Fisheries Research 49(3): 303-307.

Middleton, J. F. and Cirano, M. (2002). "A northern boundary current along Australia's southern shelves: The Flinders Current." Journal of Geophysical Research C: Oceans 107(9): 3129–3143.

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

Pepin, P., Snelgrove, P. V. R. and Carter, K. P. (2005). "Accuracy and precision of the continuous underway fish egg sampler (CUFES) and bongo nets: a comparison of three species of temperate fish." Fisheries Oceanography 14(6): 432-447.

Picquelle, S. and Stauffer, G. (1985). Parameter estimation for an egg production method of anchovy biomass assessment. National Marine Fisheries Services. National Oceanic and Atmospheric Administration Technical Report. 36. 7-17.

Rogers, P. J. and Ward, T. M. (2007). "Application of a 'case-building approach' to investigate the age distributions and growth dynamics of Australian sardine (*Sardinops sagax*) off South Australia." Marine and Freshwater Research 58(5): 461-474.

Simonoff, J. S. (1998). Smoothing Methods in Statistics. USA, Springer-Verlag.

Smith, P. E. and Richardson, S. L. (1977). Standard techniques for pelagic fish egg and larva surveys. Food and Agriculture Organisation. Fisheries Technical Paper No. 100.

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(1): 35-57.

Swartzman, G., Huang, C. and Kaluzny, S. (1992). Spatial Analysis of Bering Sea Groundfish Survey Data Using Generalized Additive Models. Canadian Journal of Fisheries and Aquatic Science. 49: 1366--1378.

van der Lingen, C. D., Checkley, J., D., Barange, M., Hutchings, L. and Osgood, K. (1998). "Assessing the abundance and distribution of eggs of sardine, *Sardinops sagax*, and round herring, *Etrumeus whiteheadi*, on the western Agulhas Bank, South Africa, using a continuous underway fish egg sampler." Fisheries Oceanography 7(1): 35-47.

Ward, T. M., Hoedt, F., McLeay, L., Dimmlich, W. F., Jackson, G., Rogers, P. J. and Jones, K. (2001a). "Have recent mass mortalities of the sardine *Sardinops sagax* facilitated an expansion in the distribution and abundance of the anchovy *Engraulis australis* in South Australia?" Marine Ecology-Progress Series 220: 241-251.

Ward, T. M., Hoedt, F., McLeay, L., Dimmlich, W. F., Kinloch, M., Jackson, G., McGarvey, R., Rogers, P. J. and Jones, K. (2001b). "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(4): 830-841.

Ward, T. M., Kinloch, M., G.K., J. and Neira, F. J. (1998). A Collaborative Investigation of the Usage and Stock Assessment of Baitfish in Southern and Eastern Australian Waters, with Special Reference to Pilchards (*Sardinops sagax*). South Australian Research and Development Institute (Aquatic Sciences). Final Report to Fisheries Research and Development Corporation. 324pp.

Ward, T. M., Rogers, P. J., McLeay, L. J. and McGarvey, R. (2009). "Evaluating the use of the Daily Egg Production Method for stock assessment os blue mackerel, *Scomber austrasicius*." Journal of Marine and Freshwater Research 62(2): 112-128.

Watson, D. F. (1981). Computing the n-dimensional Delaunay tessellation with application to Voronoi polytopes. The Computer Journal. 24: 167-172.

White, K. V. and Fletcher, W. J. (1996). Identifying the developmental stages for eggs of the Australian pilchard, *Sardinops sagax*. Fisheries Western Australia. Fisheries Research Report No.103. 21pp.

Wood, S. N. (2006). Generalized Additive Models An Introduction with R, Chapman and Hall/CRC.