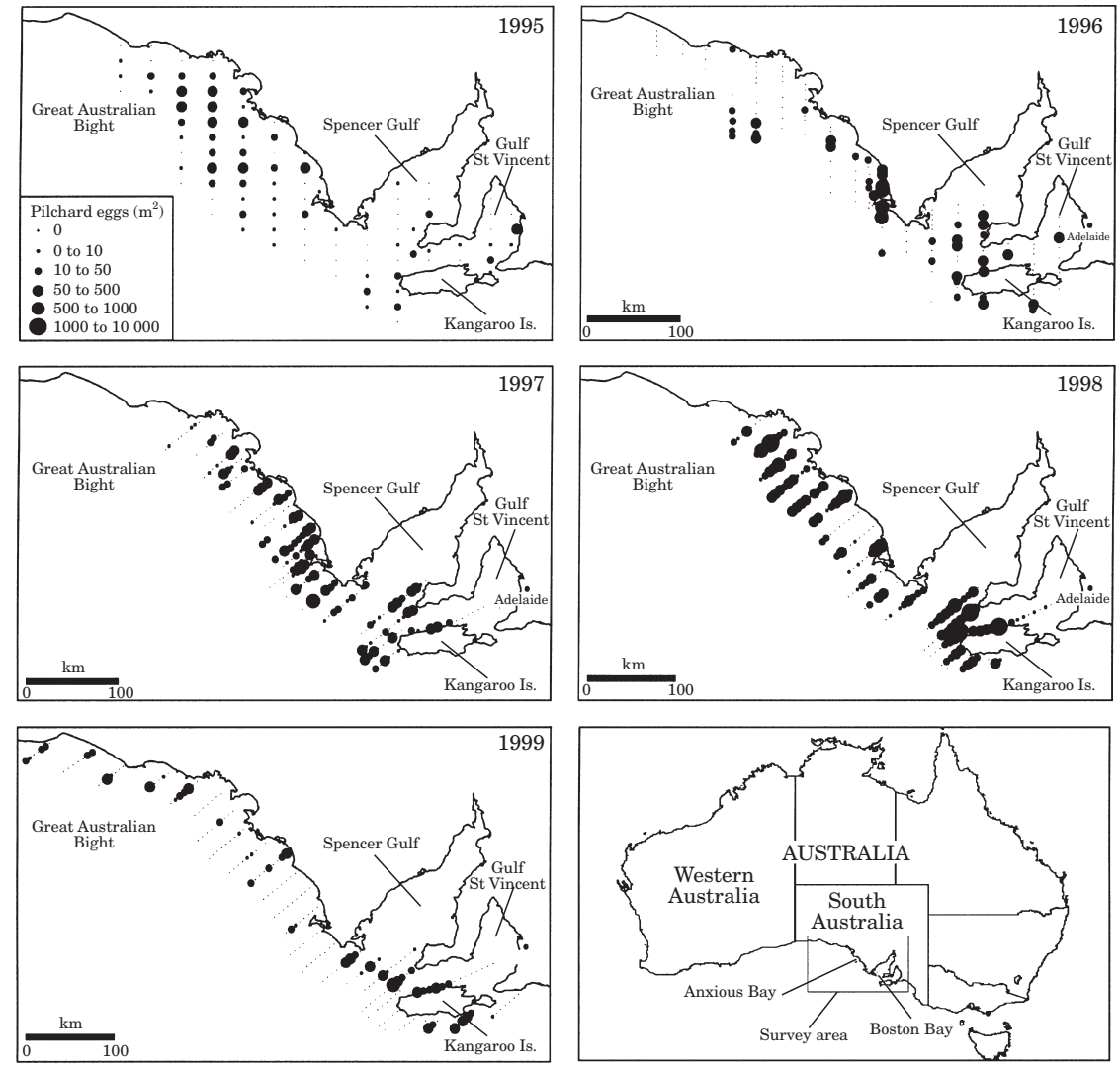

Figure 1. Maps showing locations mentioned in the text and sites at which plankton samples were collected in South Australia between 1995 and 1999.

of 10–15 d duration were conducted each year from the RV "Ngerin" during the spawning season of *S. sagax* in South Australian waters (January–March). The sampling design varied between years. In 1995 and 1996 transects were orientated north-south whereas in 1997–1999 transects were orientated northeast-southwest to improve sampling efficiency (Figure 1). The number of sites and area sampled are shown in Table 1.

Paired conical plankton nets with an internal diameter of 0.255 m were deployed to within 10 m of the seabed in depths of less than 80 m, or 70 m in waters deeper than 80 m. The net was retrieved vertically at a speed of approximately 1 m s^{-1}. Flowmeters (one in each net) were used to estimate the distance travelled by the net. Samples taken from the two nets at each site were pooled and stored in 5% buffered formaldehyde solution.

Spawning time

Sardine eggs in each sample were counted and staged according to their internal features (White and Fletcher, 1996). The age of each stage of development was estimated using temperature-development keys of White and Fletcher (1996) and assuming the sea surface temperature was the ambient temperature for egg development. The time of spawning of eggs of each stage was estimated by subtracting the age of eggs in hours from the time when each sample was collected.

Table 1. Information on plankton surveys including estimates of egg production and spawning area. Bold signifies parameters used in biomass calculations.

	1995	1996	1997	1998	1999
No. sites	97	154	189	164	212
No. sites with eggs (%)	59 (61)	43 (28)	97 (51)	110 (67)	49 (23)
No. eggs collected	1 197	116	721	2 738	292
Sampling area	111 719	76 070	49 825	49 405	67 653
Spawning area (km^2)	68 260	17 990	26 276	32 232	16 301
Egg production (eggs m^{-2})	26.35	22.16	47.32	99.56	53.66

Spawning area

After each survey was completed, the sampling area was divided into a series of contiguous polygons approximately centred on each site. The area of each polygon (km^2) was calculated using GIS software. The spawning area (A) was defined as the total area of polygons where eggs were found.

Egg production

The number of eggs of each stage under one square metre of water (P_t) was estimated for each site according to the equation:

$$P_t = A^{-1}B^{-1}CD \quad (2)$$

where A is the combined area of the net opening (m^2), B is the calibrated tow length determined from the flowmeter (m), C is the number of eggs of each age in each sample, D is wire length (m).

The mean weighted age of day-1 (<24 h old) and day-2 eggs (>24 h old) was calculated for each sample from the densities of eggs of different ages. The densities of day-1 and day-2 eggs were then weighted by the area of the polygon in which they were caught.

As residuals were not normally distributed, egg production was estimated using the linear version of the exponential mortality model:

$$\ln P = \ln (P_t) + Zt \quad (3)$$

where P is the daily egg production, P_t is egg density at age t, and Z is the instantaneous rate of egg mortality.

Negative biases in the estimates of egg production (P_{biased}) obtained using the linear version of the exponential mortality model were corrected using the following equation:

$$P = e^{(\ln P_{biased} + \sigma/2)} \quad (4)$$

where σ is the variance of the estimate of P (Lasker, 1985).

In 1997, problems with preservation of the eggs precluded application of the temperature-development key, and therefore could not be staged. This necessitated use of the technique for estimating initial daily egg production based on the mean density of eggs of all ages ($\bar{P}$) and an assumed value of egg mortality (Z) described by McGarvey and Kinloch (2000).

The mean density of eggs of all ages (i.e. day-1 and day-2 eggs) present in samples can be expressed as:

$$\bar{P} = \frac{\int_{t=0}^{1} P_t dt + \int_{t=1}^{2} P_t dt}{\int_{t=0}^{1} dt} \quad (5)$$

where t=0 is the time of spawning, and the average is calculated over a full day of sampling.

The formula for initial egg production (P) was obtained by substituting the exponential mortality equation describing the change in egg density with age [Equation (3)] into Equation (5) and solving,

$$P = \frac{\bar{P}}{\int_{t=0}^{1} (e^{-zt} + e^{-z(t+1)}) dt} \quad (6)$$

Initial egg production was calculated from Equation 6 by entering measured mean egg density ($\bar{P}$) and a value of Z equal to the mean values estimated in 1995 and 1998, when the largest numbers of eggs were collected and most reliable estimates of Z were obtained. The sensitivity of initial egg density (P) to different assumed levels of Z is low and the use of prior estimates of Z induces relatively low imprecision into estimates of P (McGarvey and Kinloch, 2000).

In 1999, mean egg production was calculated using day-1 eggs only as a few sites contained an unusually high number of day-2 eggs, which produced a negative egg mortality (Z) value.

Adult reproductive parameters

Adult sampling

In 1995, frozen samples of *S. sagax* were collected from catches of the South Australian sardine fishery operating

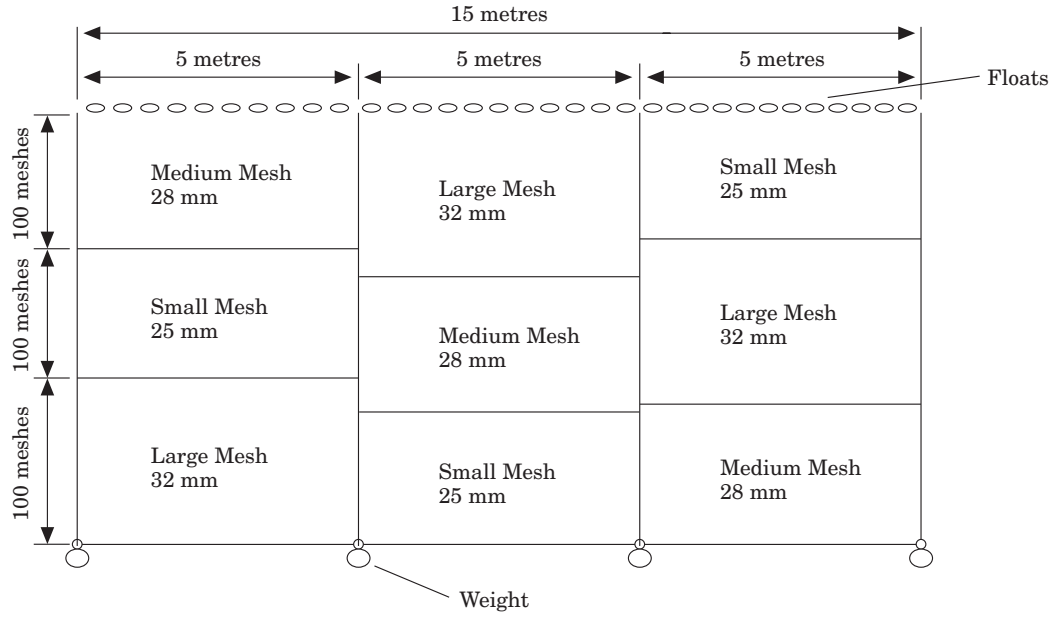

Figure 2. Schematic diagram of gillnet used to sample adult *S. sagax* in 1998 and 1999.

in southern Spencer Gulf by onboard observers. In 1996, samples were collected from southern Spencer Gulf and around the Coffin Bay Peninsula between January and April using chartered commercial purse seine vessels and preserved in formaldehyde *in situ*.

In 1997, a few samples from waters around offshore islands were obtained by deploying a mid-water trawl net similar to that described by Dotson and Griffith (1996) from the RV "Ngerin". Trawling was done at night in areas where sardine schools could be seen either at the surface or on the ship's echo sounder. The net was towed at approximately four knots for periods of one to two hours.

In 1998 and 1999, research cruises were deliberately scheduled to coincide with the two weeks around the new moon. Areas in which sardine schools were likely to be present were searched each afternoon using a dual frequency echo sounder (Furuno – 85 and 200 megahertz). The vessel was anchored in an area where several schools were found. If wind or currents were strong or running in different directions, a rope was attached from a stern bollard to the anchor line to form a "bridle". The movement and orientation of the vessel were controlled by altering the relative length of the anchor chain and the stern rope.

Samples were collected using a gillnet (Figure 2) comprised of three panels of each of three sizes of multi-filament nylon mesh (Double Diamond: ply-210/4; meshes: 25 mm, 28 mm and 32 mm). Small floats were attached to the headrope and the footrope was made of leaded rope (10 mm; 200 g m^{-1}). Weights ranging from 0.5–2.0 kg, depending on the wind and current conditions, were attached to each end of the footrope and smaller weights were attached below each seam (Figure 2).

The net was deployed from the leeward side of the vessel and lowered to the desired depth (usually 5–20 m) using droplines attached to the headrope and footrope. One or two surface lights of approximately 1000–1500 watts and one or two sub-surface lights of approximately 500–1000 watts were illuminated after the net was set. Soak time varied between approximately ten minutes and two hours, and was determined by the abundance and catch rates of fish.

Preservation of samples

Captured fish were dissected by mid-ventral incision. The numbers of male and female mature and immature fish were counted. Mature females were fixed in 5% buffered formaldehyde solution. Immature females and males were frozen. When large numbers of fish were caught only portion of the catch was sorted and preserved.

Female weight

Preserved mature females in each sample were weighed ($\pm$ 0.01 g). The mean weight of mature females in the population was calculated from the average of sample means weighted by sample size.

Sex ratio

Mature males in each sample were thawed and weighed ($\pm$ 0.01 g). The sex ratio for each sample was calculated

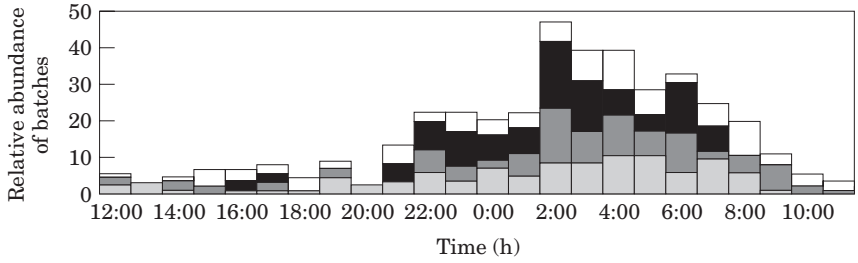

Figure 3. Estimated spawning times of S. *sagax* in South Australia between 1995 and 1999.

from the weight of mature females divided by the sum of the total weight of mature females and mature males. The mean sex ratio for the population was calculated from the average of sample values weighted by sample size.

Spawning fraction

Ovaries of mature females were sectioned and stained with haematoxylin and eosin. Several sections from each ovary were examined in order 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 (i) hydrated oocytes plus day-0 POFs, (ii) day-1 POFs and (iii) day-2 POFs. The mean spawning fraction of the population was calculated from the average of sample means weighted by sample size.

Batch fecundity

The batch fecundity of females with hydrated oocytes was estimated using the gravimetric method of Hunter *et al*. (1985). Both ovaries of each female were weighed. Three sub-sections were cut from each ovary, weighed, and the number of hydrated oocytes in each counted. The total batch fecundity for each female was calculated by multiplying the mean number of oocytes per gram of ovary segment by the total weight of the ovaries. The relationship between female weight (ovaries removed) and batch fecundity was determined by linear regression. This relationship was used to estimate the mean batch fecundity of mature females in each sample. The mean batch fecundity of the population was calculated from the average of sample means weighted by sample size.

Estimates of confidence intervals and spawning biomass

The coefficient of variation (CV) for each parameter was calculated using bootstrapping procedures. Each parameter was randomly estimated 10 000 times from data sets containing randomly chosen individuals from randomly chosen samples. The number of samples and the sample sizes used in each calculation were the same as in the original dataset. Where regressions were used to calculate parameters (P and F), bootstrap data sets consisted of randomly chosen residuals from the original regression added to each expected value. CVs were not calculated for parameters that were estimated from values obtained in other years. The 95% confidence intervals of spawning biomass estimates obtained in 1998 and 1999 (when all parameters were estimated directly) were calculated using percentile technique and 10 000 bootstrapped estimates of each parameter.

Results

Spawning time

Over 81% of batches of eggs were spawned between 2100 and 0800 h. Peak spawning time was between 0200 and 0500 h in 1995, at 0200 h in 1996 and 1998, and at 0400 h in 1999. The peak spawning time calculated from combined data (0200 h) was used as the "time of spawning" in calculations of egg production and biomass (Figure 3).

Spawning area and egg production

In 1995 the spawning area was approximately 68 260 km^2 and mean egg production was 26.35 eggs m^{-2} (CV=0.22) (Figure 4; Table 1). In 1996, following the first mass mortality, the spawning area was reduced to only 17 990 km^2, and mean egg production was 22.16 eggs m^{-2} (CV=0.11). In 1997 estimates of spawning area and mean egg production were 26 276 km^2 and 47.32 eggs m^{-2} respectively. In 1998, prior to the second mass mortality, the spawning area increased to 32 232 km^2 and mean egg production was 99.56 eggs m^{-2} (CV=0.15). In 1999, after the second mass mortality, spawning area fell to 16 301 km^2 and mean egg production was 53.66 egg m^{-2} (CV=0.19) (Figure 4; Table 1).

Female weight

Estimates of mean female weight ranged from 45.29 g (CV=0.08) in 1998 to 52.28 g (CV=0.04) in 1999 (Table 2).

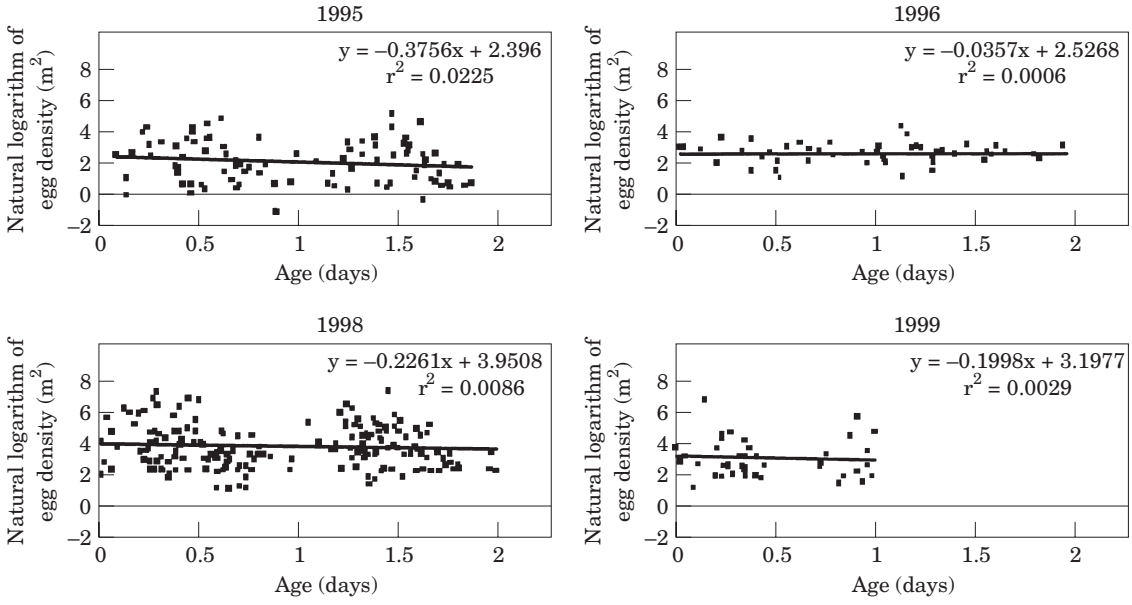

Figure 4. Graphs of egg age against the natural logarithm of mean egg density for 1995, 1996, 1998 and 1999, including regression equations and correlation coefficients. Note that day-2 eggs were excluded from the analysis for 1999.

Table 2. Estimates of egg production and adult reproductive parameters. Parameters in italics are those used in biomass calculations that were estimated from the average of data obtained in other years. Estimates in brackets are those which are considered unreliable and replaced by mean values. Bold signifies parameters used in biomass calculations.

	1995	1996	1997	1998	1999
Number of adult samples	12	32	7	11	13
No. fish	100	850	—	933	1 563
No. females	55	496	386	455	690
No. males	45	341	—	482	873
Mean female weight	**46.42**	**45.29**	**51.94**	**45.18**	**52.28**
Sex ratio (females by weight)	***0.50*** **(0.59)**	***0.50*** **(0.61)**	***0.50***	**0.52**	**0.47**
No. females with ovaries containing:					
Day 0 POFs or hydrated oocytes	0	5	30	57	131
Day 1 POFs	0	0	16	61	106
Day 2 POFs	0	0	5	61	132
Spawning fraction	***0.08***	***0.08***	***0.16*** **(0.04)**	**0.14**	**0.18**
Female wt (ovary removed) (g)	—	—	46.72	41.31	45.18
No. females with hydrates oocytes	—	5	2	55	144
Batch fecundity (no. eggs)	***14 433***	***14 433***	***14 433***	**13 615**	**15 252**

Sex ratio

The estimates of sex ratio for 1995 (0.59) and 1996 (0.61) (Table 2) are high, and as purse-seine nets tend to over-sample females (Fletcher 1990) were not used to calculate spawning biomass. Estimates of sex ration obtained for 1998 and 1999 of 0.52 (CV=0.15) and 0.47 (CV=0.12) appear to be unbiased. The mean sex ratio for 1998 and 1999 (0.50) was thus used to calculate spawning biomass for 1995–1997.

Spawning fraction

POFs were not found in samples obtained from purse-seine vessels in 1995 and 1996. Few mature fish were collected in 1997 and the estimate of mean spawning fraction for 1997 was suspiciously low (0.04). The 1997 survey was conducted at approximately the same time as the 1998–1999 surveys, and the mean spawning fraction for 1998–1999 (0.16) was used as the estimate of spawning biomass for 1997. The 1995 and 1996 surveys were