

**Abstract**—In 1996, using the continuous underway fish egg sampler (CUFES), we carried out a preliminary study with the daily egg production method (DEPM) for estimating fish biomass of Pacific sardine, *Sardinops sagax*. Full-water-column abundance of sardine eggs was correlated with the abundance of eggs taken at 3-m depth with a CUFES; however direct conversion of CUFES samples to the full-water-column abundance, required by the DEPM, would add considerable variance to an estimate of daily egg production. Our preliminary study also indicated that the average size of an egg patch for Pacific sardine was 22 km diameter and that all stages of sardine eggs were not equally vulnerable to a CUFES at 3-m depth.

Using these findings as a guide, in 1997 we carried out an adaptive allocation DEPM survey in which the numbers of sardine eggs collected with the CUFES were used to determine subsequent locations of vertical net tows. All the vertical net tows were taken in a high-density stratum where the CUFES collected at least two eggs per minute; egg density in this stratum was computed by using only vertical tows. The remaining survey area, where the CUFES collected fewer than two eggs per minute, constituted a low-density stratum where egg density was estimated by using the ratio of CUFES egg abundance in these two strata multiplied by the egg density in the high-density stratum. Conventional statistics were used because the sampling units were survey lines spaced at 22-km or greater intervals. An acceptable level of precision (CV=21%) for a daily production of 2.57 eggs 0.05 m<sup>2</sup>/d (51.4 eggs m<sup>2</sup>/d), was achieved by using only 141 vertical net tows. Therefore, the CUFES will enhance the DEPM by increasing precision of the estimates, or by reducing costs in relation to a survey composed of only vertical water-column tows, when the CUFES is used adaptively to establish sampling strata for vertical water column tows.

## Use of a continuous egg sampler for ichthyoplankton surveys: application to the estimation of daily egg production of Pacific sardine (*Sardinops sagax*) off California

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The continuous underway fish egg sampler (CUFES) (Checkley et al., 1997; van der Lingen et al., 1998; Checkley et al., 1999; Watson et al., 1999) is a new device that provides high-resolution spatial maps of fish eggs by sieving the eggs from water pumped from a fixed depth while a survey ship is underway. These data may be used as an index of fish abundance, or to study spawning habitats, and if converted to the numbers of eggs in the full-water-column, they may be used to estimate fish biomass by using one of the egg production methods (Hunter and Lo, 1997).

The objective of our study was to evaluate the use of the CUFES in the daily egg production method (DEPM) of estimating the spawning biomass of pelagic fishes (Lasker et al., 1985; Parker, 1985). In the DEPM, biomass is calculated from the number of staged eggs taken in plankton samples and the daily fecundity of the parents. In a standard DEPM estimate, eggs are sampled in vertical net tows, starting from a point below the maximum depth of the eggs (typically 70 m) and ending at the surface. These vertical samples are taken within a grid of stations located 4 nmi apart, a sampling interval known to produce uncorrelated samples of anchovy eggs (Smith and Hewitt, 1985). Use of the CUFES in the DEPM has several potential advantages over the use of a standard fixed-grid survey. Continuous sampling may increase the precision of the biomass estimate because it provides an increased

spatial resolution of egg patches (Hunter and Lo, 1997). Continuous sampling may save ship time and thereby reduce the cost of sampling per transect mile, and the increased spatial resolution of egg patches provides new knowledge regarding the spawning behavior of the species. Several potential disadvantages of using a CUFES in the DEPM also exist: the gains in precision provided by continuous sampling may be diminished because of the increases in variance due to converting numbers of eggs taken in a CUFES to a full-water-column abundance; a much more complicated formula for variance of the estimate may be needed because of correlated CUFES samples; and the numbers of staged eggs taken in a CUFES may be biased, either because a CUFES damages eggs, making the staging of them more subject to error or because all stages may not be equally vulnerable to sampling at the 3-m depth. Clearly these issues need to be resolved if a CUFES is to be used in a DEPM survey.

We used the following approach to evaluate the use of a CUFES in the estimation of daily egg production. We conducted a pilot CUFES survey in 1996 with the objective of examining the spatial properties of Pacific sardine (*Sardinops sagax*) eggs sampled by a CUFES. We determined how the numbers of eggs taken in a CUFES were related to their abundance in the full-water-column, the spatial correlation of egg samples, and the condition of eggs after passing through a CUFES. Our

**Table 1**

Total number of samples and positive samples of sardine eggs sampled in CUFES and CalVET surveys. The range and mean of duration (minutes) for CUFES collections for 1994, 1996, and 1997 surveys.

	1996 pilot CUFES survey			1997 DEPM survey	
	1994 (18 Apr–11 May)	leg 1 (15–21 Mar)	leg 2 (21 Mar–6 Apr)	leg 1 (11 Mar–27 Mar)	leg 2 (28 Mar–7 Apr)
CUFES <sup>1</sup> (positive)	—	1396 (889)	905 (568)	896 (550)	331 (137)
Duration mean range (min)	—	3.5 2–5	18.13 3–35	30 1–54	26.9 1–34
CalVET <sup>2</sup> (positive)	684 (74)	91 (66)	— —	141 (102)	— —
Survey area (km <sup>2</sup> )	380,175 (253,850 in USA)		157,000	174,196	

<sup>1</sup> Total collection in leg 1, 1996, was 1437. The first 41 tows were experimental.

<sup>2</sup> CalVET surveys in 1996 and 1997 were taken in the high-density stratum; in 1994 they were taken in a fixed grid over the whole survey area.

analysis of the 1996 cruise was used to develop an adaptive allocation survey design, similar to that proposed by Thompson et al. (1992), to estimate the biomass of sardine that incorporates a CUFES into the DEPM. Using the adaptive allocation survey design, we conducted a DEPM survey for Pacific sardine with a CUFES in 1997, and the precision of our estimate of daily production of eggs was compared with the precision of the estimate provided by a DEPM survey carried out in 1994 under standard methods (Lo et al., 1996). We also considered the accuracy of shipboard counts of eggs collected in a CUFES in relation to counts of preserved samples made after cruise end. We present our results in chronological order, beginning with those from the 1996 pilot survey which provided the rationale for the new survey design in 1997; we next describe the new survey design, and end with a comparison of the 1997 CUFES and DEPM survey with the conventional DEPM survey in 1994.

## Survey data and spatial models

### Survey data

The data used in our study were taken from three ichthyoplankton surveys (Table 1):

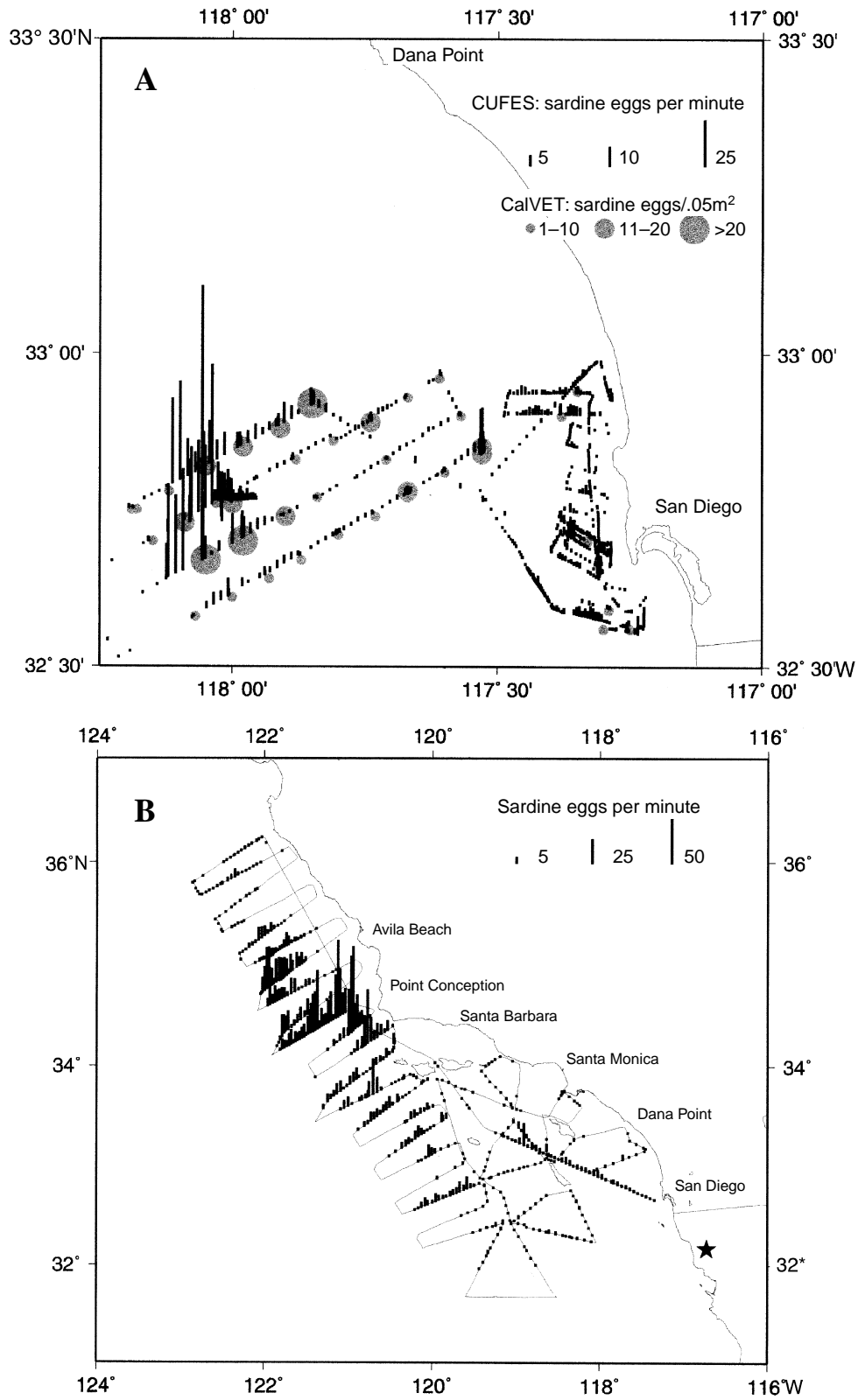
- 1 A pilot CUFES cruise in 1996. This cruise consisted of leg 1 (during which both a CUFES was used and full-water-column tows were taken) and leg 2 (during which only a CUFES was used to survey the large geographic area of spawning sardine (Fig. 1));
- 2 A DEPM survey in 1997. This survey for Pacific sardine employed a new survey design with the CUFES and the California vertical tow (CalVET, see below) (Smith et al., 1985) (Fig. 2). The allocation of CalVETs was determined by the egg density observed from the CUFES.

- 3 Results of the 1994 DEPM survey off California, U.S., and Baja California, Mexico (Lo et al., 1996). This was a conventional fixed-grid DEPM survey employing only CalVETs. This cruise was used as a standard for comparing DEPM surveys with and without the CUFES.

The CalVET net consisted of 150- $\mu$ m nylon netting. The diameter of the CalVET net frame was 25 cm; the tow was lowered to a depth of 70 m and was retrieved vertically. The CUFES was installed midship on the NOAA vessel *David Starr Jordan* onto the intake pipe over the side of the vessel; it extended 3 m below the water surface (see illustration in Checkley et al., 1997). Eggs were sieved from the water flow with the 500- $\mu$ m nylon mesh of the CUFES concentrator.

The density of eggs taken in the CalVET net was expressed as the number of eggs/0.05 m<sup>2</sup> of sea surface water, a standard procedure in the DEPM, where 0.05 m<sup>2</sup> is the area of the mouth opening of the CalVET net. All eggs taken in the CalVET samples, regardless of survey, were counted and staged in the laboratory. The density of eggs taken in the CUFES was expressed as the number of eggs taken per minute. The interval over which eggs accumulated in CUFES samples varied depending on their abundance. When abundance was low, samples were collected over intervals of 0.5 h equivalent to 7.4 km or 4 nmi on the transect line; when abundance was high, they were collected over intervals of at least 1 minute (0.24 km or 0.13 nmi). All CUFES samples were counted at sea, preserved, and recounted in the laboratory. All eggs taken by the CUFES in the pilot survey, but not the subsequent DEPM survey, were staged in the laboratory. We used the system detailed in Lo et al. (1996) and grouped eggs by their ages into half-day age classes for egg mortality computation and one-day age classes for spatial statistical analysis.

In our study, we considered the estimates of only the daily production of eggs,  $P_0$ , one of the key parameters in



**Figure 1**

Sardine eggs/minute from CUFES samples and survey pattern in cruise 9603. Leg 1: 13–21 March 1996 (**A**) and leg 2 (**B**): 6–21 March 1996. The star on the lower right corner of section B is the reference point (0,0) to compute variograms (see text).

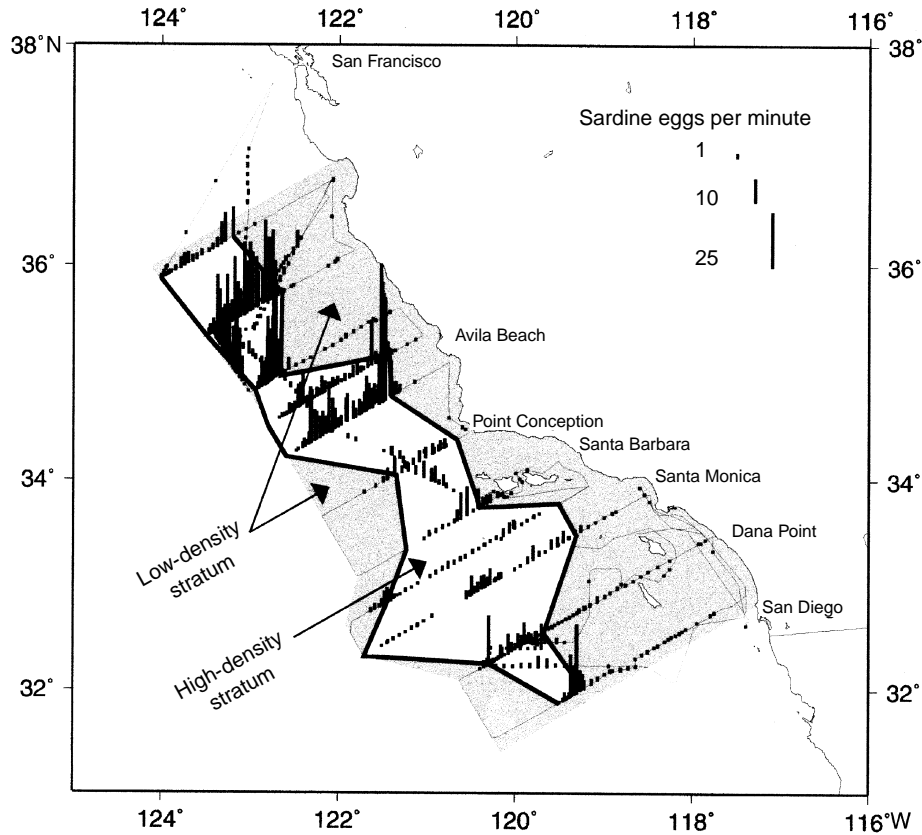


Figure 2

Sardine eggs/minute from CUFES samples and survey pattern in cruise 9703, 11 March–6 April 1997, with two strata: a high-density stratum (open area) and a low-density stratum (shaded area).

the DEPM.  $P_0$  is only one of six parameters used in estimating biomass with the DEPM. In the DEPM, biomass is related to egg production using the model  $B_s = P_0 A / (R/W_f) SF$ , where  $B_s$  = biomass for area  $A$ ;  $P_0$  = the daily egg production at age 0 day per unit sea surface area;  $W_f$  = the average female weight;  $S$  = the fraction of females spawning per day;  $F$  = batch fecundity; and  $R$  = the fraction of the biomass that is female (Alheit, 1993; Hunter and Lo, 1997). The denominator,  $(R/W_f)SF$ , is the number of eggs/biomass in grams and is also called the daily specific fecundity. In the 1994 survey all parameters were estimated but in the 1997 survey we estimated only  $P_0$  and  $A$  and used historical data for the other parameters (Hill et al.<sup>1</sup>).

### Variograms of sardine density (eggs/minute)

As the first step in developing a DEPM survey design for the CUFES, we used geostatistical techniques (Cressie,

1991; Barange and Hampton, 1997; and Fletcher and Sumner, 1999) to describe the spatial structure of the egg distribution and estimated the major diameters of sardines egg patches from the 1996 survey. We applied variogram models (Cressie, 1991; Petitgas, 1993) to CUFES samples (in units of eggs/minute) grouped into three age groups: 1-day (4–27 h), 2-day (28–51 h), and 3-day (52–75 h).

Variogram ( $\gamma(h)$ ) is defined as the variance of difference between values that are  $h$  units apart and is a function of variance and covariance:

$$2\gamma(h) = \text{var}[u(x) - u(x+h)] = 2(\text{var}(u) - \text{cov}(h)) \quad \text{if } \text{var}[u(x)] = \text{var}[u(x+h)], \quad (1)$$

where  $u(x)$  = the eggs/min at location  $x$ ;  
 $u(x+h)$  = the eggs/min at the location  $h$  (nmi) away from  $x$ ;  
 $\text{var}(u)$  = the variance; and  
 $\text{cov}(h)$  = the covariance of eggs/min that are  $h$  (nmi) apart.

$\gamma(h)$  is the semivariogram. For simplicity, we refer to  $\gamma(h)$  as the variogram. We used S+SpatialStats (Kaluzny et al.,

<sup>1</sup> Hill, Kevin, T. M. Yaremko, L. D. Jacobson, N. C. H. Lo, and D. A. Hannan. 1998. Stock assessment and management recommendations for Pacific sardine (*Sardinops sagax*) in 1997. Marine region, Admin. Rep. 98-5, Cal. Dep. of Fish and Game, 8604 La Jolla Shores Dr., La Jolla, CA 92037.

1996) and EVA software (Petitgas and Prampant<sup>2</sup>) to analyze and visualize spatial distributions of sardine eggs/min and to compute a variogram for each of the three age groups of sardine eggs. Two basic assumptions of the variogram (Eq. 1) are intrinsic stationarity and isotropy. Intrinsic stationarity means that a constant mean exists and the variance of egg density is defined by the magnitude of  $h$ . Isotropy means that spatial correlation and the range of correlation do not change with direction. The variogram is normally expressed as a function of three parameters: range, sill, and nugget effect. The range is the distance beyond which the observations are not correlated. The sill is the variance of the random field and is the asymptotic value of  $\gamma(h)$ . The nugget effect measures the micromasurement error and the white noise for  $h$  close to 0 (Cressie, 1991).

Ideally, for  $h$  close to 0, the variogram ( $\gamma(h)$ ) will be close to zero because the observations tend to be similar. As  $h$  increases, the observations become  $h$  units apart and tend to be different, or  $\text{cov}(h)$  decreases, and the variogram increases. At a certain distance,  $h^*$ ,  $\text{cov}(h)$  approaches zero, and for  $h > h^*$ , the variogram approaches its asymptote (sill). The distance,  $h^*$ , is the range. The range ( $h^*$ ) was estimated from a model that best fits the data and was used to estimate the diameter of the patch of sardine eggs because eggs whose distances are less than  $h^*$  nmi are correlated and thus are likely to be in the same patch. Conversely, eggs that are more than  $h^*$  nmi apart are no longer correlated and thus are assumed to be in different patches. We chose the robust (or stable) estimator of the variogram (Cressie and Howkins, 1980; Cressie, 1991):

$$2\hat{\gamma}(h) = \frac{\left[ \sum_{N(h)} |u(x) - u(x+h)|^{1/2} \right]^4}{[0.457 + 0.494/|N(h)|] N(h)^4}, \quad (2)$$

where  $|N(h)|$  = the number of distinctive pairs; and  
 $h$  = the distance (nmi) between any two locations.

To avoid possible trends, we first ran a local regression model (LOESS) of  $\ln(\text{eggs}/\text{min}+1)$  against line distance (the distance computed from the survey lines,  $y$ -axis) and station distance ( $x$ -axis) (Chamber and Hastie, 1992) where the reference point (0,0) is a pseudo station in Mexico (station 260 on CalCOFI line 980; Fig. 1B). We then constructed a variogram for the residuals from the local regression model (LOESS) in four directions clockwise from the transect ( $0^\circ$ ,  $45^\circ$ ,  $90^\circ$ , and  $135^\circ$ ) to examine possible anisotropy. We chose natural logarithm ( $\ln$ ) transformed data because the distribution of eggs was skewed. Finally, we used an interactive S+ function (model.variogram function) to determine the estimates of parameters for each vario-

gram: range, sill and nugget effect. The range was then used as the estimate of the diameter of the patch for each of the three age groups and total number of eggs.

## Results of the 1996 pilot CUFES survey

### Spatial correlation and patch size of sardine eggs

For each of the three age groups and the total number of eggs, the four-directional variograms of the residuals of  $\ln(\text{eggs}/\text{min}+1)$  from LOESS (Fig. 3) indicated that the variograms for transects at  $0^\circ$ ,  $45^\circ$ , and  $135^\circ$  were clearer than the cross-transect ( $90^\circ$ ), particularly for the 1-day-old eggs, 2-day-old eggs, and total egg category. The variogram in the direction of within-transect ( $0$  degree) had the clearest signal because intervals between adjacent collections were the shortest. Because the variograms for each direction looked similar, we used the variogram in the within-transect direction to assess the spatial correlation of sardine eggs.

The spherical model was chosen to fit the variogram (Cressie, 1991):

$$\gamma(h, \theta) = c_0 + c_s \begin{cases} 0 & h = 0 \\ (3/2) (\|h\|/a_s) - (1/2) (\|h\|/a_s)^3 & 0 < \|h\| < a_s, \\ C_0 + C_s, & \|h\| \geq a_s. \end{cases} \quad (3)$$

where  $c_0$  = the nugget effect;

$c_s$  = the practical sill (variance - nugget);

$a_s$  = the range; and

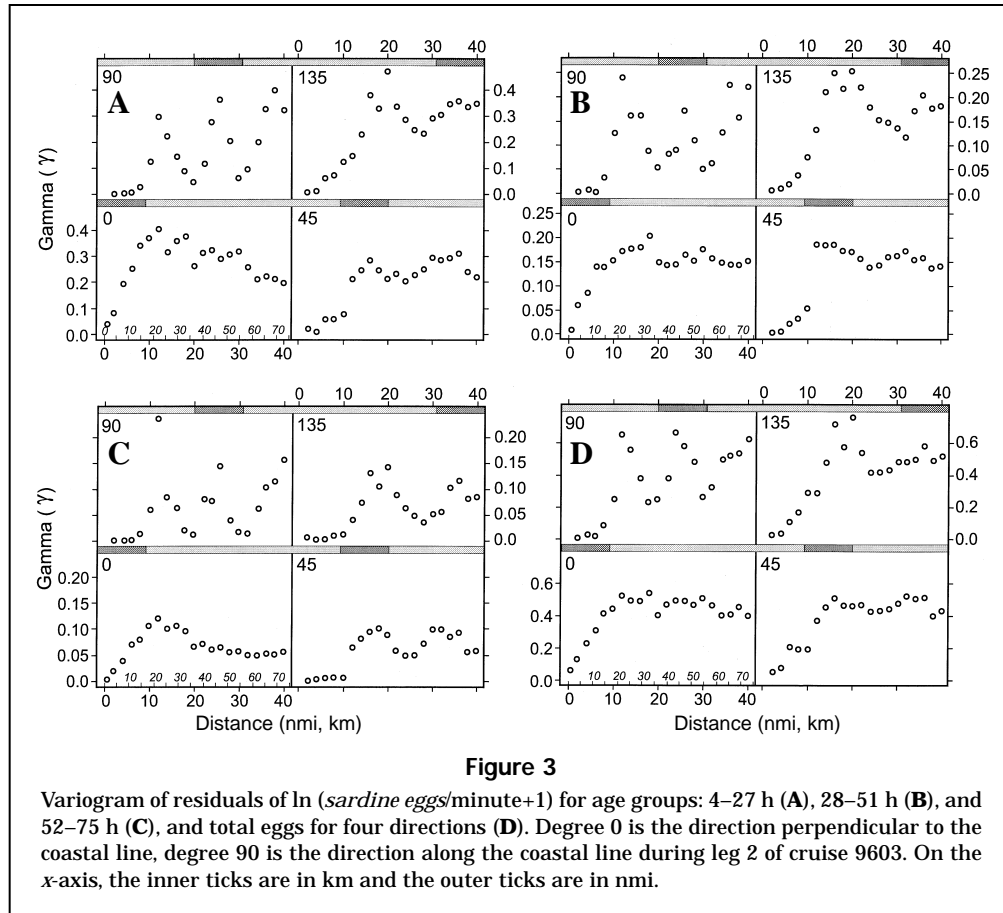
$\|h\|$  = Euclidean distance.

For total number of eggs (all eggs combined), the range of the residuals of  $\ln(\text{eggs}/\text{min}+1)$  was 22.2 km (12 nmi), sill (variance in the random field) was 0.4, and nugget was 0.05. For 1-day-old eggs, the range was 14.8 km (8 nmi) and the sill was 0.3. For 2-day-old eggs, the range was 18.5 km (10 nmi), the sill was 0.15, and nugget was 0.005, and for 3-day-old eggs, range was 22.2 km (12 nmi), the sill was 0.065, and nugget was 0.005 (Fig. 4). Because the maximum range was 22.2 km (12 nmi), eggs collected more than 22.2 km (12 nmi) apart were considered uncorrelated; therefore, transect lines spaced intervals of 12 nmi or greater were considered independent. The data also indicated the gradual dispersion of egg sardine patches with time as described by Smith (1973) because the diameter of sardine egg patches increased from 14.8 km for 4–27 h old eggs to 22.2 km for eggs 52–75 h old.

### Conversion of CUFES egg density to full-water-column abundance and distribution of egg stages

Egg counts from 91 paired samples collected with the CUFES and CalVETs during leg 1 of the 1996 survey (Table 1) were used to derive a conversion factor from eggs/minute of CUFES sample to CalVET catch ( $R$ ). We used a regression estimator to compute the ratio of eggs/minute from the CUFES to eggs/tows from CalVETs,  $R = \mu_y/\mu_x$ , where  $y$  = eggs/minute;  $x$  = eggs/tow; and  $R$  = the catch ratio. The estimator of  $R$  is  $\hat{R} = \Sigma(x \times y) / \Sigma(x^2)$ .

<sup>2</sup> Petitgas, P., and A. Prampant. 1993. EVA (estimation variance), a geostatistical software on IBM-PC for structure characterization and variance computation. CM1993/D:65, 81<sup>st</sup> meeting of ICES. IFREMER laboratoire d'Ecologie Halieutique, BP 21105, 44311 Nantes (France), Cedex 03, 33 p.



Paired samples were taken wherever high abundance of eggs appeared in samples collected with the CUFES. We obtained egg/minute = 0.73 eggs/0.05m<sup>2</sup> (CV=0.16) (Fig. 5). This means that for one egg observed from a CalVET, one would expect to see, on average, 0.73 eggs/min. Or for one egg/min from a CUFES, one would expect to see 1.5 eggs/tow. A striking difference existed between the data from the 1996 pilot survey and the full survey carried out in 1997. In 1997, the catch ratio of eggs/minute to eggs/tows was 0.25 (CV=0.08) from 110 pairs of CalVET and CUFES of which at least one sample was positive (Fig. 5). This means that one egg/tow from a CalVET tow was equivalent to approximately 0.25 egg/min from a CUFES, or one egg/minute from the CUFES was equivalent to 4 eggs/tow from a CalVET sample. The ephemeral nature of such conversion coefficients was not known to us when we developed the design for the 1997 survey. However, the variance associated with the direct 1996 conversions was a strong incentive to reduce the effects of direct conversions in the design of the 1997 survey and in the calculation of biomass.

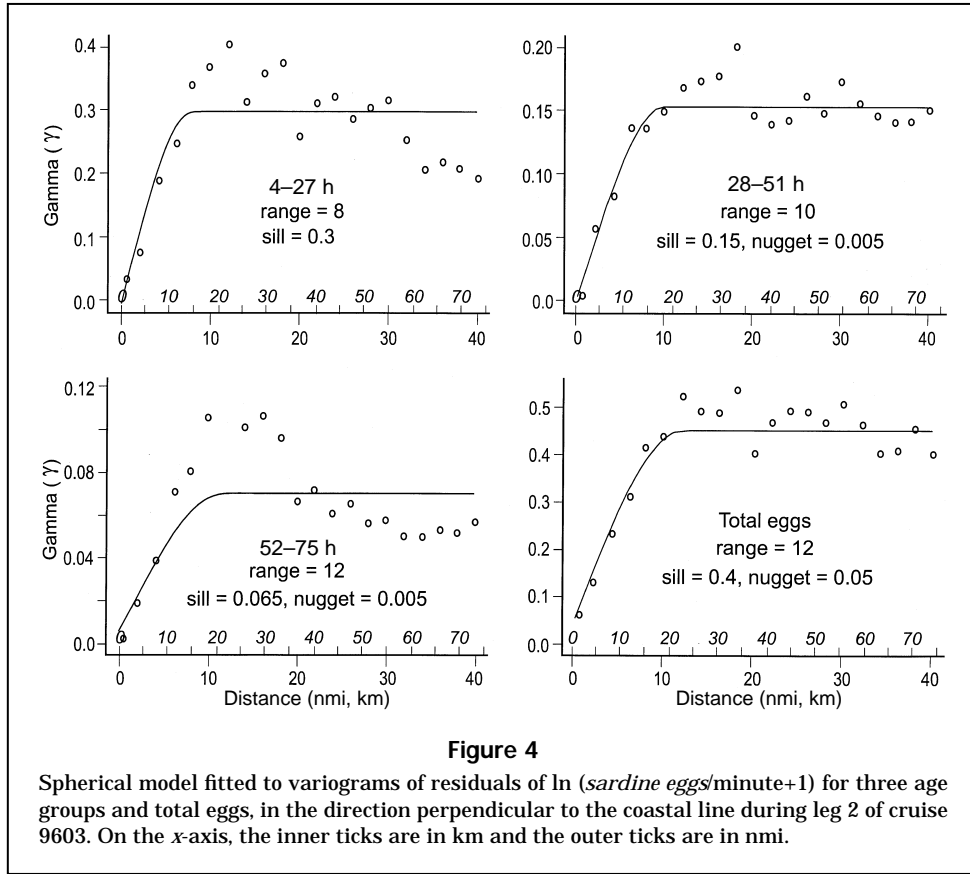
To determine if the CUFES provides an unbiased sample of all sardine eggs stages, we compared the distributions of developmental stages between the two samplers taken in 91 paired CUFES and CalVET samples during leg 1 of the 1996 survey (Fig. 1). A 2 × 11 contingency ta-

ble was constructed for total counts of each of 11 stages of eggs collected with the CalVET and the CUFES. A chi-square statistic was computed to test the null hypothesis that the distribution of stages was independent of the samplers.

The chi-square ( $\chi^2$ ) analysis showed that the distribution of stages was not the same between the two samplers ( $\chi^2=188.47$ , df =10,  $P$ -value <0.01) (Table 2). The difference was primarily due to eggs of stages I, III, V, and VI. The CUFES caught only two stage-I eggs; therefore we decided to run a  $\chi^2$  test with stage-I and stage-II eggs collapsed into one group. A similar conclusion was reached for the later case ( $\chi^2=160.31$ , df =9,  $P$ -value <0.01). A large  $\chi^2$  value indicates that staged sardine eggs in the upper 3 m may not be representative of the sardine egg stages in the whole-water-column and that the egg production computed from the CUFES survey would be biased.

#### Design for the 1997 CUFES and DEPM survey

The foregoing analysis of the 1996 survey data indicated that adequate correlation exists in overall egg abundance between the CUFES and the full-water-column egg samples, but a direct conversion of CUFES data to full-water-column abundance would add considerable variance to an estimate of daily egg production. The foregoing analysis



**Figure 4**  
Spherical model fitted to variograms of residuals of  $\ln(\text{sardine eggs/minute} + 1)$  for three age groups and total eggs, in the direction perpendicular to the coastal line during leg 2 of cruise 9603. On the x-axis, the inner ticks are in km and the outer ticks are in nmi.

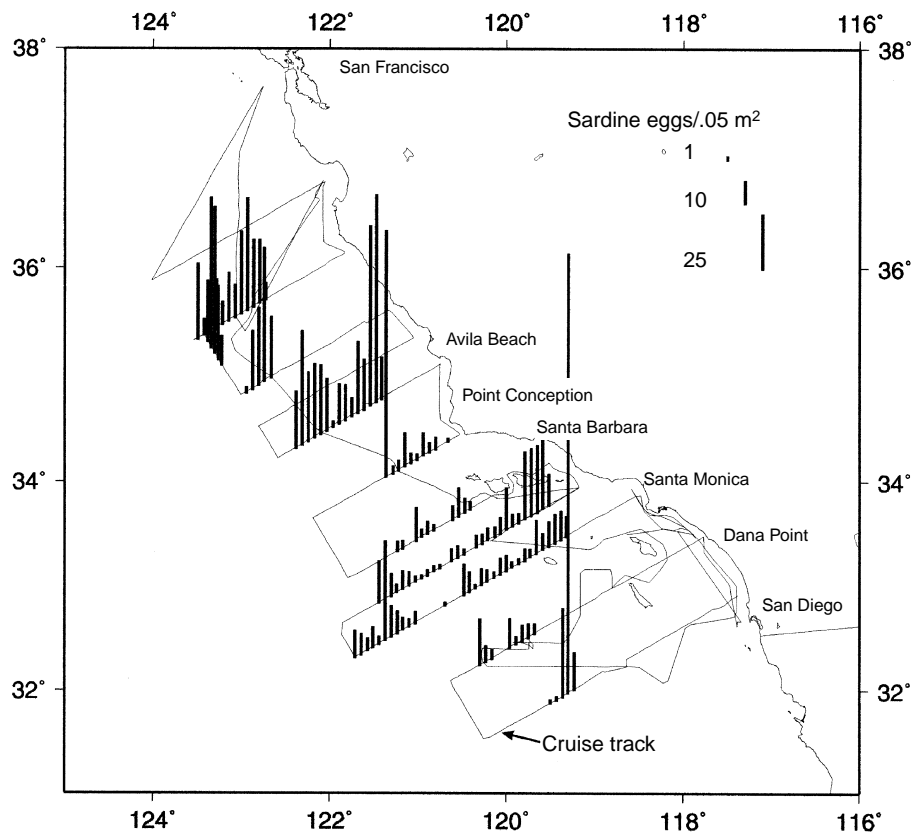
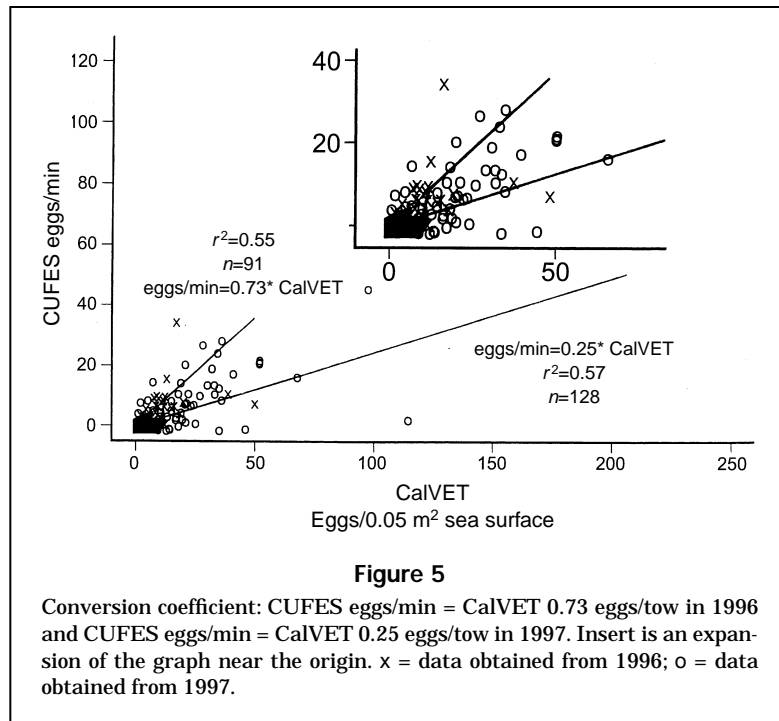
**Table 2**  
Contingency table of sardine egg counts by developmental stages and two samplers: the CalVET and the CUFES, leg 1, 1996.

	Egg stages										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
<b>Total count</b>											
CalVET	31	63	116	46	36	95	23	27	37	15	13
CUFES	2	74	466	44	148	104	103	22	75	21	14
<b>Percent</b>											
CalVET	6	13	23	9	7	9	5	5	7	3	3
CUFES	0	7	43	4	14	10	10	2	7	2	1

also indicated that a potential bias may exist if staged CUFES eggs are used to estimate daily egg production ( $P_0$ ) because all stages of eggs may not be equally vulnerable to the CUFES. A further complication was found, in that egg samples taken in the CUFES were strongly correlated up to distances of 22.2 km, necessitating the use of geostatistics if individual samples were to be used for the estimates. We concluded from these findings that the most effective way to use the CUFES in the DEPM design was to use it to allocate full-water-column tows with a CalVET. This adaptive allocation design, similar to one proposed by Thompson et al. (1992), takes advantage of the rapid con-

tinuous monitoring capability of a CUFES, while preserving the quantitative features of the CalVET.

We decided to allocate a CalVET at 4-nmi intervals, whenever catch from the CUFES was greater than or equal to 2 eggs/min<sup>1</sup>. When the catch from the CUFES began to decrease to fewer than 2 eggs/min, then CalVETs were stopped (Fig. 6). This critical value for the allocation of CalVETs was equivalent to slightly more than three eggs per CalVET tow and was based on the conversion factor from the pilot study of CUFES eggs/min = CalVET 0.73 eggs/tow. The CUFES was in operation continuously along each transect line, and samples were taken at an interval





ranging from 1 to 54 minutes with a mean of 30 minutes and median of 15 minutes (Table 1). Only samples from CalVETs were used to model the sardine embryonic mortality curve, thereby avoiding a possible stage-specific bias with the CUFES.

Spacing of transect lines was also determined to some extent by samples from the CUFES. The scheduled spacing of lines was 40 nmi apart but if a high density of sardine eggs was encountered, an additional transect line was added between the two scheduled lines. As a result of this procedure, two lines were added (lines 4 and 8) in the 1997 DEPM survey (Fig. 2).

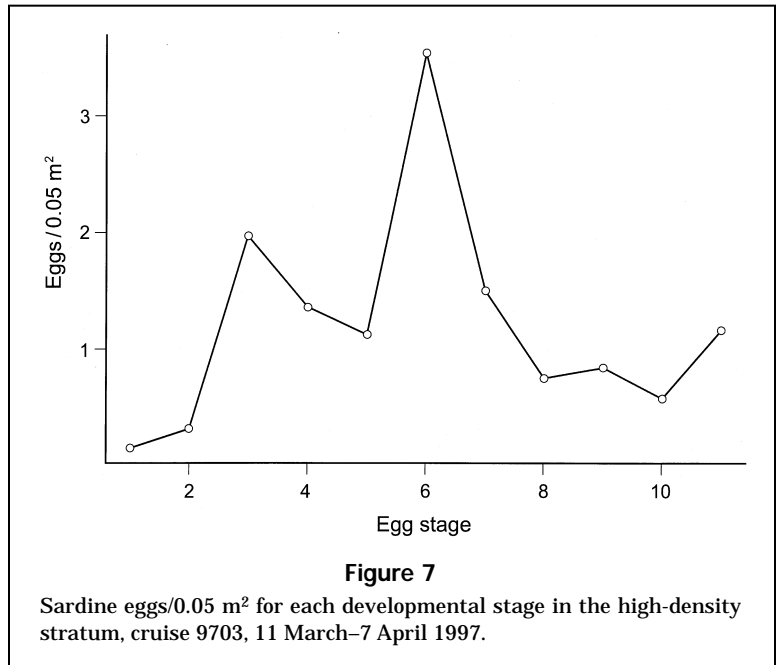
To estimate  $P_0$ , the survey area was then stratified into two strata: a high-density stratum where the density of eggs taken by the CUFES was at least 2 eggs/min; and a low-density stratum (Fig. 2) where only CalVET samples were taken and where the density of eggs was less than 2 eggs per minute. Within each stratum, the transect line was selected as the sampling unit both for CalVET samples and for CUFES samples. Because lines were spaced at intervals greater than 12 nmi, statistical procedures based on uncorrelated data were used (correlation should not be ignored if the distances are less than 12 nmi). We describe below the two different estimation methods used to estimate  $P_0$ : one for the high-density stratum and the other for the low-density stratum; we combined the two methods to estimate  $P_0$  combined for the total survey area.

We used transects as the primary sampling units and applied conventional statistical methods to construct the mortality curve from CalVET samples (Armstrong, 1988). CalVETs, the secondary sampling units, were taken for the first ten transects, except transect number eight. The  $P_0$  was estimated for each of two strata by using different methods, and an weighted average was obtained for the whole survey.

**Egg production in the high-density stratum ( $P_{0,1}$ )**

Ages of staged sardine eggs (Fig. 7) were assigned according to a temperature-dependent time-to-stage model (Lo et al., 1996). Sardine eggs were grouped in half-day categories excluding those eggs that were newly spawned during the first three hours. An average eggs-per-tow and an average age were obtained for each half-day category within each transect. A weighted average number of eggs in each half-day category was obtained where the weight is duration of the CUFES sampling interval for each transect line (Eq. 4, Table 3).

$$P_t = \frac{\sum_i P_{it} m_i}{\sum_i m_i} \quad \text{var}(P_t) = \frac{n / (n - 1) \sum_i m_i^2 (P_{it} - P_t)^2}{\left( \sum_i m_i \right)^2} \quad (4)$$



**Figure 7**  
Sardine eggs/0.05 m<sup>2</sup> for each developmental stage in the high-density stratum, cruise 9703, 11 March–7 April 1997.

**Table 3**  
Daily sardine egg and yolksac larval production and their standard error in the high-density stratum in 1997.

	Age (day)	Daily production/ 0.05 m <sup>2</sup>	Standard error
Eggs	0.32	3.29	1.32
	0.76	4.48	2.10
	1.17	4.32	2.05
	1.71	6.12	2.88
	1.92	3.21	1.60
	2.52	1.86	0.66
Yolksac larvae	5.62	0.65	0.16

where  $P_{it}$  = the eggs or yolksac larvae/0.05 m<sup>2</sup>; and  $m_i$  = the total CUFES sampling time (minutes) for the  $i$ th transect for  $i = 1, \dots, 7, 9$ , and 10.

For a weighted nonlinear regression, the weight is  $1/SE(p_i)$ . The yolksac larval stage includes larvae from time of hatching to the time they form functional jaws, i.e. larvae  $\leq 6$  mm live length or 5 mm captured length (Zweifel and Lasker, 1976). As with the egg data, the average number of yolksac larvae per tow was calculated for each transect and the overall mean catch of yolksac larvae was computed as a weighted average. Yolksac larva production was the mean catch of larvae  $\leq 5.00$  mmi (captured size) per CalVET divided by its temperature-dependent stage duration (Zweifer and Lasker, 1976). The age of yolksac larvae was estimated by the average of minimum age and maximum age, i.e. hatching time + age of forming functional jaw)/2 (Lo et al., 1996) (Table 3).

With these data, an embryonic mortality was modeled by an exponential curve (Eq. 5) (Lo et al., 1996). The mortality curve was fitted to eggs/0.05 m<sup>2</sup> in each age class and numbers of yolk sac larvae/0.05 m<sup>2</sup> with an assigned age based on observed mean water temperature. The estimates of the intercept ( $P_{0,1}$ ) and the instantaneous mortality rate from the mortality curve were obtained by a nonlinear regression of S+ nonlinear regression function (nls()).

The exponential embryonic mortality curve is

$$P_t = P_{0,1} \exp(-zt), \quad (5)$$

where  $P_t$  = a weighted average of eggs – yolk sac larvae /0.05m<sup>2</sup>; and

$t$  = the mean age (d) for each of 6 half-day age groups of eggs and yolk sac larvae. The weights are total CUFES sampling time (minutes) for each transect (Eq. 4).

### Daily egg production in the low-density stratum ( $P_{0,2}$ )

Because no CalVET samples were taken in the low-density stratum, we estimated daily egg production ( $P_{0,2}$ ) in that stratum as the product of the egg production in the high-density stratum ( $P_{0,1}$ ) and the ratio ( $q$ ) of egg/min in the low-density stratum to that in the high density stratum from the CUFES samples. Here we assumed that  $q$  is the same no matter whether it was computed from eggs/min by the CUFES or from eggs/tow by the CalVET:

$$P_{0,2} = P_{0,1} q, \quad (6)$$

$$q = \frac{\sum_i \frac{\bar{x}_{2,i}}{\bar{x}_{1,i}} m_i}{\sum_i m_i}, \quad (7)$$

where  $m_i$  = the total CUFES time (minutes) in the  $i$ th transect; and

$x_{ji}$  = was eggs/min in the  $j$ th stratum and  $i$ th transect.

The variance of  $q$  was computed according to that of a ratio estimator (Eq. 4).

### Daily egg production for the total survey area ( $P_0$ )

$P_0$  was computed as a weighted average of  $P_{0,1}$  and  $P_{0,2}$ , where

$$\begin{aligned} P_0 &= \frac{P_{0,1} A_1 + P_{0,2} A_2}{A_1 + A_2} \\ &= P_{0,1} w_1 + P_{0,2} w_2 \\ &= P_{0,1} [w_1 + qw_2] \end{aligned} \quad (8)$$

and according to Goodman (1960), the unbiased estimate of  $\text{var}(P_0)$  is

$$v(P_0) = v(P_{0,1}) (w_1 + w_2 q)^2 + P_{0,1}^2 w_2^2 v(q) - v(P_{0,1}) w_2^2 v(q), \quad (9)$$

where  $w_i = \frac{A_i}{A_1 + A_2}$ ,  $i = 1, 2$ , and  $A_i$  = the area size.

### Simulation

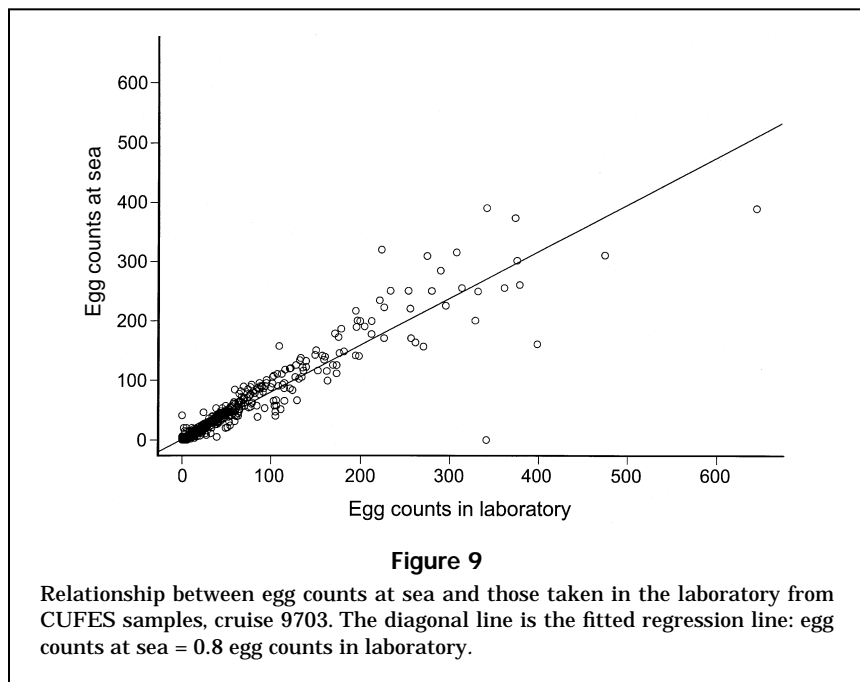
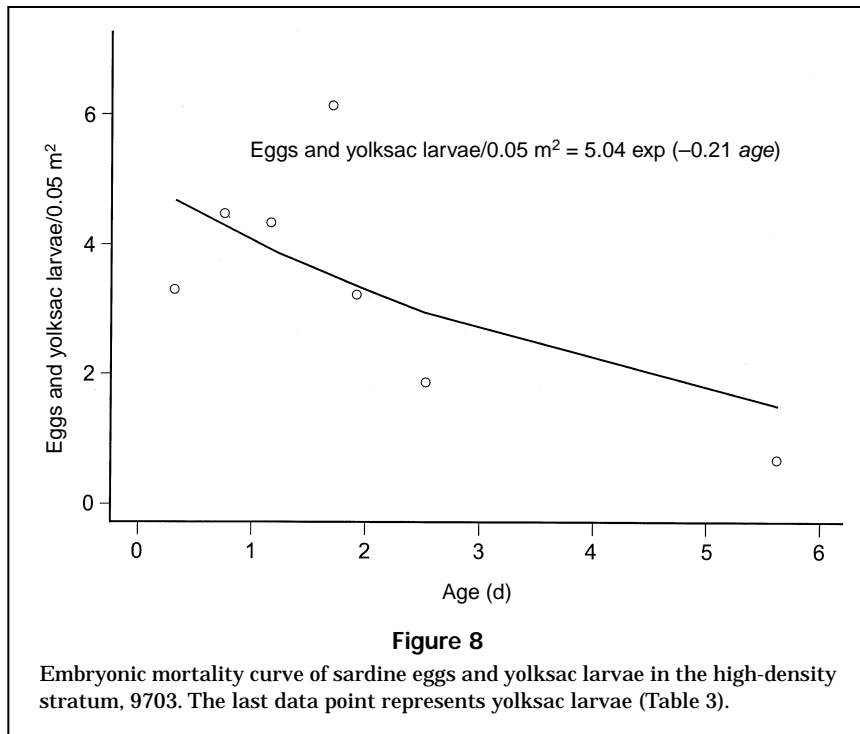
Bootstrap simulations were conducted to provide the possible biases and another estimate of the standard error of daily egg production ( $P_0$ ) and the instantaneous mortality rate ( $z$ ) for each stratum and the entire survey area under the adaptive allocation sampling scheme. As mentioned earlier, CalVETs were taken on nine transect lines and not on line 8 and line 11. In the simulation, nine transects with CalVETs were sampled with replacement and estimation procedures described in previous section were followed. To evaluate the effect of weighting, we included weighted and unweighted nonlinear regression where the weight was the inverse of the standard error of egg production of each age group and yolk sac larvae. One thousand iterations were run, and the standard deviation of 1000 estimates was the bootstrapped standard error of the estimates. Bias was the difference between the average of 1000 estimates and the estimate from the original data. The bias-corrected estimate was the original estimate minus the bias.

## Results of the 1997 CUFES and DEPM survey

### Daily egg production

The daily egg production for each half-day category and yolk sac larval production and their ages (d) were used to construct an embryonic mortality curve for the high-density stratum (Eq. 5, Fig. 8, Table 3). The daily egg production in the high-density stratum ( $P_{0,1}$ ) based on unweighted nonlinear regression was 5.04 eggs/0.05 m<sup>2</sup>/d (100.8 eggs/m<sup>2</sup>/d, CV=0.25) and egg mortality was  $z=0.21$  (CV=0.73) for an area ( $A_1$ ) of 66,841 km<sup>2</sup> (19,530 nmi<sup>2</sup>) (Eq. 8, Fig. 9). The ratio ( $q$ ) of egg density between the low-density stratum and high-density stratum from CUFES samples was 0.211 (CV=0.43) (Eq. 7). Therefore, in the low-density stratum, the egg production ( $P_{0,2}$ ) was 1.064 eggs/0.05 m<sup>2</sup>/d (21.28 eggs/m<sup>2</sup>/d, CV=0.49) for an area ( $A_2$ ) of 107,255 km<sup>2</sup> (31,338 nmi<sup>2</sup>). The estimate of the daily egg production for the entire survey area was 2.57/0.05 m<sup>2</sup> (51.4/m<sup>2</sup>, CV=0.27) (Eq. 8, Table 4). The weighted nonlinear regression produced estimates slightly different from those with unweighted nonlinear regression:  $P_{0,1} = 4.76/0.05$  m<sup>2</sup> (59.2/m<sup>2</sup>, CV=0.18),  $z = 0.35$  (CV=0.14), and the  $P_0$  for the entire survey area was 2.43/0.05 m<sup>2</sup> (48.6/m<sup>2</sup>, CV=0.21).

The bootstrap estimate of  $P_0$  (5.10) was similar to the original estimate  $P_0$  (5.04) in the high-density stratum. The standard error of  $P_0$  (1.6) from the bootstrap analysis was higher than the estimate from the original data (1.28). The bias of  $P_0$  (0.06) was negligible because the ratio



of bias to standard error was less than 0.25 (Efron and Tibshirani, 1993). However, the daily instantaneous mortality rate ( $z=0.29$ ) from the bootstrap was higher than 0.21 from the original data. The bias-corrected  $z$  was 0.13 (SE=0.12). For the entire survey area, the bootstrapped  $P_0$  was 2.60/0.05 m<sup>2</sup> (SE=0.71; CV=0.27), similar to the original estimate (2.57/0.05 m<sup>2</sup>).

The bootstrap results indicated that the weighted non-linear regression produced a downward biased estimate of  $P_0$  (bias=-1.26=3.50-4.76) in the high-density stratum. The standard error (1.60) of  $P_0$  from bootstrap was also higher than that computed from the original data (0.86). The fact that the ratio of bias to standard error (1.26:1.60) was greater than 0.25 may indicate that the weighted non-

Table 4

Estimates of daily egg production ( $P_0$ ; number of eggs/0.05 m<sup>2</sup>/day at age 0), daily instantaneous mortality rate ( $z$ ) for the high-density stratum (66,841 km<sup>2</sup>) and low-density stratum (107,255 km<sup>2</sup>),  $P_0$  for the entire survey area (174,096 km<sup>2</sup>), the catch ratio of eggs/min in the low-density stratum to eggs/min in the high-density stratum ( $q$ ), their standard errors (SE), estimated bias, and the bias-corrected estimates for the unweighted and weighted nonlinear regression from 1000 iterations of bootstrap simulation.

	High-density stratum				Low-density stratum		Entire survey area			
	$P_{0,1}$	SE ( $P_{0,1}$ )	$z$	SE ( $z$ )	$P_{0,2}$	SE ( $P_{0,2}$ )	$P_0$	SE ( $P_0$ )	$q$	SE ( $q$ )
Unweighted nonlinear regression										
survey	5.04	1.28	-0.21	0.16	1.06	0.52	2.57	0.72	0.211	0.09
bootstrap										
mean	5.10	1.60	-0.29	0.28	1.10	0.53	2.60	0.85	0.22	0.08
SE	1.60	0.72	0.12	0.11	0.48	0.28	0.71	0.34	0.09	0.03
CV	0.31	0.46	-0.40	0.38	0.45	0.52	0.27	0.41	0.41	0.40
bias	0.06	0.32	-0.08	0.12	0.03	0.01	0.03	0.13	0.009	-0.01
bias corrected	4.98	0.96	-0.13	0.04	1.04	0.51	2.54	0.58	0.202	0.10
Weighted nonlinear regression										
survey	4.76	0.86	-0.35	0.050	1.004	0.45	2.43	0.51	0.211	0.09
bootstrap										
mean	3.50	0.72	-0.27	0.056	0.730	0.33	1.80	0.41	0.22	0.08
SE	1.60	0.35	0.10	0.025	0.400	0.21	0.76	0.20	0.09	0.03
CV	0.46	0.49	-0.36	0.440	0.550	0.63	0.42	0.48	0.42	0.40
bias	-1.26	-0.14	0.08	0.006	-0.274	-0.12	-0.63	-0.10	0.009	0.01
bias corrected	6.02	1.00	-0.43	0.044	1.278	0.57	3.06	0.61	0.202	0.10

linear regression was unwarranted in our case. One possible reason is that the standard errors (SE) of egg production in each age group were between 1.3 and 2.8, whereas the SE for yolksac larvae was 0.16 (Table 3). Although the weighted regression should be used when the variances of data are unequal, we believe that there is too large a disparity between variance of eggs and yolksac larvae, and too much weight was assigned to yolksac larvae for a weighted nonlinear regression.

The spawning biomass was computed from the daily egg production from the 1997 survey, and the historical daily specific fecundity (number of eggs/gram of biomass) was 23.55 (Macewicz et al., 1996) assuming the daily specific fecundity was the same as that for 1994–96. Sardine spawning biomass in 1997 would be 379,940 t<sup>3</sup> for an area of 174,096 km<sup>2</sup> (50,868 nmi<sup>2</sup>) from San Diego to San Francisco. No variance of  $B_s$  was computed because no variance of the number of eggs per population weight (g)/day was available.

#### Comparison of results from the 1997 CUFES and DEPM survey with results from a conventional DEPM survey

We compared the results of the 1997 CUFES and DEPM design with those of a conventional DEPM (1994), in which only CalVET samples were taken, to illustrate how the

CUFES allocation design may affect the performance of a DEPM survey of Pacific sardine. We believe the comparison is instructive, even though the two surveys differed somewhat in area and population size; the conventional 1994 survey covered a larger area than the 1997 survey (380,175 km<sup>2</sup> vs. 174,096 km<sup>2</sup>), and, the total biomass of sardine was smaller in 1994 than in 1997 (111,493 t (Lo et al., 1996) vs. 379,940 t, respectively). In both surveys staged eggs and yolksac larvae from CalVET samples were used in the calculation of  $P_0$ .

An obvious difference in the results of the two surveys was that only 11% (74/684) of CalVET samples were positive for sardine eggs in the 1994 conventional survey, whereas in the 1997 survey 72% (102/141) were positive (Table 5). These results indicate that CUFES was effective in allocating CalVET samples and thereby reducing ship-time costs per survey mile. The coefficients of variation (CV) for the estimates of  $P_0$  were similar: 0.22 for the conventional survey compared with 0.27 for the CUFES-based DEPM. Thus, variance penalty for using the ratio estimator  $q$  did not greatly diminish the benefit in using the CUFES in the DEPM. This simple statistical comparison, however, does not reveal the greatest potential benefits in using a CUFES. The allocation of CalVETs would be most useful when the population is at a lower level, as it was in 1994, because at such levels one must cover a large survey area to assure an unbiased estimate, but the population is probably concentrated in a very small fraction of the area where CalVET samples will be allocated. In addition, the high-resolution maps of the spatial distribution

<sup>3</sup> The spawning biomass would be 359,280 metric tons if  $P_0$  of 2.43/0.05 m<sup>2</sup> from weighted nonlinear regression was used.

Table 5

Sardine daily egg production ( $P_0$ ) from a conventional survey (1994), compared with a CUFES and DEPM survey (1997).

1994 Conventional DEPM survey Area: 380,175 km <sup>2</sup>		1997 CUFES and DEPM survey Area: 174,096 km <sup>2</sup>	
CalVET samples		CalVET samples	
total	684	total	141
positive for eggs	72	positive for eggs	102
positive percent	11%	percent positive	72%
CUFES samples	None	CUFES samples	
		total	1227
		total positive	687
		percent positive	56%
		high-density stratum	84%
		low-density stratum	40%
Daily egg production		Daily egg production	
$P_0$	0.169/0.05 m <sup>2</sup>	$P_0$	2.57/0.05 m <sup>2</sup>
CV	0.22	CV	0.27
spawning biomass	111,493 t	spawning biomass	379,940 t

of eggs provided by the CUFES have not as yet been incorporated into the DEPM design, but we believe in the long-term it will be possible to improve the accuracy of surveys by doing so, as well as possible to develop new insights into the processes involved in selection of spawning habitats by parent fish.

### Comparison of shipboard egg counts with preserved egg counts

A key element of the allocation design was that the allocation of CalVET samples was based on near real-time counts and on the identification of eggs by CUFES operators. After having been counted by CUFES operators at sea, the eggs were preserved in vials, and later recounted and identified in the laboratory by experts. We compared egg counts in the laboratory with those taken at sea from the 1997 survey to determine the reliability of shipboard counting and identification and to indicate the extent to which a difference affected the final estimate of the daily egg production.

Within each stratum, we first computed an overall mean eggs/min for the laboratory and ship count by using a ratio estimator (where  $y$ =total number of eggs and  $x$ =the total minutes for each transect summed over all transects) (Table 6). We also computed a ratio of eggs/min from the laboratory to that from the ship for each transect line and obtained a weighted ratio for each stratum, where weight was the duration for each transect within a stratum (Eq. 7). The ratio for the entire survey area was a weighted average of two ratios, one from each stratum, and weight was the area of each stratum, as in Equation 5.

In the high-density stratum, the ratio from the laboratory counts to the ship counts was 1.19:1 (CV=0.03). In the low-density stratum, the ratio from the laboratory to the ship was 1.22:1 (CV=0.05) (Table 6). The overall ratio for

the entire survey area was 1.20:1 (CV=0.10); therefore the laboratory count was higher than the ship count by 20% (Table 6).

Of a total 1227 collections, 687 were positive according to laboratory counts. There were 16 collections where ship counts were positive but counts in the laboratory were zero. Eggs of other species in those 16 CUFES collections were obviously misidentified as sardine eggs. (Table 6). A total of 658 pairs had positive counts, out of which 130 pairs had equal positive counts. A total of 524 pairs had zero counts. Therefore there were 654 equal counts between laboratory and ship (524 zeros and 130 positive counts) and 573 pairs (=1227-654) with a mismatch. The relationship between the total counts from the laboratory and the ship confirmed the undercount from the ship (Fig. 9). The variance of undercounts increased with the total counts from the laboratory. One outlier was a collection where the ship count was zero and the laboratory count was 341. Although the absolute undercounts increased with the total number of eggs, the percent of undercount decreased with the total egg count.

### Conversion coefficient between two strata ( $q$ )

In the 1997 CUFES and DEPM survey, one of the primary functions of the CUFES collections was to provide a conversion coefficient ( $q$ ) of egg density between two strata to convert the egg production in the high-density stratum ( $P_{0,1}$ ) to the low-density stratum ( $P_{0,2}$ ), i.e.  $P_{0,2} = P_{0,1} \times q$  (Eq. 6). The conversion coefficients ( $q$ ) computed from the laboratory and ship counts were similar: 0.213 (CV=0.44) from the laboratory and 0.211 (CV=0.43) from the ship counts. Therefore, the bias of using egg counts at sea to calibrate egg production per unit area in the low-density stratum would be small: if the ratio of 0.213:1 were used, the egg production for the entire survey area would be 2.60/0.05 m<sup>2</sup> instead of 2.57/0.05 m<sup>2</sup>.

Table 6

Comparison of egg density and distribution of zero and positive egg counts from CUFES samples with those taken in the laboratory and at sea (ship), 1997. CVs are in shown in parentheses.

Egg density (eggs/min)	High-density stratum	Low-density stratum	Overall	$q$
ship	4.16 (0.41)	0.47 (0.45)		0.213 (0.44)
laboratory	4.91 (0.4)	0.57 (0.45)		0.211 (0.43)
ratio	1.19 (0.03)	1.22 (0.05)	1.20 (0.10)	

Distribution of zero and positive egg counts			
Ship	Laboratory		
	zero	positive	total
zero	524	29	553
positive	16	658 <sup>1</sup>	674
total	540	687	1,227

<sup>1</sup> 658 = 130 with exact match + 428 without match.

## Discussion

Presently the most productive use of the CUFES in the DEPM is to use the CUFES as an efficient auxiliary information provider and the CalVET, or another full-water-column tow, as the primary sampler. Given our present level of knowledge of egg distributions and accuracy of predicting them, it would be folly to depend only on the CUFES for an estimate of  $P_0$ . Predictive egg-distribution models (Sundby, 1983; Westgard, 1989) are promising and sometime in the future might make exclusive use of the CUFES practical in a DEPM. Exclusive use of the CUFES as a sampler in the DEPM also would require detailed knowledge of the stage-specific vulnerability of eggs to the sampler because a bias seemed to exist for sardine. In short, full-water-column tows are essential for accurately estimating egg production today, but the CUFES can greatly facilitate the allocation of such tows.

The CUFES enhancement of the basic DEPM design (Lasker, 1985) may have broad application because the DEPM is used world-wide for estimating the spawning biomass of sardines, anchovies, and other species of fishes (Priede and Watson, 1993; Zeldis, 1993; Lo, 1997). Thus, we feel it is useful to discuss some of the new features of the DEPM survey that we developed for estimating  $P_0$  with the CUFES.

### Critical value for allocation sampling

To use the CUFES in our DEPM survey design requires selecting an egg density that triggers full-water-column sampling (CalVET sampling). We used a critical value of 2 eggs/min, which was equivalent to 3 or 8 eggs/tow, depending on the conversion factor. If the 1997 conversion factor (eggs/min=0.25 egg/tow) was correct, we could lower the critical value to 1 egg/min, equivalent to 4 eggs/tow.

This would create a larger high-density stratum and more CalVET tows would be allocated. This range of critical values (3–8 eggs/tow) was similar to the value (5 eggs/tow) used in a stratified sampling design for an anchovy survey in Biscay Bay in Spain (Petitgas, 1997). As a result, the precision of  $P_0$  and  $z$  would likely be improved. Increasing the area for the high-density sampling also reduces the potential for bias from the assumption of a constant egg mortality between strata. On the other hand, lowering the critical value diminishes the gain from using the CUFES. Clearly, an optimal critical value exists for each species and survey area. The critical value can be determined prior to the survey or during the survey by using order statistics (Thompson and Seber, 1996; Quinn et al., 1999).

The extent that the critical value can be fine tuned to deliver an optimum balance between the CUFES and CalVET samples for a particular region, species, and season is unknown. The large difference in catch ratios between our 1996 (0.73) and 1997 (0.25) surveys certainly does not support the idea of fine tuning. These differences may overstate the expected variability for sardine because the areas were different; the 1996 samples were taken over a very limited portion of the survey area, whereas in 1997, the sample pairs were taken where high-density spawning occurred (Figs. 1A, 2, and 6). Interestingly, our 1997 estimate (0.25) is similar to that computed by us for sardine off South Africa (van der Lingen et al., 1998).

### Variability in catch ratios

The extent of vertical mixing of eggs is probably the main factor affecting the variation of the catch ratio between surveys. Because the selection of the optimal critical value for CalVET sample allocation depends upon on the relationship between catches in the CUFES and those in the CalVET, or the catch ratio, it seems useful to consider what

the ratio would be if water were perfectly mixed in the whole-water-column. If the water were perfectly mixed, fish eggs would be distributed randomly and the catch ratio (between CalVET and CUFES) would be a constant, because egg count would be proportional to the volume of water filtered through the two samplers. A CUFES filters on the average  $0.64 \text{ m}^3$  of water/min and the CalVET filters  $3.5 \text{ m}^3$  of water. Therefore under perfect mixing, the ratio of eggs/min to eggs/tow =  $0.64 \text{ m}^3/3.5 \text{ m}^3 = 0.18$  for the daytime when fish schools are in deeper water, assuming the vertical distribution of sardine eggs was similar to that of anchovy eggs (Moser and Pommeranz, 1999). At night, when fish schools are in the upper 50 meters,<sup>4</sup> the ratio would be  $0.64 \text{ m}^3/2.5 \text{ m}^3 = 0.26$ . This means that on the average, for four to six eggs seen in a CalVET catch, we would expect one egg/min in the CUFES. The fact that the estimated ratio for two years were 0.76 in 1996 and 0.25 in 1997, indicates that more sardine eggs appeared in the upper 3 m than would be expected under perfect mixing and less mixing occurred in 1996 than in 1997. Clearly, if all the eggs were in the upper 3 m of water column, the ratio would be 1:1 for the same surface area. As the extent of vertical mixing in the sea is highly variable, we believe that calibration tows are always needed, even if the CUFES is used only as an index of egg abundance. Vertical egg mixing models might eventually help to reduce calibration requirements.

### Identification and counting of eggs at sea

Identification and counting of fish eggs while the ship is underway was an essential ingredient of our adaptive allocation design. The eggs of some commercially important clupeid species are often difficult to distinguish from those of co-occurring species (Ahlstrom and Moser, 1980; Matarese and Sandknop, 1984; Watson and Sandknop, 1996). Further, many melanostomiin species have eggs with characteristics (e.g. large diameter, wide perivitelline space, segmented yolk) that are similar to those of co-occurring sardines and other clupeid eggs. During a DEPM survey off Oregon, Bentley et al. (1996) encountered a type of melanostomiin egg similar to the egg of Pacific sardine at 24 of 46 stations. Our experience has shown that the risk of misidentification increases when identifications are made at sea; during our CUFES and DEPM survey in 1997, about 1% of the eggs initially identified as sardines turned out to be melanostomiin eggs after examination in the laboratory. Fortunately, melanostomiin eggs were in such low abundance that they had no effect on the critical values of density. The possibility of misidentification differs depending on season, location, and target species. Clearly all shipboard positive records for areas that lie outside of the known spawning range and season of the

target species should be checked in the laboratory after the cruise. Owing to the great abundance of sardines and anchovy eggs, the effect of misidentification on biomass estimation is probably trivial if the survey is conducted during peak spawning months.

Egg counts on shipboard were somewhat lower than shoreside counts. Even though the effect of the difference is negligible from the standpoint of biomass estimation, we recommend that shoreside measurement be maintained. In particular, for collections that contain a large amount of other organisms (e.g. salps) which makes it difficult to count the eggs on aboard the ship, shoreside counting would be necessary.

### Stratified design

An important feature of the survey design was the stratification of sampling by egg density and sampler type. In the high-density stratum, we used only staged eggs from CalVET samples to estimate  $z$  and  $P_0$ , whereas in the low-density stratum we collected only CUFES samples. We believe it would not be useful to use staged eggs from CUFES samples in the low-density stratum to estimate  $z$  and  $P_0$  directly because of the low egg abundance, possibility of stage-specific bias, and lack of yolksac larvae (the CUFES does not sample yolksac larvae well). It seemed preferable to use, for the low-density stratum, the estimate of  $P_0$  for the high-density stratum, adjusted by the ratio of egg densities taken in CUFES at high and low strata. This, of course, requires the assumption that egg mortality did not differ between strata. A direct test of this assumption is impractical because of the large sampling effort needed at low density to obtain a sufficient number of positive samples to detect a difference in mortality rates. Fortunately, the effect of this potential bias is diminished because the low-density stratum contributes fewer eggs. In our example, the low-density stratum contributed about 25%<sup>5</sup> of the daily production. An alternative approach would be to allocate CalVET sampling to the low-density stratum. We believe this approach would not be cost effective because the number of positive CalVETs would be so low.

Another important element of the stratified design was the use of transect lines as the sampling unit. Model-based geostatistics are needed (Fletcher and Sumner, 1999) for data from continuous samplers such as echo-sounders and CUFESs, unless sampling units are defined such that data are uncorrelated among sampling units in the survey design (Armstrong et al., 1988). Because conventional design-based statistical procedures are easier to apply, we preferred using a transect line as our sampling unit, which requires that the minimum sample size allows a between-transect distance greater than the diameter of the egg patch. Fortunately the within-transect CUFES collections provided the information needed on the spatial structure to determine the distance of CalVET lines to insure samples are uncorrelated. In our case, tows, a minimum of 22.2 km

<sup>4</sup> Castillo Valderrama, P. R. 1995. Distribucion de los principales recursos pelagicos durante los veranos de 1992 a 1994. Instituto Del Mar Del Peru, Informe No 114. Instituto del Mar del Peru (IMARPE), Esquina Gamarra Y General Valle Chucuito, Callao Peru, 24 p.

<sup>5</sup>  $25\% = 100 \times (1.064 \times 107,255) / (2.57 \times 174,096)$  (Table 4).

(12 nmi) apart, would ensure uncorrelated samples and provide the unbiased estimates of abundance of eggs in each development stage of sardine eggs.

### Use of yolksac larvae

Although yolksac larvae have been used to estimate  $P_0$  with the DEPM method (Lo, 1985; 1986; Lo et al., 1996; Hunter and Lo, 1997), but are not a requirement for using the CUFES, we included yolksac larvae to estimate  $P_0$  because the mortality rate of eggs and yolksac larvae are similar for northern anchovy (Lo, 1985, 1986) and because the development of early stages of anchovy and sardine is similar (Ahlstrom, 1943; Zweifel and Lasker, 1976; Moser and Ahlstrom, 1985; Lo et al., 1996). Our threshold for taking CalVET samples, 2 eggs per minute, generated 102 positive CalVETs (72% were positive). This number is far fewer positive tows than the number needed to be assured a significant slope for the regression of numbers of eggs on their ages. In anchovy, where the eggs are less patchy than sardine, about 500 positive tows were required to assure a CV of the estimate of mortality rate close to 0.6 (Lo, 1997). To obtain a significant slope in the 1997 survey, we used the number and average age of yolksac larvae (adjusted for observed mean temperature) as well as staged eggs. This introduced a potential bias, because by doing this we assumed that yolksac larvae have the same mortality rate as eggs. To avoid this bias, the number of staged egg samples should be increased. One approach would be to stage the eggs taken with a CUFES in the high-density stratum and combine them with the CalVET samples from the same stratum. This would greatly increase the number of staged eggs available but requires the assumption that CUFES staged eggs are an unbiased sample of the full-water-column, a condition not met in our 1996 pilot study. Another approach would be to increase the number of CalVET samples per mile in the high-density stratum from the present one sample per 4 nmi to a higher frequency. A doubling of the CalVET sampling rate would significantly increase survey costs and may not increase the number of positive samples sufficiently to be able to use only eggs for estimation of  $P_0$ . Considering the relative risks and costs of these approaches, we feel that the use of the yolksac larvae in the estimation of  $P_0$  was preferable. In other species, the distribution of eggs may be less concentrated than they are for sardine and sampling at the 4 nmi sampling rate may be adequate. Clearly, other solutions may exist, and we recommend considering these issues with each new application.

### Conclusions

We conclude that the CUFES is a useful tool with the DEPM when it is used adaptively to establish sampling strata for CalVETs. In this mode, the CUFES increases precision and reduces cost per transect mile. Perhaps one of the major benefits of using a CUFES in the DEPM is that one can better afford to expand survey boundaries and thereby reduce the potential bias of not enclosing the

entire population, a very common bias in field surveys in general (Gunderson, 1993). Clearly, the more contiguous the distribution of spawned eggs within the surveyed habitat, the greater the benefits in using the CUFES.

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### Literature cited

- Alheit, J.  
1993. Use of the daily egg production method for estimating biomass of clupeoid fishes, a review and evaluation. *Bull. Mar. Sci.* 53(2):750–767.
- Ahlstrom, E. H.  
1943. Studies on the Pacific pilchard or sardine (*Sardinops caerulea*). 4. Influence of temperature on the rate of development of pilchard eggs in nature. *U.S. Fish. Wildl. Serv., Spec. Sci. Rep.* 23, 26 p.
- Ahlstrom, E. H., and H. G. Moser.  
1980. Characters useful in identification of pelagic marine fish eggs. *Calif. Coop. Oceanic Fish. Investig. Rep.* 21:121–131.
- Armstrong, M., P. Shelton, I. Hampton, G. Jolly, and Y. Melo.  
1988. Egg production estimates of anchovy biomass in the southern Benguela. *Calif. Coop. Oceanic Fish. Investig. Rep.* 29:137–157.
- Barange, M., and I. Hampton.  
1997. Spatial structure of co-occurring anchovy and sardine populations from acoustic data: implications for survey design. *Fish. Oceanogr.* 6:294–108.
- Bentley, P. J., R. L. Emmett, N. C. H. Lo, and H. G. Moser.  
1996. Egg production of Pacific sardine (*Sardinops sagax*) off Oregon in 1994. *Calif. Coop. Oceanic Fish. Investig. Rep.* 37:193–200.
- Chambers, J. M., and T. J. Hastie (ed).  
1992. Statistical models in S. Wadsworth and Brooks/Cole Advanced Books and Software, Pacific Grove, CA, 608 p.
- Checkley, D. M., Jr, R. C. Dotson, and D. A. Griffith.  
1999. Continuous, Underway sampling of eggs of northern anchovy (*Engraulis mordax*) and Pacific sardine (*Sardinops sagax*) in spring 1996 and 1997 off southern and central California. *Deep-Sea Res. (special rep.)* II 47(2000) 1139–1155.
- Checkley, D. M., Jr., P. B. Ortner, L. R. Settle, L.R., and S. R. Cummings.  
1997. A continuous, underway fish egg sampler. *Fish. Oceanogr.* 6:58–73.
- Cressie, N.  
1991. Statistics for spatial data. John Wiley and Sons, Inc. New York, Chichester, Toronto, Brisbane, Singapore, 900 p.
- Cressie, N., and D. M. Howkins.  
1980. Robust estimation of the variogram. I. *J. Int. Assoc. Math. Geology* 12:115–125.



- Efron, B., and R. J. Tibshirani.  
1993. An introduction to the bootstrap. Monographs on statistics and applied probability 57. Chapman and Hall, New York, London, 436 p.
- Fletcher, W. J., and N. R. Sumner.  
1999. Spatial distribution of sardine (*Sardinops sagax*) eggs and larvae: an application of geostatistics and resampling to survey data. *Can. J. Aquat. Sci.* 56:907–914.
- Gunderson, D. R.  
1993. Surveys of fisheries resources. John Wiley and Sons, Inc., New York, Chichester, Brisbane, Toronto, Singapore, 248 p.
- Goodman, L. A.  
1960. On the exact variance of products. *J. Am. Stat. Assoc.* 55(N292):708–713.
- Hunter, J. R., and N. C. H. Lo.  
1997. The daily egg production methods of biomass estimation: some problems and potential improvements. OZEANOGRAFIKA, Boletín De La Sociedad De oceanografica De Gipuzkoa, Medalla De Oro De La Ciudad De San Sebastian 2:9–40.
- Kaluzny, S. P., C. Vega, T. P. Cardoso, and A. A. Shelly.  
1996. S+ spatialstats. User's manual, version 1. Mathsoft, Inc., Seattle, WA, 226 p.
- Lasker, R. (ed).  
1985. An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*). U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36, 99 p.
- Lo, N. C. H.  
1985. Egg production of the central stock off northern anchovy, *Engraulis mordax*, 1951–82. *Fish. Bull.* 83(2):137–150.  
1986. Modeling life-stage-specific instantaneous mortality rates, an application to northern anchovy, *Engraulis mordax*, eggs and larvae. *Fish. Bull.* 84(2):395–406.  
1997. Empirical analyses of the precision of regression estimates of daily egg production and egg mortality of pelagic fish for daily egg production method. OZEANOGRAFIKA, Boletín De La Sociedad De Oceanografica De Gipuzkoa, Medalla De Oro De La Ciudad De San Sebastian 2:71–89.
- Lo, N. C. H., Y. A. Geen Ruiz, M. Jaacob Cervantes, H. G. Moser, and R. J. Lynn.  
1996. Egg production and spawning biomass of Pacific sardine (*Sardinops sagax*) in 1994, determined by the daily egg production method. *Calif. Coop. Oceanic. Fish. Invest. Rep.* 37:160–174.
- Macewicz, B. J., J. J. Castro Gonzalez, C. E. Coterio Altamirano, and J. R. Hunter,  
1996. Adult reproductive parameters of Pacific sardine, *sardinops sagax*, during 1994. *Calif. Coop. Oceanic. Fish. Invest. Rep.* 37:140–151.
- Matarese, A. C., and E. M. Sandknop.  
1984. Identification of fish eggs. *In* Ontogeny and systematics of fishes (H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, eds.), p. 27–31. *Am. Soc. Ichthyol. Herpetol. Spec. Publ.* 1.
- Moser, H. G., and E. H. Ahlstrom.  
1985. Staging anchovy eggs. *In* An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax* (R. Lasker, ed.), p. 37–42. U.S. Dep. Commer., NOAA Technical Report NMFS 36.
- Moser, H. G., and T. Pommeranz.  
1999. Vertical distribution of eggs and larvae of northern anchovy, *Engraulis mordax*, and of the larvae of associated fishes at two sites in the Southern California Bight. *Fish. Bull.* 97:920–943.
- Parker, K.  
1985. Biomass model for the egg production method. *In* An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax* (R. Lasker, ed.), p. 5–6. U.S. Dep. Commer., NOAA Technical Rep. NMFS 36.
- Petitgas, P.  
1993. Geostatistics for fish stock assessments: a review and an acoustic application. *ICES J. Mar. Sci.* 50:285–298.  
1997. Use of disjunctive Kriging to analyse an adaptive survey design for anchovy, *Engraulis encraicolus*, eggs in Biscay Bay. OZEANOGRAFIKA, Boletín De La Sociedad De Oceanografica De Gipuzkoa, Medalla De Oro De La Ciudad De San Sebastian 2:121–132.
- Priede, I. G., and J. J. Watson.  
1993. An evaluation of the daily egg production method for estimating biomass of Atlantic mackerel (*Scomber scombrus*). *Bull. Mar. Sci.* 53(2):891–911.
- Quinn, T. J., II, D. Hanselman, D. Clausen, C. Lunsford, J. Heifetz.  
1999. Adaptive cluster sampling of rockfish populations: proceedings of the American Statistical Association, 1999 Joint Statistical Meeting, Biometrics section. *Am. Stat. Assoc.*, Alexandria, VA.
- Smith, P. E.  
1973. The mortality and dispersal of sardine eggs and larvae. *Rapp. P.-V. Reun. Cons. Perm. Int. Explor. Mer* 164:282–292.  
1985. Anchovy egg dispersal and mortality as interred from close interval observations. *Calif. Coop. Oceanic. Fish. Invest. Rep.* 26:97–112.
- Smith, P. E., W. Flerx, and R. P. Hewitt.  
1985. The CalCOFI vertical egg tow (CalVET). *In* An egg production method for estimating spawning spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*) (R. Lasker, ed.), p. 27–32. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- Sundby, B.  
1983. A one-dimensional model for the vertical distribution of pelagic fish eggs in the mixed layer. *Deep-Sea Res.* 30: 645–661.
- Thompson, S. K., F. L. Ramsey, and G. A. F. Seber.  
1992. An adaptive procedure for sampling animal populations. *Biometrics* 48:1195–1199.
- Thompson, S. K., and G. A. F. Seber.  
1996. Adaptive sampling. John Wiley and Sons, Inc., New York, Chichester, Brisbane, Toronto, Singapore, 265 p.
- van der Lingen, C. D., D. Checkley Jr, M. Barange, L. Hutchings, and K. Osgood.  
1998. Assessing the abundance and distribution of eggs of sardine, *Sardinops sagax*, and round herring, *Etrumenus whiteheadi*, on the western Agulhas Bank, South Africa, using a continuous, underway fish egg sampler. *Fish. Oceanogr.* 7(1):35–47.
- Watson, W., and E. M. Sandknop.  
1996. Clupeidae. *In* The early stages of fishes in the California Current region (H. G. Moser, ed.), 159–171 p. *Calif. Coop. Oceanic Fish. Invest. Atlas* 33.
- Watson, W., R. L. Charter, H. G. Moser, R. D. Vetter, D. A. Ambrose, S. R. Charter, L. I. Robertson, E. M. Sandknop, E. A. Lynn, and J. Stannard.  
1999. Fine-scale distributions of planktonic fish eggs in the

- vicinities of Big Sycamore Canyon and Vandenberg Ecological Reserves, and Anacapa and San Miguel Island, California. Calif. Coop. Oceanic. Fish. Investig. Rep. 40:128–153.
- Westgard, T.  
1989. Two models of the vertical distribution of pelagic fish egg in the turbulent layer of the ocean. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 191:195–200.
- Zeldis, J. R.  
1993. Applicability to egg surveys for spawning-stock biomass estimation of snapper, orange roughy, and hoki in New Zealand. Bull. Mar. Sci.:53(2) 864–890.
- Zweifel, J. R., and R. Lasker.  
1976. Prehatch and posthatch growth of fishes—a general model. Fish. Bull. 74(3):609–621.