Assessing Options for Using a Continuous Underway Fish Egg Sampler to Enhance Dedicated Daily Egg Production Studies Conducted from Small Research Vessels

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Abstract: This study was undertaken to identify options for using a Continuous Underway Fish Egg Sampler (CUFES) to enhance the application of the Daily Egg Production Method (DEPM) in situations where targeted surveys are conducted from small research vessels (e.g. <25m) and the time spent at each sampling site is short (e.g. <10 min). To do this, we conducted experiments to: 1) determine the reliability of information obtained from onboard examination of CUFES samples; 2) assess the sampling efficiency of CUFES while on-site and underway and 3) measure the effectiveness of underway CUFES samples in predicting the presence/absence of sardine eggs in the following CalVET net sample. We also compared the accuracy, precision and costs of estimates of total daily egg production obtained using data obtained from CalVET net samples taken at 1) predetermined sites (low and high sampling intensities) and 2) CUFES-determined sites. The accuracy of onboard estimates of egg abundance appeared to be affected by sea conditions. The efficiency of CUFES as an underway sampler was also lower when sea conditions were rough. Data from laboratory analyses of underway CUFES samples were a good predictor of egg abundance in the following CalVET net sample. Estimates of total egg production using CalVET samples from CUFES-determined sites based on laboratory analyses of CUFES samples differed by less than 10% from those obtained from pre-determined sites. However, estimates based on onboard analyses of CUFES samples differed from those obtained from pre-determined sites by up to 50%. When the time spent taking each CalVET sample is low (e.g. less than 10 minutes), using data from CUFES to reduce the number of samples taken using CalVET nets is not the optimal use of this technology. This is mainly because of difficulties associated with sorting plankton samples at sea, but also because in situations where the time spent at each site is short, the small decreases in field (cruise duration) and laboratory (sorting) costs do not outweigh the reductions in the reliability of estimates of egg production. However, CUFES data does appear to provide a potentially important source of information to increase the reliability of estimates of total daily egg production. Numerical techniques (e.g. analogous to integrated fishery assessment models) need to be developed to integrate data obtained from CUFES and CalVET nets to provide more accurate and precise estimates of total daily egg production for DEPM studies.

Keywords: CUFES, DEPM, Mean daily egg production, Spawning area, Total daily egg production, Sardine, *Sardinops sagax*.

INTRODUCTION

The Daily Egg Production Method (DEPM) was developed in the late 1970s for direct stock assessment of northern anchovy, *Engraulis mordax* [1, 2]. It is now widely used for stock assessment of several small pelagic (and some demersal) fishes, especially anchovies, *Engraulis* spp and sardine, *Sardinops sagax* [3]. The method relies on the premise that spawning biomass can be calculated from estimates of the number of pelagic eggs produced per day in the spawning area, i.e. total daily egg production, and the number of eggs produced per unit mass of population, i.e. daily fecundity [1-7]. Total daily egg production is calculated from the product of estimates of mean daily egg production per unit area and total spawning area. Traditionally, these two parameters have

been estimated from samples obtained at predetermined sites using paired CalVET (Californian Vertical Egg Tow) or bongo nets during research cruises conducted at the peak spawning season [2].

In the early 1990s, the Continuous Underway Fish Egg Sampler (CUFES), which sieves eggs from water pumped aboard the research vessel, was devised to facilitate characterisation of the spawning habitat of pelagic fishes [8]. Several studies have assessed the accuracy and precision of CUFES data [8-11] by comparing estimates of egg density obtained using CalVET or bongo nets and a CUFES. For example, Checkley *et al.,* [8] and van der Lingen *et al.,* [9] showed that estimates of sardine egg density obtained from a CUFES while on-site tend to be higher than those obtained from CalVET nets, apparently due to the concentration of buoyant sardine eggs in surface water. In addition, van der Lingen *et al.,* [9] showed that the precision of estimates of sardine egg density from CalVET nets and CUFES were similar at moderate to high densities, but that CUFES esti-

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mates had higher coefficients of variation (CVs) at low densities. In contrast, Pepin *et al.,* [11] found that the precision of estimates of egg density for three species of temperate fishes (not including sardine) obtained using CUFES were much lower than those obtained using bongo nets. It has been shown that CUFES under-sample early stage eggs of some species, including sardine, although the significance of this effect appears to vary among species [8, 11].

Since the early 1990s the CUFES has been used as a secondary sampler in some DEPM surveys [12, 13] with the aim of improving the precision and reducing the costs of estimating total daily egg production. In these situations, the CUFES has mainly been used to adjust CalVET net sampling intensity to reflect levels of egg density, with the aim of reducing field and laboratory (sorting) costs [10, 12]. For example, in a situation where CalVET samples were collected as part of extensive multi-disciplinary sampling program (CalCOFI cruises) and considerable time was expended at each sampling site, Lo *et al.,* [10] concluded that the most effective way to use a CUFES was to determine where/when CalVET nets should be deployed. In some cases, the ratio of egg density in CUFES/CalVET net samples has also been used to estimate egg production in areas with low egg densities where CalVET net samples were not taken; however spatial and temporal variations in this ratio have limited the further development of this approach [10].

Pepin *et al.,* [11] suggested that the most effective way to determining the total abundance of fish eggs may be to use a CUFES to map horizontal distribution and a vertical plankton sampler to provide high resolution, unbiased estimates of density. Although the idea that CUFES can significantly enhance DEPM assessments is widely accepted [3], relatively few studies have compared the accuracy and precision of estimates of mean daily egg production and spawning area obtained with and without a CUFES. Importantly, the cost-savings resulting from the use of a CUFES, although commonly acclaimed, have not been assessed objectively. Checkley *et al.,* [13] noted that under some circumstances there may be no advantage in using the CUFES in DEPM studies, but no clear guidelines have been developed to indicate when or how a CUFES can be used most effectively to support DEPM-based stock assessments of small pelagic fishes. In particular, there has been no consideration of the benefits of, or best approach to, using CUFES in dedicated surveys (i.e. where CalVET sampling is not conducted as part of extensive multi-disciplinary sampling program (e.g. CalCOFI cruises) and the time expended at each sampling site is short).

The DEPM has been used for routine stock assessment of sardine, *Sardinops sagax,* in waters off South Australia since 1998 [14, 15]. The (previously annual, now biennial) estimate of spawning biomass is the primary biological performance indicator which underpins the sustainable management of the fishery. Surveys are conducted with the primary purpose of collecting egg and adult samples required to apply the DEPM. Sampling design for collecting egg samples is designed to maximise accuracy and precision of estimates of egg production and spawning area. Hence a large number of sites are sampled each year and these represent relatively small, similar-sized areas (grids). The costs of

conducting DEPM-based stock assessments were initially shared by the South Australian Government and participants in the South Australian Sardine Fishery. However, in recent years the full costs of these assessments have been recovered from industry participants through annual licensing fees paid to the State's fisheries management agency (PIRSA Fisheries) which, in turn, contracts the State's research provider (SARDI Aquatic Sciences) to undertake the assessments. Hence, there is a clear need to ensure that estimates of spawning biomass are not only as accurate and precise as practicable, but are also obtained as cost-effectively as possible.

This study was undertaken to identify the best approach to utilising a CUFES in future applications of the DEPM to sardine in waters off South Australia. Results are likely to be relevant to other jurisdictions where dedicated DEPM surveys are conducted from small research vessels and the time taken to collect each sample is short. We conducted experiments to: 1) determine the reliability of information obtained from onboard examination of CUFES samples; 2) assess the sampling efficiency of CUFES while on-site and underway; and 3) determine the effectiveness of underway CUFES samples in predicting the presence/absence and abundance of sardine eggs in the following CalVET net sample. The benefits/costs of using a CUFES in DEPM applications were assessed by comparing the accuracy, precision and costs of parameter estimates obtained from a) pre-determined CalVET net samples and b) CalVET net samples selected because of the presence of at least one egg in the preceding underway CUFES sample (CUFES-determined sites). A context for assessing variations in accuracy, precision and costs was established by calculating parameter estimates from two levels of CalVET net sampling intensity (high and low).

METHODS

Survey Design

We conducted two ichthyoplankton surveys of southern Spencer Gulf during the 2008 sardine spawning season (22-25 February and 26-28 March). During each survey, we collected CalVET net samples from a subset of the standard ichthyoplankton sites that we sample during each annual DEPM survey, as well as additional sites located mid-way between each standard site (Fig. **1**). An "on-site CUFES" sample was taken concurrently with each CalVET net sample. An "underway CUFES sample" was taken between each site.

Rationale

The reliability of information obtained from onboard examination of CUFES samples was determined by comparing estimates of presence/absence and counts of sardine eggs obtained in onboard examinations with those obtained in the laboratory analyses.

The sampling efficiency and effectiveness of the CUFES was assessed by comparing the number, densities and stages of eggs obtained in each:

   (i).   On-site CUFES sample with the sample obtained concurrently with the CalVET net;

Fig. (1). Location of standard (solid circles) and additional (open circles) CalVet net sites sampled during Cruises 1 and 2 in 2008. On-site and underway CUFES samples were taken at and between each CalVet net site, respectively. Densities of sardine eggs sampled with CalVET net (A), on-site CUFES (B) and underway CUFES (C).

(ii). Underway CUFES sample with the following on site CUFES sample;

(iii). Underway CUFES sample and the following CalVET net sample.

The benefits/costs of using a CUFES to estimate DEPM parameters were assessed by comparing precision, accuracy and costs of estimating key parameters using (i) data predetermined and CUFES-determined samples. The effects of enumeration errors made during onboard examination of CUFES samples were estimated by comparing estimates obtained using data from onboard examinations and laboratory analyses. All calculations were made for both the low and high sampling intensities so that the effects of using a CUFES could be compared to the effects associated with normal variations in the design of CalVET net surveys.

Sampling Methods

At each standard and additional site (Fig. **1**), a paired CalVET net (0.3 m diameter, 333 µm mesh) was deployed to within 10 m of the seabed in waters less than 80 m deep and to a depth of 70 m in waters deeper than 80 m. The net was then retrieved vertically 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.

The RV Ngerin is 24.8 m in length with a displacement weight of 197 t. The installation of a CUFES on the RV Ngerin was overseen by Mr Tim Mares in June 2007 (Ocean Instruments Incorporated, San Diego, California, U.S.A.).

The structure and function of the CUFES system is described in detail by Checkley *et al.,* [8].

The CUFES installed on the RV *Ngerin* is comprised of a submersed semi-vortex pump with a flow rate of 640 L.min⁻¹ that intakes water through the hull, a concentrator consisting of a vertical oscillating cylinder of 500 µm Nitex mesh; and a sample collector. The opening in the hull is located 17.1 m behind the bow 1.6 m to the starboard side of the keel. Large particles including fish eggs are carried from the pump to the concentrator in an 8 cm diameter hose. A concentrated flow of approximately 30 L min⁻¹ is directed into the sample collector. The filtrate is directed overboard through a flexible hose. Oceanographic and spatial data is logged continuously while the CUFES is running using a shipboard thermosalinograph (SBE 45) that was linked that to the drainage port of the concentrator. GPS data was logged using a TripNav™ TN-200 USB GPS receiver.

Sample Analysis

Each CUFES sample was examined onboard using a Leica MS5 dissecting microscope. The number of sardine eggs in each sample was counted or estimated, depending on the number of eggs present (i.e. large numbers of eggs were estimated rather than counted due to logistical constraints). Samples were preserved in 5% buffered formaldehyde and seawater.

All CalVET net and CUFES samples were sorted in the laboratory at SARDI Aquatic Sciences. Sardine eggs were counted and staged based on the descriptions of White and Fletcher [16].

Data Analysis

Egg Density

Egg density in one cubic metre of water (P_v) in samples obtained using the CalVET net was estimated at each site according to Equation 1:

$$P_v = \frac{C}{V} \qquad \text{Eq. (1)}$$

where C is the number of eggs in each sample and V in the volume filtered (m^3).

The number of eggs of each stage under one square metre of water (P_t) was estimated at each site according to Equation 2:

$$P_t = \frac{C.D}{V} \qquad \text{Eq. (2)}$$

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.

Egg density in one cubic metre of water (P_v) in samples obtained using CUFES was estimated at each site according to Equation 3:

$$P_v = \frac{C}{t.V} \qquad \text{Eq. (3)}$$

where C in the number of eggs in each sample, *t* is the time over which the sample was collected and *V* is the rate of flow through the CUFES collector (640 L.min^{-1}).

Mean Daily Egg Production

Biased mean daily egg production (P_b) was calculated from CalVET net samples by fitting the linear version of the exponential egg mortality model to estimates of egg age and density at each site [17]. The linear version of the exponential egg mortality model is:

$$lnP_b = \ln(P_i) - Zt \qquad \text{Eq. (4)}$$

where P_i is the density of eggs of age *t* at site *i* and *Z* is the instantaneous rate of egg mortality.

Estimates of mean egg production (P_b) obtained using the linear version of the exponential mortality model have a strong negative bias, therefore a bias correction factor was applied following the equation of Picquelle and Stauffer [18]:

$$P = e^{(\ln P_b + \sigma^2/2)} \qquad \text{Eq. (5)}$$

As the data were over-dispersed, confidence intervals were calculated using bootstrap methods [19]. A total of 500,000 bootstrap samples were generated by resampling from the data pairs to estimate confidence intervals.

Spawning Area

After the cruises were completed, the survey area was divided into a series of contiguous grids approximately centred on each standard (low sampling intensity) station. The area represented by each station (km^2) was calculated using MAPINFO® software. For high intensity sampling, the addi-

tional stations were assigned an area equal to the sum of the area of one quarter of each of the adjacent standard grids, which were assigned an area equal to half the normal value. Blocks on the end of transects were assigned an area equal to three quarters the normal value. The same grids were used for CUFES-determined analysis as for predetermined sites. The spawning area (*A*) was defined as the total area of grids in which *S. sagax* eggs were found.

Total Egg Production

Total egg production was calculated according to the equation:

$$P_T = P_o \times 10^6 \times A \qquad \text{Eq. (6)}$$

where P_o is mean egg production (eggs.m^{-2}) and A is the spawning area (km^2).

Accuracy, Precision and Costs of Using a CUFES

We assessed the relative accuracy of parameter estimates obtained using a CUFES to determine where CalVET net samples would be taken by measuring the difference from estimates obtained using pre-determined sites at high sampling intensity.

The precision of estimates obtained with and without a CUFES were compared using the relative 95% Confidence Interval (95% CI/mean). Coefficients of Variation (CVs) were not calculated as the bootstrapped estimates of 95% CIs were not symmetrical (i.e. normally distributed).

Field costs were estimated by standardising the time taken to complete Cruises 1 and 2 by removing time lost due to bad weather and other extraneous factors. The marginal costs of running the RV Ngerin (i.e. including crew, fuel, maintenance, etc but excluding overheads, capital and depreciation costs) were estimated at $3,600/day. It was assumed that five scientific staff at the cost of $270 per day per person (this estimate also excludes salary on-costs, overheads, etc) were required to undertake each ichthyoplankton survey. Based on data obtained in the present study, it was assumed to take 5 minutes to collect each CalVET net sample and 30 minutes to travel between each sampling site.

Direct laboratory costs (including consumables, electricity, etc but excluding capital and depreciation costs) were assumed to be $10 per day. The cost for a technician to sort samples was assumed to be $190 for a seven hour day (excluding salary on-costs, overheads, etc). Based on data from the present study, it was assumed that it took 30 minutes to sort a CalVET net or underway CUFES sample and 15 minutes to sort an on-site CUFES sample.

RESULTS

Total Samples

This study is based on 606 samples containing 6,347 sardine eggs collected during two research cruises (Table **1**). The CalVET net, on-site CUFES and underway CUFES samples collected Cruise 1 each contained more eggs than samples collected during the second cruise using the same method (Table **1**).

Table 1. Number of Samples and Sardine (*Sardinops Sagax*) Eggs Collected Obtained Using CalVET Nets and on Site and Underway CUFES During Ichthyoplankton Surveys from the RV Ngerin in Southern Spencer During February and March 2008. Estimates Based on Laboratory Analyses of Samples

	Cruise 1		Cruise 2		Total	
	No. of Samples	No. of Eggs	No. of Samples	No. of Eggs	No. of Samples	No. of Eggs
CalVET	104	1905	104	784	208	2689
CUFES on site	104	456	103	205	207	661
CUFES underway	95	2096	96	901	191	2997
Total	303	4457	303	1890	606	6347

Table 2. Comparison of Counts of Sardine (*Sardinops Sagax*) Eggs Made in Onboard Examinations and Laboratory Analyses of Samples Taken During Ichthyoplankton Surveys Conducted from the RV *Ngerin* in Southern Spencer Gulf During February and March 2008

	Cruise 1 (N = 199)		Cruise 2 (N = 200)		Total (N = 399)	
	Onboard # (%)	Lab #	Onboard # (%)	Lab #	Onboard # (%)	Lab #
Total stations		199		200		399
Positive stations	89	90	83	110	172	200
Negative	110	109	117	90	227	199
False positive	7 (3.5)		3 (1.5)		10 (2.5)	
False negative	8 (4.0)		30 (15.0)		38 (9.5)	
Equal counts	133 (66.8)		114 (57.0)		247 (61.9)	
Double zero counts	102 (51.3)		87 (43.5)		189 (47.4)	
Underestimated	42 (21.1)		76 (38.0)		118 (29.6)	
Overestimated	24 (12.1)		10 (5.0)		34 (8.5)	

Reliability of Onboard Examinations of CUFES Samples

Laboratory analyses indicated 50.1% of the CUFES samples (both on-site and underway samples) contained sardine eggs, whereas onboard examinations suggested that sardine eggs were present in 43.1% of these samples (Table **2**). The laboratory analyses were assumed to be 100% accurate, as they are in all DEPM studies, because re-examination of samples confirmed that all sardine eggs were removed during sorting. Eggs were not observed during onboard examinations but were identified during laboratory analyses in 9.5% of samples. Conversely, onboard examinations identified eggs as being present in 2.5 % of samples in which no eggs were later observed (Table **2**).

Onboard examinations of presence/absence of eggs differed from the results of laboratory analyses in 15 samples (7.5%) from Cruise 1 (7 false positives, 8 false negatives), whereas 33 (16.5%) onboard counts made during Cruise 2 (3 false positives, 30 false negatives) differed from laboratory results (Table **2**).

There were strong correlations between counts of sardine eggs made onboard the vessel and in the laboratory (Fig. **2**, Table **3**). Onboard examinations and laboratory analyses resulted in equal egg counts for 247 samples (61.9 %, Table

2), of which 189 samples (47.4 %) were double zero counts. Onboard counts underestimated the number of eggs in 118 samples (29.6 %) and over-estimated eggs in 34 samples (8.5 %) compared to laboratory analyses. Correlation coefficients for counts made onboard the vessel and in the laboratory were reduced by the effects of samples which contained large numbers of eggs, for which onboard data were estimated (e.g. approximately 100 eggs) rather than counted accurately as they were in the laboratory.

Sampling efficiency of on-site CUFES

On-site CUFES samples included approximately one quarter as many eggs as the CalVET net samples during both Cruise 1 and Cruise 2 (Table **1**).

The time taken to collect CUFES and on-site CalVET net samples were virtually identical. However, the CUFES filtered approximately one quarter of the water filtered by the CalVET nets during this period (e.g. 0.5-4.4 m^3 for on-site CUFES versus 1.5-17.3 m^3 for CalVET nets).

Mean egg density for on-site CUFES samples was not significantly different from the estimate for the CalVET net samples during Cruise 1 (Table **3**). However, estimates of mean density in on-site CUFES and CalVET net samples
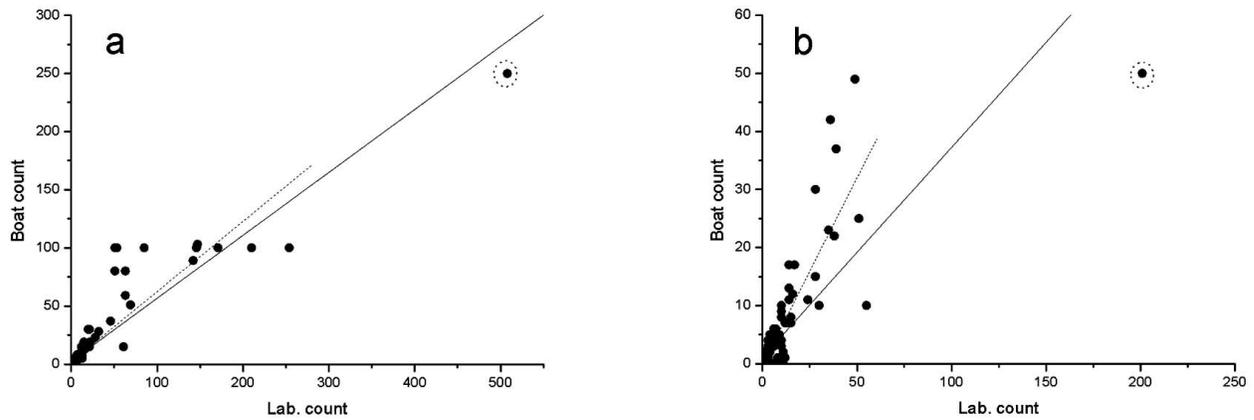
Fig. (2). Comparison of counts of sardine (*Sardinops sagax*) eggs made in laboratory analyses and onboard examinations of CUFES samples collected during ichthyoplankton surveys conducted from RV *Ngerin* in southern Spencer Gulf in February and March 2008. The solid line shows the linear relationship between untransformed counts for a) Cruise 1 (y = 0.38 x + 0.09, R^2 = 0.88, n = 195, p < 0.0001) and b) Cruise 2 (y = 0.77x +0.06, R^2 = 0.76, n = 196, p < 0.0001) 2008. The dotted line is the linear relationship with the circled outlier removed. The descriptive statistics for regressions with outliers removed and for ln-transformed data are shown in Table **3**.

Table 3. Sampling Efficiency of On-Site and Underway CUFES and Effectiveness of Underway CUFES in Predicting Mean Egg Density in Following the CalVET Net Sample

		Mean Egg Density (SE)	Mean Egg Density (SE)	
Sampling Efficiency of On-Site CUFES		**On-Site CUFES**	**CalVET**	**Wilcoxon Signed Ranks Test**
	Cruise 1 n = 103	2.45 (0.70)	2.08 (0.87)	Z = -1.812, p = 0.07
	Cruise 2 n = 103	1.35 (0.29)	1.23 (0.33)	Z = -2.672, p = 0.008
Sampling efficiency of underway CUFES		Underway CUFES	Following on-site CUFES	
	Cruise 1 n = 93	1.67 (0.55)	2.31 (0.73)	Z = -1.188, p = 0.235
	Cruise 2 n = 95	0.64 (0.16)	1.37 (0.30)	Z = -3.367, p = 0.001
Underway CUFES as predictor of following CalVET		Following CalVET	Underway CUFES	
	Cruise 1 n = 95	2.16 (0.94)	1.81 (0.56)	Z = -1.009, p = 0.313
	Cruise 2 n = 95	1.01 (0.24)	0.64 (0.16)	Z = -2.126, p = 0.034

were significantly different (CalVet lower) during Cruise 2 (Table **3**).

Estimates of egg density obtained in on-site CUFES and CalVET net samples were correlated during both Cruises (Fig. **3**, Table **4**). For Cruise 1, the relationship (Table **4**) suggests that for every 10.0 eggs m^{-3} collected in the CalVET net, 5.81 eggs m^{-3} were collected with the CUFES. Similarly, the relationship for every 10 egg m^{-3} collected in the CalVET net during Cruise 2 approximately 6.18 eggs m^{-3} were collected using the CUFES (Table **4**). However, the average fits for both cruises were strongly affected by one or two CalVET net samples that included large numbers of early stage (I and V) eggs. Removing the obvious outlier from these analyses (Fig. **3**, Table **4**), changes these relationships considerably, suggesting that 21.0 and 14.1 eggs m^{-3} were obtained in on-site CUFES for every 10 eggs m^{-3} collected in the CalVET nets during Cruise 1 and 2, respectively.

Samples obtained using on-site CUFES included approximately half as many egg stages as samples obtained using CalVET nets. Over 40% of eggs collected using the CalVET net during Cruise 1 were Stage II eggs (Fig. **4**). No single egg stage comprised more than 30% of eggs collected (on-site) using the CUFES during either cruise. The most abundant egg stage obtained in on-site CUFES samples during Cruises 1 and 2 were Stages XI and VII, respectively. Stages Va and Vb were rarely recorded in on-site CUFES samples.

Sampling Efficiency of Underway CUFES

A total of 2796 eggs was collected in underway CUFES samples whereas only 561 eggs were collected in following on-site CUFES samples. This pattern was observed in Cruise 1 and Cruise 2. Underway CUFES samples were filtered from a larger volume of water than the samples from the following on-site CUFES sample (ranges of 9.1-17.8 m^3 and 0.5-4.3 m^3, respectively).
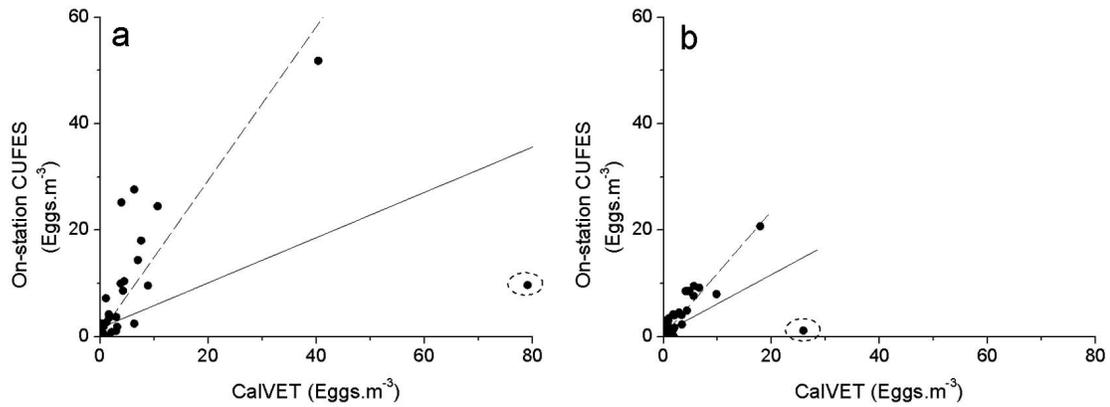
Fig. (3). Comparison of egg density in on site CUFES and CalVET net samples collected during ichthyoplankton surveys conducted from RV *Ngerin* in southern Spencer Gulf in February and March 2008. The solid line shows the linear relationship between untransformed densities for Cruise 1 (a. $y = 0.43 x + 1.56$, $R^2 = 0.28$, n = 103, p < 0.0001) and Cruise 2 (b, $y = 0.55x + 0.68$, $R^2 = 0.41$, n = 103, p < 0.0001). The dotted line is the linear relationship with the circled outliers removed. The descriptive statistics for regressions with outliers removed are shown in Table **3**.

Table 4. Regression Equations, R^2, N and p for Plots of Egg Density in Samples Taken Using CalVET Nets and on Site and Underway CUFES During Ichthyoplankton Surveys Conducted from the RV *Ngerin* in Southern Spencer Gulf During February and March 2008. Scatterplots of Untransformed Data are Provided in Figures Listed in the Table

	Untransformed Egg Densities (Eggs.m^{-2})	Untransformed Egg Densities (Eggs.m^{-2}) 1 Outlier Removed
Onboard Examination (y) Versus Laboratory Analysis (x) Fig. (2)		
Cruise 1	$y = 0.54x + 2.45$ $R^2 = 0.86$ N = 195, p <0.0001	$y = 0.60x + 1.93$ $R^2 = 0.78$ N = 194, p <0.0001
Cruise 2	$y = 0.36x + 1.09$ $R^2 = 0.63$ N = 196, p <0.0001	$y = 0.64x - 0.09$ $R^2 = 0.75$ N = 195, p <0.0001
On site CUFES (y) versus CalVET (x) Fig. (3)		
Cruise 1	$y = 0.43x + 1.56$ $R^2 = 0.28$ N = 103, p <0.0001	$y = 1.44x + 0.47$ $R^2 = 0.79$ N = 102, p <0.0001
Cruise 2	$y = 0.55x + 0.68$ $R^2 = 0.41$ N=103 P<0.0001	$y = 1.16x + 0.19$ $R^2 = 0.88$ N = 102, p <0.0001
Underway CUFES (y) proceeding on site CUFES (x) Fig. (5)		
Cruise 1	$y = 0.57x + 0.34$ $R^2 = 0.57$ N = 93, p <0.0001	$y = 0.47x + 0.48$ $R^2 = 0.29$ N = 92, p <0.0001
Cruise 2	$y = 0.42x + 0.06$ $R^2 = 0.64$ N = 95, p <0.0001	$y = 0.21x + 0.25$ $R^2 = 0.31$ N = 94, p <0.0001
CalVET (y) versus preceding underway CUFES (x) Fig. (6)		
Cruise 1	$y = 1.31x - 0.21$ $R^2 = 0.61$ N = 95, p <0.0001	$y = 0.81x + 0.08$ $R^2 = 0.77$ N = 94 P<0.0001
Cruise 2	$y = 1.31x + 0.18$ $R^2 = 0.72$ N = 95, p <0.0001	No outlier

Mean egg density in underway CUFES samples and the following on-site CUFES samples were not significantly different for Cruise 1 (Table **3**). However, mean density in underway CUFES samples was significantly different (lower) than the following on-site CUFES samples for Cruise 2.

The estimate of egg density obtained in each underway CUFES sample was strongly correlated with the estimate of egg density for the following on-site CUFES sample during both cruises (Table **4**). On average, approximately 6.0 and 4.3 eggs m^{-3} were present in underway CUFES samples for each 10.0 eggs m^{-3} obtained in the following on-site CUFES sample during Cruises 1 and 2, respectively.
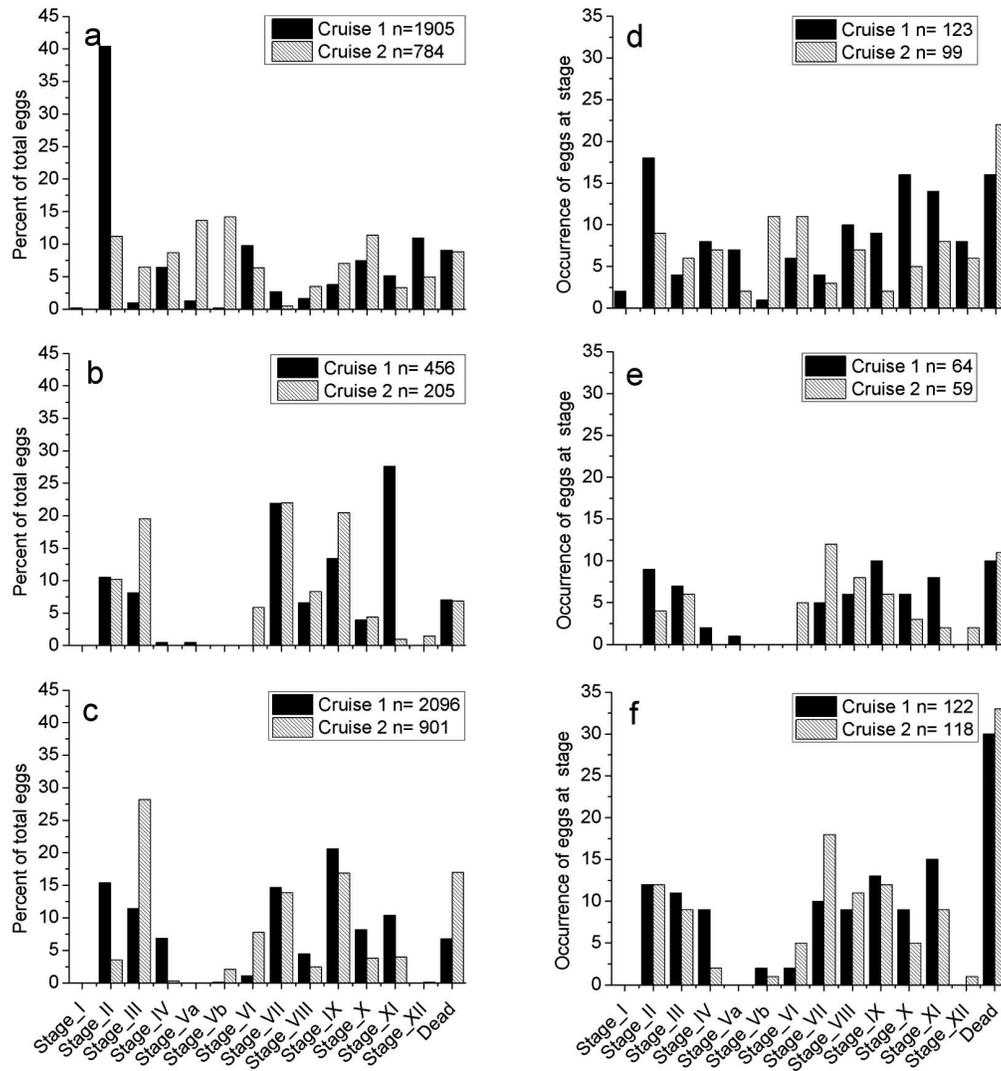
Fig. (4). Egg stage histograms for (a and d) CalVET, (b and e) on-station CUFES, and (c and f) underway CUFES collected during ichthyo-plankton surveys conducted from RV *Ngerin* in southern Spencer Gulf in February and March 2008. (a, b and c show the percentage of the total number of eggs at each stage (n) collected during each cruise. d, e and f show the number of times each stage occurred in a sample, n is the total number of times an egg of each stage was observed in a sample.)

Approximately twice as many egg stages were obtained in underway CUFES samples (Fig. **4**) compared to the following on-site CUFES samples. Underway CUFES samples included more eggs that were classified as dead than onsite CUFES samples. Stages Va and Vb were rarely recorded in underway or on-site CUFES samples.

Effectiveness of Underway CUFES in Predicting Following CalVET net Sample

Similar total numbers of eggs were collected in the underway CUFES samples and the following CalVET net sample during both Cruise 1 and 2.

The volume of water filtered to collect underway CUFES samples was less variable than the volume of water filtered to obtain a CalVET net sample from the following site, because the distance between sites varied less than the depth of sites. Underway CUFES samples were filtered from 9.1-17.8 m^3 of water whereas CalVET net samples from the following site were filtered from 1.5-17.3 m^3.

Mean egg density in underway CUFES samples and the following CaVET samples were not significantly different for Cruise 1 (Table **3**). However, mean density in underway CUFES samples and the following CalVET net sample were significantly different (CUFES lower) for Cruise 2 (Table **3**).

Estimates of egg density from underway CUFES samples and the following on-site CalVET net sample were highly correlated during both cruises (Fig. **6**, Table **4**). During Cruise 1, the relationship indicated that on average for every 10 eggs m^{-3} present in an underway CUFES sample approximately 12.9 eggs m^{-3} was present in the following CalVET net sample. Similarly, during Cruise 2 for every 10 eggs m^{-3} collected in an underway CUFES sample, an average of 13.3 eggs m^{-3} was obtained in the following CalVET net sample.

Similar numbers of eggs of each stage were obtained in underway CUFES samples (Fig. **4**) and the following on CalVET net sample. Stages Va and Vb were more common in CalVET net samples than the preceding CUFES sample.
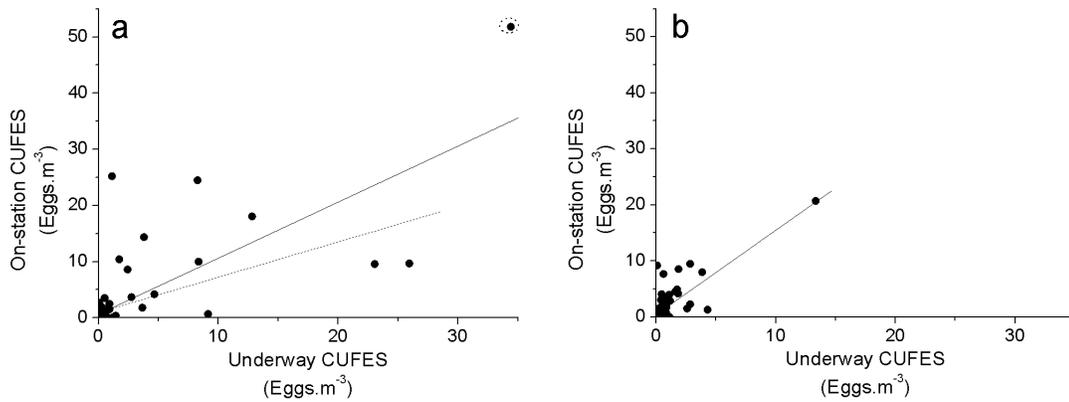
Fig. (5). Comparison of egg density in underway CUFES and following on site CUFES samples collected during ichthyoplankton surveys conducted from RV *Ngerin* in southern Spencer Gulf in February and March 2008. The solid line shows the linear relationship between untransformed densities for Cruise 1 (a, $y = 0.57x +0.34$, $R^2 = 0.57$, n =93, $p < 0.0001$) and Cruise 2 (b, $y = 0.42x +0.06$, $R^2 = 0.64$, n =95, $p < 0.0001$). The dotted line is the linear relationship with the circled outlier removed. The descriptive statistics for regressions with outlier removed and for ln-transformed data are shown in Table **3**.

Fig. (6). Comparison of egg density in underway CUFES and following ClaVET net samples collected during ichthyoplankton surveys conducted from RV *Ngerin* in southern Spencer Gulf in February and March 2008. The solid line shows the linear relationship between untransformed densities for Cruise 1 ($y = 1.31x -0.21$, $R^2 = 0.61$, n = 95, $p < 0.0001$) and Cruise 2 ($y = 1.31x +0.18$, $R^2 = 0.72$, n =, $p < 0.0001$). The dotted line is the linear relationship with the circled outliers removed. The descriptive statistics for regressions with outliers removed and for ln-transformed data are shown in Table **3**.
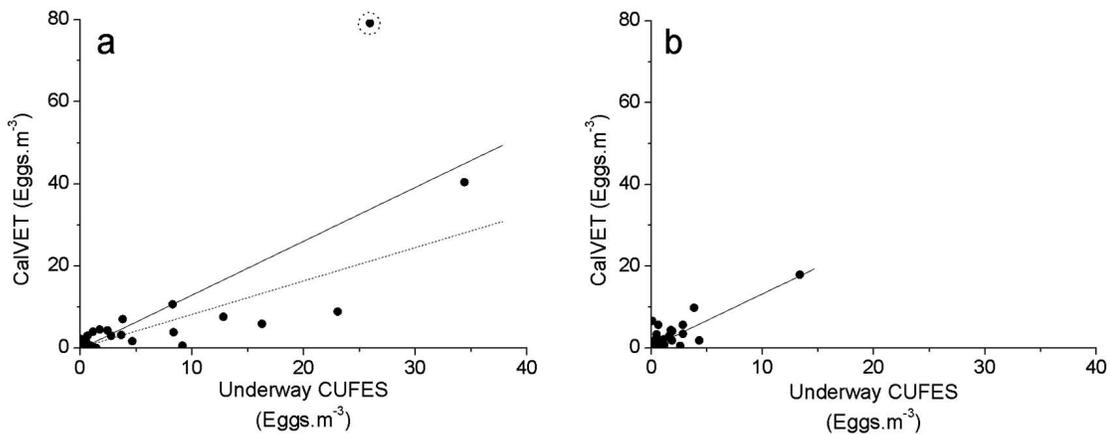
Estimates of DEPM Parameters from Pre-Determined and CUFES-Determined Sites

Number of Sites Sampled

Using data from laboratory analyses of CUFES samples to determine which CalVET net samples would have been collected reduced the total sample sizes by approximately one-fifth to one-third. For example, during Cruise 1, 75.0% (Table **5**) of low density sites and 63.5% of high density sites would have been sampled based on the laboratory analyses of underway CUFES samples. Similarly, during Cruise 2, 80.4% and 70.2% of low and high density sites, respectively, would have been sampled.

Based on the onboard examination of CUFES samples, the number of CalVET net samples that would have been collected would have been reduced by approximately one-third to one-half. For example, during Cruise 1 67.8% (Table **5**) of low density sites and 58.6% of high density sites would have been sampled. Similarly, during Cruise 2 63.0% and 53.8% of low and high density sites, respectively, would have been sampled (based on data collected onboard).

Number of Positive Sites

The number of predetermined sites at which eggs were collected at the high sampling intensity was slightly less than twice the number of positive sites identified at low sampling intensity during Cruise 1 and 2 (Table **5**).

The reduction in the proportion of positive sites that resulted from using the preceding underway CUFES sample to determine whether a CalVET net sample would be taken was lower than the reduction in the number of sites sampled. Based on laboratory analyses of the CUFES samples, the number of positive CalVET net samples obtained during Cruise 1 would have been reduced by 7.1% and 10.0% at low and high sampling intensities, respectively. Similarly, during Cruise 2 the number of positive CalVET net samples would have been reduced by 3.4% at low sampling intensity and 9.3% at high sampling intensity.

Based on the onboard examinations of the CUFES samples, the number of positive CalVET net samples obtained during Cruise 1 would have been reduced by only 3.6% (Table **5**) and 12.0% at low and high sampling

Table 5. Estimates of Mean Daily Egg Production, Spawning Area and Total Daily Egg Production Obtained Using Data From All Predetermined CalVET Sites and Those that Would Have Been Sampled Based on Data Obtained Using Underway CUFES (Laboratory Analyses and Onboard Examinations Separately). Estimates are for Two (High and Low) Sampling Intensities During Ichthyoplankton Surveys Conducted From the RV *Ngerin* in Southern Spencer Gulf During February and March 2008 (see Fig. 1)

	Cruise 1		Cruise 2	
	Low Density	**High Density**	**Low Density**	**High Density**
Predetermined CalVET Sites				
Number of Stations Sampled	56	104	56	104
Number of Positive Stations	28	50	29	54
Mean Egg Density (eggs m^{-2})	255.8	212.3	87.7	84.8
Standard Error	±156.6	±93.1	±27.9	±20.3
Mean Daily Egg Production (eggs m^{-2})	118.48	107.13	73.33	65.96
% Difference	+10.6	0.0	+6.9	0.0
95 % Confidence Intervals	24.4-547.3	41.1–269.0	32.6-158.5	34.4-123.1
Precision (95%CI/mean)	4.4	2.1	1.7	1.3
Spawning area (km^2) (live egg presence)	6677	6217	6483	6095
% Difference	+7.4	0.0	+0.1	0.0
Total daily egg production (10^6 eggs)	791,113	666,086	475,382	402,072
% Difference	+18.8	0.0	+18.2	0.0
Laboratory analysis of CUFES to determine CalVET sites sampled				
Number of Stations Sampled	42	66	45	73
Number of Positive Stations	26	45	28	49
Mean Egg Density (eggs m^{-2})	275.1	235.0	87.7	90.8
Standard Error	±168.2	±103.3	±27.9	±21.8
Mean Daily Egg Production (eggs m^{-2})	131.34	113.74	73.33	67.19
% Difference	+22.6	+6.2	+6.9	+1.2
95 % Confidence Intervals	26.9-602.0	41.8-297.0	32.5-158.1	34.2-127.1
Precision (95%CI/mean)	4.4	2.2	1.7	1.4
Spawning area (km^2)	6218	5611	6483	5455
% Difference	+0.1	-9.7	+0.1	-0.1
Total daily egg production (10^6 eggs)	816,768	638,226	475,382	366,492
	+22.6	-4.2	+18.2	-8.8
CUFES Onboard examination determined CalVET sites				
Number of Stations Sampled	38	61	35	56
Number of Positive Stations	27	44	22	34
Mean Egg Density (eggs m^{-2})	265.1	238.6	104.4	117.3
Standard Error	±162.2	±105.7	±34.7	±28.9
Mean Daily Egg Production (eggs m^{-2})	129.29	170.20	144.39	80.43
% Difference	+20.7	+58.9	+73.2	+13.5
95 % Confidence Intervals	26.5-593.0	53.1-501.9	58.1-327.8	35.9-164.5
Precision (95%CI/mean)	4.3	2.6	1.9	1.6
Spawning area (km^2)	6433	5514	4954	3892
% Difference	+3.5	-11.3	-0.2	-0.5
Total daily egg production (10^6 eggs)	831,627	938,486	715,344	313,095
% Difference	+24.9	+40.9	+77.9	-22.1

intensities, respectively. However, during Cruise 2, the number of positive on-site CalVET net samples would have been reduced by 24.1% at low sampling intensity and 37.0% at high sampling intensity.

Spawning Area

The estimates of spawning area obtained using data from high sampling intensity sites were 6.9% and 6.0% lower

(Table **5**) than those obtained for low sampling intensity sites for Cruise 1 and Cruise 2, respectively.

Using data from the laboratory analyses of underway CUFES samples to determine which CalVET net samples would be collected would have reduced the estimates of spawning area for Cruise 1 by 6.9% (Table **5**) and 9.7% at the low and high sampling intensities, respectively. Similarly, during Cruise 2 the estimate of spawning area would

Table 6. Summary of Time Requirements and Costs of Alternatively Designed Cruises Based on the Partitioned Time Allocated to Aspects of the Cruise 1 Discussed in this Study

	Time (hours)	$
DEPM based on predetermined CalVET samples		
At Sea (Marginal at sea costs = $4950 day^{-1} or $206.25 hr^{-1})		
Travel to sampling area	12:00	2,475
Travel between sites and transects – see Fig. (**1**)	59:00	12,169
Collect predetermined CalVET samples for low intensity sampling	4:40	963
Additional time to collect predetermined CalVET samples for high intensity sampling	4:00	825
Return to port	12:00	2,475
At sea sub-total (low sampling intensity)	87:40	18,082
At sea sub-total (high sampling intensity)	92:20	18,907
Laboratory (Marginal laboratory costs = $200 day^{-1} or $28.57 hr^{-1})		
Time taken to sort predetermined CalVET samples for low intensity sampling	28:00	800
Additional time to sort predetermined CalVET samples for high intensity sampling	24:00	686
Total		
Total to collect and sort samples from predetermined CalVET samples (low intensity)	115:40	**18,882**
Total to collect and sort samples from predetermined CalVET samples (high intensity)	144:40	**20,393**
CUFES-determined CalVET samples		
At Sea (Marginal at sea costs = $4950 day^{-1} or $206.25 hr^{-1})		
Travel to sampling area	12:00	2,475
Travel between sites and transects – see Fig. (**1**)	59:00	12,169
Collect CUFES-determined CalVET samples for low intensity sampling	3:10	653
Return to port	12:00	2,475
Additional time to collect CUFES-determined CalVET samples for high intensity sampling	1:55	395
At sea sub-total (low sampling intensity)	86:10	17,772
At sea sub-total (high sampling intensity)	88:05	18,167
Laboratory		
Time taken to sort CUFES-determined CalVET samples for low intensity sampling	19:00	543
Additional time to sort CUFES-determined CalVET samples for high intensity sampling	11:30	329
Total		
Total to collect and sort samples from CUFES-determined CalVET samples (low intensity)	121:35	**18,315**
Total to collect and sort samples from CUFES-determined CalVET samples (high intensity)	133:05	**19,073**
Other Variables		
Time taken to sort on-site CUFES (15 min. each)(high intensity)	26:00	743
Time taken to sort underway CUFES (30 min. each)(high intensity)	47:30	1357
Maximum Cost/Data (high density sampling sort all predetermined CalVET and CUFES	217:50	**22,493**
Minimum Cost (only collect and sort CUFES-determined samples at low sampling intensity	121:35	**18,315**

have been unchanged at low sampling intensity and reduced by 10.3% at the high sampling intensity.

Based on the onboard examination of CUFES samples, the estimate of spawning area for Cruise 1 would have been reduced by 3.7% (Table **5**) and 11.3% (km^2) at low and high sampling intensities, respectively. However, the large reduction in the number of positive samples identified during the second cruise would have reduced the estimates of spawning area by 23.6% at the low sampling intensity and 36.1% at the high sampling intensity.

Mean Daily Egg Production

The estimates of mean daily egg production calculated for the high sampling intensities were similar to (slightly lower than) those obtained at low sampling densities for Cruise 1 (-9.6%, Table **5**) or 2 (-10.0%), respectively,).

Estimates of mean daily egg production obtained from laboratory examination of CUFES samples to determine which CalVET net samples would be analysed were similar to, or slightly higher than, those for pre-determined sites in both cruises. During Cruise 1, the difference was 10.9% and

6.2% at low and high sampling intensities, respectively (Table **5**). Similarly, during Cruise 2 the estimates were identical at low sampling intensity and 1.9% higher at high sampling intensity.

Estimates of mean daily egg production using onboard examinations to determine which CalVET net samples would be analysed were higher than those estimated for predetermined sites in both cruises. During Cruise 1, this difference was 9.1% and 58.9% at low and high sampling intensities respectively (Table **5**). During Cruise 2 this difference was 96.9% at low sampling intensity and 21.9% at high sampling intensity.

Total Daily Egg Production

The estimates of total egg production obtained from high density predetermined sites were 15.8% and 15.4% lower (Table **5**) than those obtained from high density predetermined sites for Cruise 1 and 2, respectively.

The estimates of total daily egg production obtained from laboratory examined CUFES-determined sites sampled during Cruise 1 were 3.2% higher (Table **5**) than those obtained from predetermined sites at low sampling intensity and 4.2% lower at high sampling intensity. The estimate of total egg production for Cruise 2 from laboratory examined CUFES-determined sites was identical to the estimate from low intensity predetermined sites and 8.8% lower for high intensity pre-determined sites.

The estimates of total egg production obtained from onboard examined CUFES-determined sites sampled during Cruise 1 were 5.1% and 40.9% higher (Table **5**) than those obtained from predetermined sites at low and high sampling intensities respectively. Similarly, the estimate of total egg production for Cruise 2 from CUFES-determined sites was 50.5% higher than the estimate from predetermined sites at low sampling intensity and 22.1% lower than the estimate obtained using pre-determined sites at high sampling intensity.

Accuracy, Precision and Costs, with and Without a CUFES

Accuracy

Based on the assumption that the estimates of egg production obtained from high sampling intensity predetermined sites were the most accurate, the next most accurate estimates were those obtained from CalVET samples selected on the basis of the laboratory analyses of CUFES samples at high sampling intensity (Table **5**). For both cruises, the least accurate estimates of egg production were those obtained from CUFES-determined sites based on onboard examinations of samples.

Precision

The precision (as measured by 95% CI/mean) of estimates of mean daily egg production was affected by sampling intensity. As expected, high sampling intensity was associated with increased precision. Estimates of mean daily egg production obtained from onboard examination of CUFES samples were less precise than other estimates in three out of four cases (Table **5**).

Costs

The standardised cruise, which included taking CalVET net samples at the 56 predetermined (low sampling intensity) sites, took approximately 87 hours (~3 days 15 hours, Table 6) to complete and cost approximately $18,082 dollars. Doubling the number of predetermined sites sampled (i.e. to 104) increased the time at sea by four hours and forty minutes and increased the cost of the cruise by 4.6% to $18,907. Collecting CalVET net samples only at CUFES-determined sites reduced the at sea costs to $17,772 and $18,167 at low (38 sites) and high (61 sites) sampling intensity, respectively. Hence, using CUFES to determine when/where CalVET net samples would be taken reduced the costs of the standardised survey by $309 (1.7%) and $740 (3.9%) at low and high sampling intensity respectively.

Approximately doubling the sampling intensity increased the laboratory time and costs of sorting CalVET net samples from 28 hours to 52 hours and $800 to $1,486, respectively. Using the onboard analysis of CUFES to determine which samples would have been taken reduced the sorting time to by 9 h and 21 h 30 m at low and sampling intensities, respectively. Combined with the savings in field time, using the CUFES to determine which samples would have been taken reduced the total cost of collecting data from $18,882 to $18,315 (3.0%) and from $20,393 to 19,073 (i.e. 6.5%) at low and high sampling intensities respectively.

The total cost of collecting and sorting all CalVET net and CUFES (on-site and underway) samples for the standardised Cruise 1 was $22,493 (Table **6**). This was $2,100 (10.3%) more than the cost of collecting and sorting all of the high density predetermined CalVET net samples and $4,411 (25.4%) higher than the costs of collecting and sorting the low intensity predetermined samples.

DISCUSSION

Reliability of Onboard Examination of CUFES Samples

One of the central tenets of the current protocols for using CUFES to determine whether or not to take a CalVET net sample during DEPM surveys is that CUFES samples can be sorted accurately at sea to provide reliable "real-time" information about local egg abundance. In the present study, there was a strong correlation between the egg counts made during onboard examinations and later laboratory analysis for the first cruise ($R^2 = 0.95$) when the weather was generally good. However, the error rate during the second cruise ($R^2 = 0.76$), when the weather was poor, was relatively high. This finding emphasises the potential significance of the difficulties associated with sorting ichthyoplankton samples at sea on small research vessels such as the *RV Ngerin*, which is only 23 m in length and is relatively unstable in rough weather. It appears that using CUFES in real-time to determine when/where to take a CalVET net sample in DEPM surveys is an approach that may be better suited to larger research vessels, such as the *David Starr Jordan* [8, 10, 13], than to the smaller vessels that are used by many marine research agencies to conduct dedicated DEPM surveys. However, Lo *et al.,* [10] also found significant differences between egg counts made onboard the *David Starr Jordan* with those made later in the laboratory. For example,

the shipboard count for one sample was zero and the laboratory count was 341 eggs. Acknowledging and/or resolving the discrepancy between onboard and laboratory egg counts is important because our results show that CUFES provides information that could significantly enhance quantification of egg abundance, but that sorting errors made onboard the vessel can significantly affect estimates of DEPM parameters.

Sampling Efficiency of On-Site CUFES

The CUFES efficiently sampled sardine eggs on-site, with egg densities in the CUFES generally higher than those obtained in the corresponding CalVET net sample. As other workers have noted, this difference presumably reflects the tendency of buoyant sardine eggs to concentrate near the surface [8]. During each of our cruises, the egg density recorded in one anomalous CalVET sample was more than four times greater than that measured in the corresponding CUFES sample, which must have been the result of a sub-surface concentration of early stage eggs. The high densities of early stage eggs bias estimates of egg production upwards because the effects of dispersal are confounded with the effects of mortality. These "outliers" reflect the over-dispersed nature of most sardine egg data-sets (many zeros, numerous moderate values and a few high values) obtained from CalVET net surveys, which complicate estimation of mean egg density and mean daily egg production [3]. One option for using on-site CUFES samples in future DEPM studies may be to adjust for the effects of these outliers in CalVET net samples on the outcome of analyses used to estimate key DEPM parameters. This could involve, for example, the addition of dispersal term to the egg production model that included the ratio of egg densities recorded in CaVET and CUFES samples from each site.

In our study, Stage 2 eggs which are used to estimate egg production (and mortality) in DEPM studies were under-represented in CUFES samples. In addition, Stages V and VI, when the embryo is in the early phases of development and may be particularly fragile, were rarely identified in onsite (or underway) CUFES. Hence, onsite CUFES samples alone do not appear to be suitable for estimating mean daily egg production of sardine, but, as previously indicated, may be suitable for enhancing the precision of estimates of egg production obtained using CalVET nets. Determining how this integration of data should be done will require careful consideration of several statistical problems, such as the spatial and temporal variations in the ratio of egg density in CUFES/CalVET net samples [10].

Sampling Efficiency of Underway CUFES

Our results suggest that the efficiency of CUFES in sampling sardine eggs while the vessel is underway may vary according to the sea conditions. This is because egg densities recorded in the underway CUFES samples taken during Cruise 1, when the weather was good, were similar to those recorded in the following on-site CUFES sample, whereas those taken during the second cruise, when the seas were rough, were lower than those recorded on-site. This result may be due to the effect of the pitching of the research vessel on the flow of water across the CUFES opening in hull. In future we will determine the effect of placing a vertical plate behind the CUFES opening in reducing the effect of rough sea conditions on the sampling efficiency of the CUFES while the vessel is underway.

The finding by Lo *et al.,* [10] that the sampling efficiency of the CUFES, compared to the CalVET net, varied between cruises/years has implications for the proposition that the CUFES could become the primary/only sampler in future DEPM surveys [3]. Our findings suggest that on-site CUFES samples may be useful for calibrating the sampling efficiency of the CUFES while the vessel is underway. This approach may be important because our underway CUFES samples contained a large proportion of eggs which were classified as dead, especially during the rough second cruise when many eggs were damaged.

Effectiveness of CUFES for Predicting Egg Density in the Following CalVET Sample

Data from our laboratory analyses show that the effectiveness of underway CUFES samples in predicting egg density in the following CalVET net sample varied between cruises, and may be affected by the prevailing weather conditions. Mean egg density in underway CUFES samples was not significantly different from mean egg density in the following CalVET net sample during Cruise 1, but was significantly lower during Cruise 2. This finding re-emphasises the difficulties of using the CUFES as the primary/only egg sampler in DEPM studies and the need for ongoing calibration of the data from underway CUFES.

Accuracy of Estimates of Total Daily Egg Production

Although several previous studies have used onboard analyses of underway CUFES samples to determine where/when CalVET net samples should be taken [10], we are not aware of any previous attempts to assess the effects of errors in onboard egg counts on estimates of DEPM parameters. Our laboratory analyses showed that using underway CUFES samples to determine which sites should be sampled with a CalVET net would have reduced the number of sites sampled by 19.0-36.5%, whilst only reducing the number of positive sites by 3.4-10.0%. However, using data collected while onboard the vessel would have reduced the number of sites sampled by 32.2-46.2% and, more importantly, reduced the number of positive samples by 3.6-37.0%, with the largest reductions occurring during the second cruise when the weather was poor.

The effects of onboard errors in estimating presence/absence of eggs on estimates of spawning area were similar to the effects on the number of positive sites. Based on the laboratory data, using underway CUFES samples to determine which CalVET net samples to take would have reduced the estimate of spawning area by less than 11.0%. Based on the onboard counts the reductions in the estimates of spawning area were similar to those from laboratory data for the first cruise (i.e. 3.7 and 11.3% at low and high sampling intensities, respectively), but much higher during the second cruise (i.e. 23.6 and 36.2%, respectively). This finding shows that relatively low rates of error in onboard counts (i.e. comparable to those of Lo *et al.,* [10]) can have significant effects on estimates of spawning area, which is a critical determinant of spawning biomass [19]. In contrast, halving the number of predetermined sites sampled had a much

lower effect on the estimate of spawning area (7.4 and 6.4% increases for Cruises 1 and 2, respectively).

Based on laboratory analyses, the use of underway CUFES data to determine which CalVET samples would have been taken resulted in estimates of mean daily egg production that were similar to or slightly (<11%) higher than those estimated from predetermined sites. However, based on onboard examinations estimates of mean daily egg production were 9.1% to 96.9% higher than those based on predetermined sites. This is partly because onboard counts were more likely to identify a site as not containing eggs when egg counts were low, which biased the estimate of egg production upwards.

The finding that estimates of total egg production obtained using predetermined CalVET net samples at low sampling intensities were 18-19% higher than those obtained at high sampling intensities in both cruises has implications for DEPM studies that use traditional (CalVET net) approaches. Specifically, it re-emphasises the benefits of collecting large numbers of samples, which represent relatively small grids, to estimate mean daily egg production and spawning area. In contrast, based on laboratory analyses, estimates of egg production from CUFES-determined sites were less than 10% different from those obtained from pre-determined sites, which shows the potential value of information obtained using CUFES for DEPM studies. However, based on the onboard analyses, the differences in the estimates of total daily egg production ranged from 5.1% to 50.5%, which shows that errors in onboard analyses of CUFES samples can have major effects on estimates of spawning biomass.

Accuracy, Precision and Costs of Estimates of Total Daily Egg Production

The potential value of using CUFES data to enhance quantification of the distribution and abundance of sardine eggs is emphasized by the finding that estimates of total egg production based on laboratory analyses are more accurate than estimates obtained from reduced sampling intensity of pre-determined sites. In contrast, estimates based on onboard analyses of CUFES samples produced the least accurate estimates of total daily egg production calculated in this study. Similarly, the precision of estimates based on laboratory analyses of CUFES determined samples were similar to those based on predetermined sites, whereas estimates based on onboard examinations were generally less precise.

Most previous accounts of the use of CUFES in DEPM studied have focused on the potential benefits of using this technology to reduce field and laboratory costs. Our studies suggest that when the time taken to collect a CalVET net sample is small (e.g. 5 min), the reduction in field time resulting from this approach is minimal (less than 4% of total cruise time), due to extended time taken to travel along transects between sites (e.g. 30 min) compared to the relatively short time (5 min) required to stop and take a sample. It should be noted, however, that this effect on total survey costs would be much different in situations where CalVET net samples are collected as part of broader ecological sampling programs and the time on-site is extended due to the need to collect additional data. For example, the benefits of using a CUFES to determine when samples should be taken

would be much greater during CalCOFI cruises where the total sampling regime at each site takes approximately 3 hours to complete (Dave Griffiths, pers. comm.).

The small reduction in costs and significant loss in accuracy associated with the onboard analysis of samples suggests that in dedicated DEPM surveys where the primary objective is to obtain robust estimates of DEPM parameters, using underway CUFES samples to determine when/where CalVET net samples should be taken may not be the most beneficial use of this technology when dedicated surveys are conducted from small research vessels (an time spent at each sampling site is low). This is because the relatively small cost reductions that accrue from this approach (e.g. less than 5% of total cruise costs) may not offset the potentially significant effects of the resulting reductions in the accuracy of estimates of total daily egg production.

The other potential cost saving that may result from using underway CUFES samples to determine where CalVET net samples should be taken is in laboratory (sorting) costs. Our analyses show that using the CUFES in the standard way has the potential to reduce laboratory time/costs by approximately 41.3% (52 h – 30 h 30 min= 21 h 30 min h or $614A) at high sampling intensity. The benefits of this 3.0% saving in total must be weighed against the 20+% reduction in the accuracy of the estimates of total daily egg production associated with use of onboard analysis of CUFES samples to determine when/where CalVET net samples should be taken. The relative cost of vessel and laboratory time is also significant factor. The marginal cost of one day at sea aboard the research vessel is approximately $5,000A whereas one day in the laboratory costs approximately $200. Hence, reducing laboratory costs by even 50% would have had a minimal effect on the total cost of our study. We suggest that using a CUFES to reduce the laboratory and field costs of DEPM surveys may not the best way to use this technology, in at least some situations (i.e. dedicated surveys from small research vessels when time spent at each sample site is short, i.e. less than 10 minutes).

Maximising Benefits of Using a CUFES in DEPM Surveys

Despite the apparent limitations of using a CUFES to determine where/when CalVET samples should be taken, the correlations between the estimates of egg density obtained in CalVET net, on-site CUFES and underway CUFES samples suggests that there is significant potential for using CUFES to enhance the quantification of egg abundance. For example, as on-site CUFES collect fewer samples with very large numbers of eggs than CalVET nets, onsite CUFES data may be useful for addressing the problem of biases in estimates of mean daily egg production that arise through the undue influence on analyses of samples contained large numbers of early stage eggs. Conversely, due to difficulties associated with staging egg collected in CUFES data from CalVET nets will continue to be required in DEPM studies to provide a basis for estimating rates of egg mortality.

A key weakness of DEPM studies based solely on CalVET net samples is that samples from a small area (0.14 m^2) are used to quantify egg abundance over a large area (tens of km^2). As underway CUFES samples are collected

over a large horizontal area (i.e. the distance between sites), these data have the potential to significantly improve the reliability of estimates of mean daily egg production obtained for each sampling unit [20]. Data from on-site CUFES may be useful for quantifying spatial and temporal variations in the effectiveness of CUFES in estimating egg density whilst the vessel is underway.

CONCLUSIONS

The DEPM is acknowledged to be an imprecise method [3]. Much of this imprecision arises from difficulties associated with estimating total daily egg production. Using data from CUFES to reduce the number of samples taken using CalVET nets does not appear to be the optimal way to use this technology in situations where dedicated surveys are undertaken from small research vessels and the time spent at each sampling station is short. This is mainly because of the difficulties associated with sorting plankton samples at sea, but also because the reduction in costs does not appear to outweigh the effects on the accuracy and precision of estimates.

Critics of the DEPM rightly point out that samples from very small areas are used characterise the abundance of eggs over very large areas. The CUFES provides a potentially important tool for increasing the reliability of estimates of egg production by increasing the area from which information on egg abundance is collected. The impacts of this approach on the total costs of DEPM surveys would be relatively low because taking CUFES samples between and on each CalVET net site, as we did in this study, does not add significantly to the field costs. Furthermore, the cost of a day in the laboratory is much lower than the cost of boat day, so the additional sorting time also has a relatively small impact on total costs. Data from CUFES samples may also be useful for addressing the problem of over-dispersal of data which complicates estimation of mean daily egg production calculated from samples obtained using CalVET nets only.

Our results suggest that suggest that CUFES samples provide valuable information on the distribution and abundance of eggs that could potentially be used in conjunction with data from CalVET nets to calculate accurate and precise estimates of total egg production. To do this effectively, sophisticated analytical methods (analogous to integrated fisheries models) will need to be developed to integrate data obtained using CalVET nets and CUFES (both on-site and underway). Curtis *et al.,* [20] showed that vertical egg models that incorporate data from CUFES have significant potential to increase the precision of stock assessments obtained using the DEPM. We are currently working to develop methods to integrate data from CalVET nets and CUFES to estimate total daily egg production in future DEPM studies of sardine in waters off South Australia.

REFERENCES

[1] Parker K. A direct method for estimating northern anchovy, *Engraulis mordax*, spawning biomass. Fish B-NOAA 1980; 84: 541-4.

[2] Lasker R. An egg production method for estimating spawning biomass of pelagic fish: Application to the Northern Anchovy, *Engraulis mordax*. NOAA; 1985: 1-99.

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

[4] Lo NCH, Hunter JR, Moser HG, Smith PE, Methot RD. The daily fecundity reduction method: a new procedure for estimating adult fish biomass. ICES J Mar Sci 1992; 49: 209-15.

[5] Alheit J. Use of the Daily Egg-Production Method for Estimating Biomass of Clupeoid Fishes - a Review and Evaluation. B Mar Sci 1993; 53(2): 750-67.

[6] Hunter JR, Lo NCH. Ichthyoplankton methods for estimating fish biomass: introduction and terminology. B Mar Sci 1993; 53: 723-7.

[7] Hunter JR, Lo NCH. The daily egg production method of biomass estimation: Some problems and potential improvements. Ozeanografika 1997; 2: 41-69

[8] Checkley DM, Ortner PB, Settle LR, Cummings SR. A continuous, underway fish egg sampler. Fish Oceanogra 1997; 6(2): 58-73.

[9] van der Lingen CD, Checkley JD, Barange M, Hutchings L, Osgood K. 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. Fish Oceanogra 1998; 7(1): 35-47.

[10] Lo NCH, Hunter JR. Charter R. Use of a continuous egg sampler for ichthyoplankton surveys: application to the estimation of daily egg production of Pacific sardine (*Sardinops sagax*) off California. Fish B-NOAA 2001; 99(4): 554-71.

[11] Pepin P, Snelgrove PVR, Carter KP. Accuracy and precision of the continuous underway fish egg sampler (CUFES) and bongo nets: a comparison of three species of temperate fish. Fish Oceanogra 2005; 14(6): 432-47.

[12] ICES The DEPM estimation of spawning-stock biomass for sardine and anchovy. ICES Coop Res Rep 2004; 268: 91.

[13] Checkley DM, Dotson RC, Griffith DA. Continuous, underway sampling of eggs of Pacific sardine (*Sardinops sagax*) and northern anchovy (*Engraulis mordax*) in spring 1996 and 1997 off southern and central California. Deep-Sea Res Pt II 2000; 47(5-6): 1139-55.

[14] Ward TM, Ferguson G, Rogers PJ. Australian Sardine (*Sardinops sagax*) Fishery. SARDI Aquat Sci 2008; F2007/000765-2. pp. 74.

[15] Ward TM, Hoedt F, McLeay L, Dimmlich WF, Kinloch M, Jackson G, McGarvey R, Rogers PJ, Jones K. Effects of the 1995 and 1998 mass mortality events on the spawning biomass of *Sardinops sagax* in South Australian waters. ICES J Mar Sci 2001; 58(4): 830-41.

[16] White KV, Fletcher WJ. Identifying the developmental stages for eggs of the Australian pilchard, *Sardinops sagax*. Fisheries Western Australia. 1996; Fish Res Rep No.103. pp. 21.

[17] Picquelle S, Stauffer G. Parameter estimation for an egg production method of anchovy biomass assessment. National Marine Fisheries Services. NOAA Tech Rep 1985; 7-17.

[18] Efron B, Tibshirani RJ. An Introduction to the Bootstrap. New York, Chapman and Hall Inc 1993.

[19] Gaughan DJ, Leary TI, Mitchell RW, Wright IW. A sudden collapse in distribution of Pacific sardine (*Sardinops sagax*) off southwestern Australia enables an objective re-assessment of biomass estimates. Fish B-NOAA 2004; 102(4): 617-33.

[20] Curtis KA, Checkley Jnr, DM, Pepin P. Predicting the vertical profiles of anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*) eggs in the California Current System. Fish Oceanogra 2007; 16(1): 68-84