Validating a new sampling technique for estimating egg production

Ward, T.M., McGarvey, R., Ivey, A., and Grammer, G.L.

March 2020

FRDC Project No 2017-027

ISBN 978-1-876007-25-6

Validating a new sampling technique for estimating egg production

FRDC Project No 2017-027

2020

This publication (and any information sourced from it) should be attributed to **Ward, T.M., McGarvey, R., Ivey, A., and Grammer, G.L., South Australian Research and Development institute (SARDI), 2019, *Validating a new sampling technique for estimating egg production*, Adelaide, March.**

Disclaimer

The authors warrant that they have taken all reasonable care in producing this report. The report has been through the SARDI internal review process, and has been formally approved for release by the Research Director, Aquatic Sciences. Although all reasonable efforts have been made to ensure quality, SARDI does not warrant that the information in this report is free from errors or omissions. SARDI and its employees do not warrant or make any representation regarding the use, or results of the use, of the information contained herein as regards to its correctness, accuracy, reliability and currency or otherwise. SARDI and its employees expressly disclaim all liability or responsibility to any person using the information or advice. Use of the information and data contained in this report is at the user's sole risk. If users rely on the information they are responsible for ensuring by independent verification its accuracy, currency or completeness.

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a readers particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the FRDC.

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

Researcher Contact Details

Name: A/Prof Tim Ward
Address: South Australian Research and Development Institute
2 Hamra Avenue, West Beach, 5024
Phone: 08 8429 2192
Email: tim.ward@sa.gov.au

FRDC Contact Details

Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6285 0400
Fax: 02 6285 0499
Email: frdc@frdc.com.au
Web: www.frdc.com.au

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

Tables

Figures

Acknowledgments

The Fisheries Research and Development Corporation (FRDC) funded this project (FRDC Project No: 2017/027). SARDI also contributed to the salaries of Ward, McGarvey and Grammer. We thank the master and crew of the RV *Ngerin* for their professional assistance during surveys. We also acknowledge the efforts of SARDI staff involved in the field surveys and laboratory processing of samples. Drs Paul Rogers, Jonathan Smart and Stephen Mayfield (SARDI Aquatic Sciences) provided comments on a draft of the report. The final report was approved for publication by Dr Michael Steer (SARDI Aquatic Sciences).

Abbreviations

AFMA	Australian Fisheries Management Authority
CalVET	Californian Vertical Egg Tow net
CTD	Conductivity Temperature Depth
CUFES	Continuous Underway Fish Egg Sampler
CV	Coefficient of Variation
DEPM	Daily Egg Production Method
FRDC	Fisheries Research and Development Corporation
GAM	Generalised Additive Models
GLM	Generalised Linear Models
GVP	Gross Value of Production
P_0	Mean daily egg production
PIRSA	Primary Industries and Regions South Australia
SASF	South Australian Sardine Fishery
SARDI	South Australian Research and Development Institute
SPF	Commonwealth Small Pelagic Fishery
TAC	Total Allowable Catch
z	Egg mortality

Executive Summary

Background and Need

Estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator in the South Australian Sardine Fishery (SASF) and Commonwealth Small Pelagic Fishery (SPF). The DEPM is also being used to assess the status of other commercially-important species, e.g. Snapper in South Australia.

Estimates of mean daily egg production (P_0) are a critical element of the DEPM. Simulation modelling conducted as part of FRDC Project No. 2014/026 suggested that the precision of estimates of P_0 may be increased if the size and shape of the sampling unit (currently a narrow vertical cylinder) could be matched more closely to the likely size and distribution of egg patches (e.g. by conducting longer oblique tows).

Objective

The objective of this study was to improve the accuracy and precision of estimates of spawning biomass obtained using the DEPM. To address this objective, we evaluated the potential benefits of using a high speed oblique sampler (Nackthai net) instead of the traditional vertically-towed CalVET and Bongo nets to measure egg density.

Methods

Field experiments were undertaken from the *RV Ngerin* in southern Spencer Gulf during 21-27 February 2018. A Continuous Underway Fish Egg Sampler (CUFES) was used to locate areas where eggs of Australian Sardine (*Sardinops sagax*) were present in moderate to high densities. A marker buoy that consisted of a drogue positioned at a depth of ~20 m and a floating pole with radar reflector, flag and light was deployed to mark the selected water body. The buoy was designed to ensure that repeated sampling was conducted in the same patch of eggs. Each hour, samples were taken near the marker buoy using three samplers (i.e. Nackthai, CalVET and Bongo). The precision of estimates of mean egg density and P_0 obtained using the Nackthai and traditional methods were compared.

Results

Estimates of egg density and P_0 derived from the Nackthai were lower and less precise than those obtained from the CalVET or Bongo. Due to its large size and cumbersome design (e.g. 22 kg depressor), the Nackthai was more difficult to deploy in rough weather and required more staff than the other samplers. Samples obtained from the Nackthai also contained more eggs and took longer to process in the laboratory than those from the other nets. There was no evidence to suggest that replacing the traditional samplers with the Nackthai would enhance application of the DEPM to Australian Sardine or other small pelagic fishes.

Implications for stakeholders

Because of its logistical advantages (easy to deploy, relatively small samples easily sorted in the laboratory), the CalVET should continue to be used as the primary tool for measuring egg density of Australian Sardine. Using a Bongo, which filters more water and captures more eggs than the CalVET may be warranted for species with lower egg densities, such as Jack Mackerel (*Trachurus declivis)*. Other opportunities for improving the precision of estimates of spawning biomass obtained using the DEPM should continue to be explored.

Keywords

Egg density, mean daily egg production, egg mortality, Australian Sardine, *Sardinops sagax*, South Australian Sardine Fishery, Small Pelagic Fishery

Introduction

Background

Estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator in Australia's largest fishery, the South Australian Sardine Fishery (SASF), as well as the Commonwealth Small Pelagic Fishery (SPF). The DEPM is also being used increasingly to support the assessment and management of other commercially-important species, e.g. Snapper and King George Whiting in South Australia.

The main reason for the increased application of the DEPM is the growing recognition that fishery-dependent assessments of many species need to be augmented by fishery-independent methods. Numerous studies have highlighted the high levels of uncertainty associated with estimates of spawning biomass obtained using the DEPM. It is widely recognised that uncertainty in estimates of mean daily egg production (P_0) is one of the key drivers of uncertainty in estimates of spawning biomass (e.g. Stratoudakis et al. 2006, Bernal et al. 2012, Dickey-Collas et al. 2012, Ward et al. 2018).

Simulation modelling conducted in a recent study funded by FRDC (Project No. 2014/026) highlighted the benefits of improving the match between the size and shape of the sampling unit (currently a narrow vertical cylinder obtained) with the size and distribution of egg patches. As a result of that study, SARDI purchased a high speed sampler (Gulf 7 or Nackthai net) that can towed obliquely behind the research vessel and collects samples over a longer distance and from a larger volume of water than the traditional vertical tows.

Need

Accurate and precise estimates of spawning biomass are needed to underpin sustainable management of Australia's largest fishery, the SASF. Estimates of P_0 that underpin application of the DEPM need to be as accurate and precise as possible.

There was a need to evaluate the potential for an alternative sampling technique to provide more precise estimates of mean egg density and P_0 than the traditional methods. The benefits/costs of using the new sampler needed to be assessed to evaluate its potential use future assessment of the SASF.

Objective

The objective of this study was to improve the accuracy and precision of estimates of spawning biomass obtained using the DEPM.

To address this objective, we evaluated the potential benefits of using a high speed oblique sampler (Gulf 7 or Nackthai net) instead of the traditional vertically-towed CalVET or Bongo nets to measure egg density and estimate P_0.

The study involved a) sea trials to establish logistical procedures for using the new sampler; and b) experimental application off South Australia.

Methods

Fieldwork

Field experiments were undertaken from the *RV Ngerin* in southern Spencer Gulf during 21-27 February 2018. A Continuous Underway Fish Egg Sampler (CUFES) was used to locate an area where eggs of Australian Sardine (*Sardinops sagax*) were present in moderate to high densities. A marker buoy that consisted of a drogue positioned at a depth of ~15 m and a floating pole with radar reflector, flag and light was deployed to mark the selected water body (Figure 1). This buoy was designed to ensure that repeated sampling was conducted in the same patch of eggs. Each hour, samples were taken near the marker buoy using the Nackthai (Figure 2) and the combination Bongo/CalVET net (Figure 3).

Table 1. Properties and dimensions of each sampler.

Net type	Tow orientation	Opening diameter (m)	Opening area (m^2)	Net length (m)	Mesh size (µm)
Paired CalVET net	Vertical	0.3 m	0.07069 m^2	1.8 m	333 µm
Paired Bongo net	Vertical	0.6 m	0.28323 m^2	3.0 m	500 µm
Nackthai	Oblique	0.2 m	0.03142 m^2	1.5 m	300 µm

Figure 1. The drogue and buoy used to follow patches of eggs.

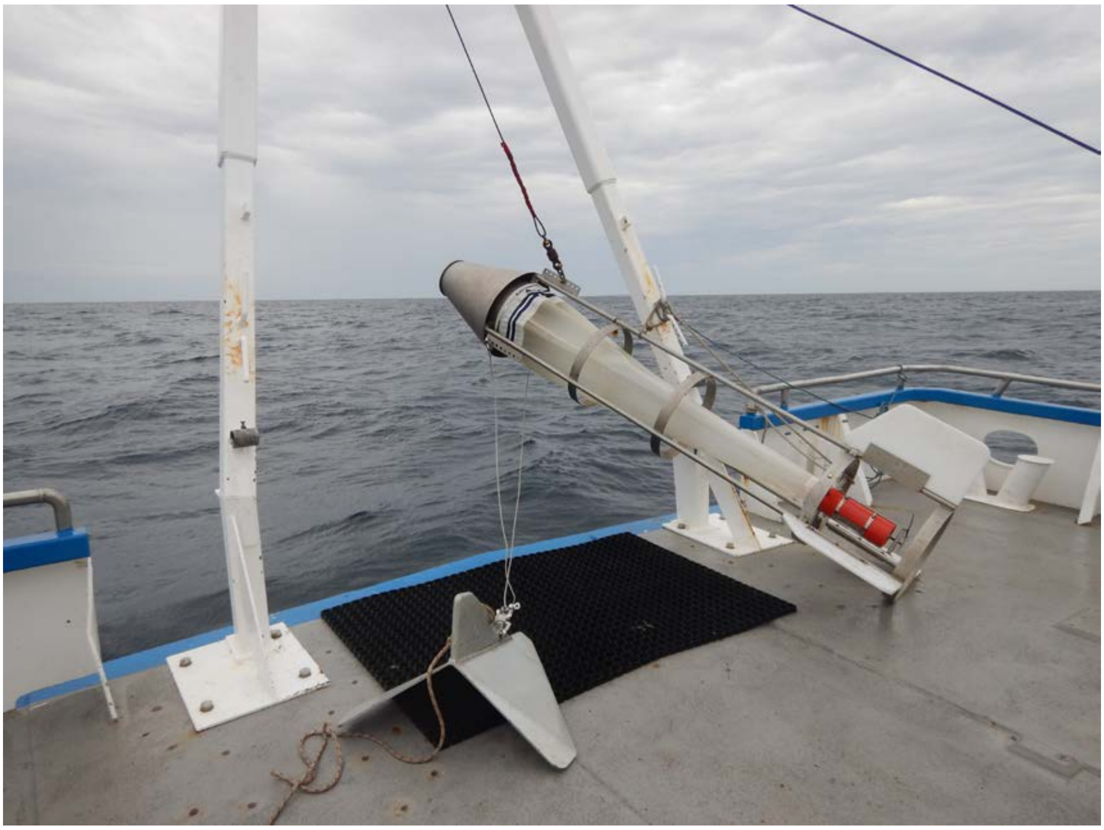

Figure 2. The Nakthai net used to conduct oblique tows.

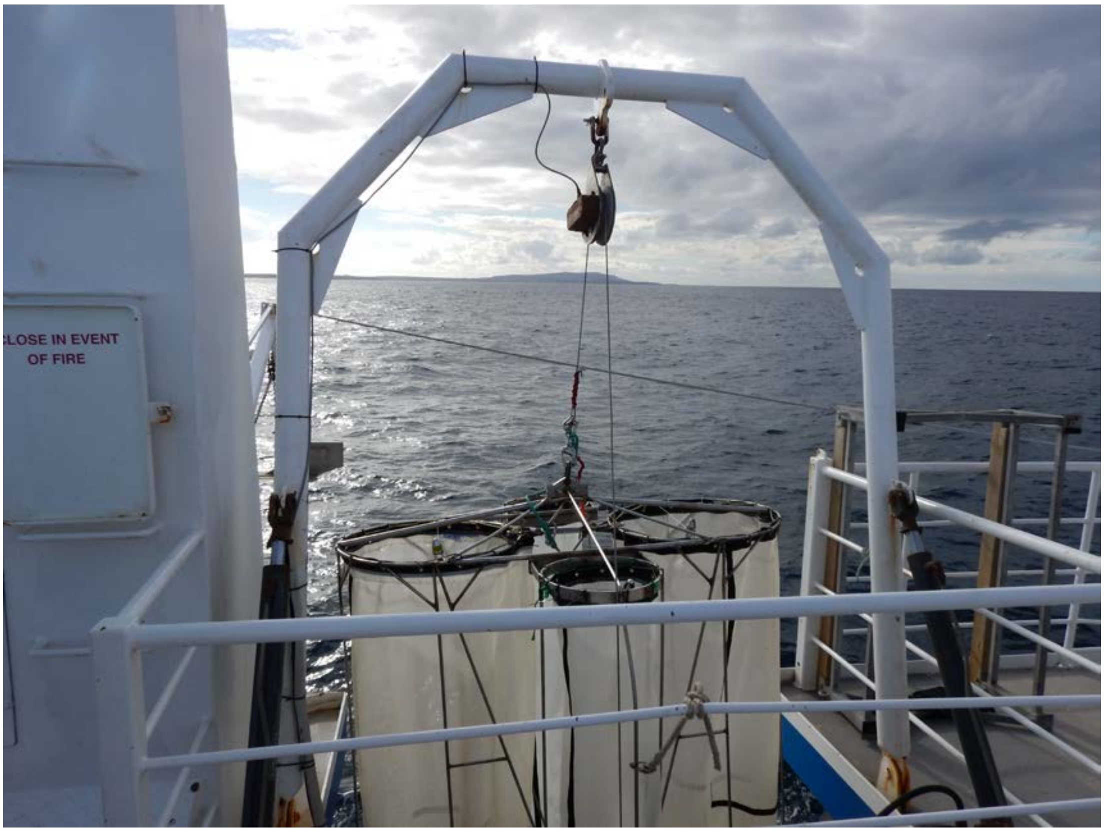

Figure 3. The combination Bongo/CalVET net used to conduct the vertical tows.

Vertical tows were deployed to five metres from the bottom, typically 50–60 m, and retrieved at 1 $m.s^{-1}$. The Nakthai was deployed to either 40 or 50 m depending on the depth. One-minute oblique tows were then conducted at ~ten metre depth intervals based on a 2:1 ratio of wire length to depth, with the boat travelling at 5 knots away from the drogue. Tows were typically ~10-15 minutes in duration and 1,500-3,000 m in length. Figure 4 shows the depth and temperature profiles of a tow with a target depth of 50 m. Once the tow was completed the vessel returned to the drogue to repeat the sampling procedure on the hour. All samples were preserved in 5% formaldehyde and sea water solution.

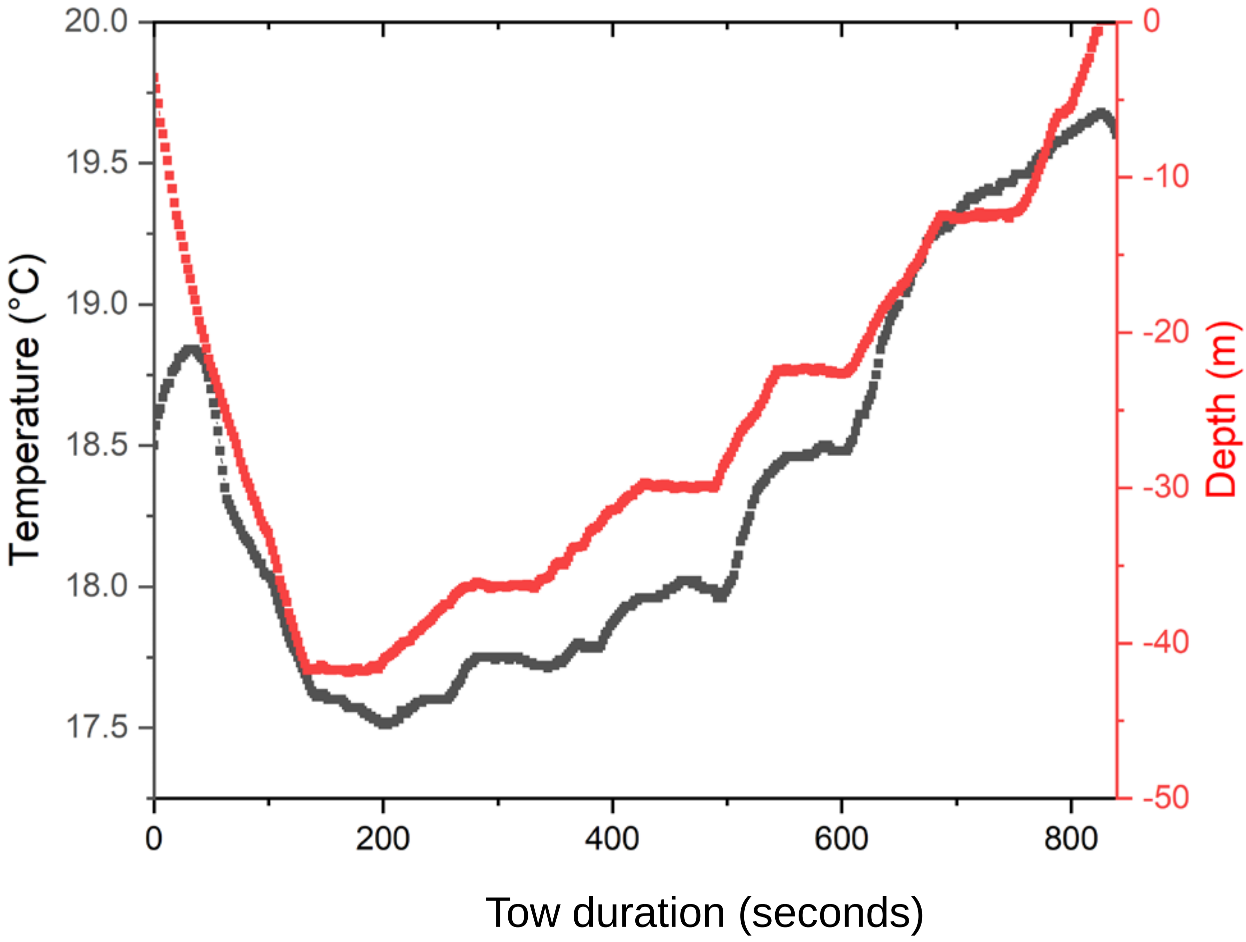

Figure 4. Depth and temperature profile of a typical Nackthai tow.

Three drifts comprising a total of 124 samples were undertaken with each sampling gear (Figure 5, Table 2). Drift 1 started on an area with high abundance of Australian Sardine eggs but was interrupted by bad weather. Drift 2 commenced where Drift 1 finished and the abundance of Australian Sardine eggs continued to be high. Drift 2 was concluded when a full cohort of egg development had been sampled. Drift 3 was undertaken further north in the Gulf in a location with lower egg abundance.

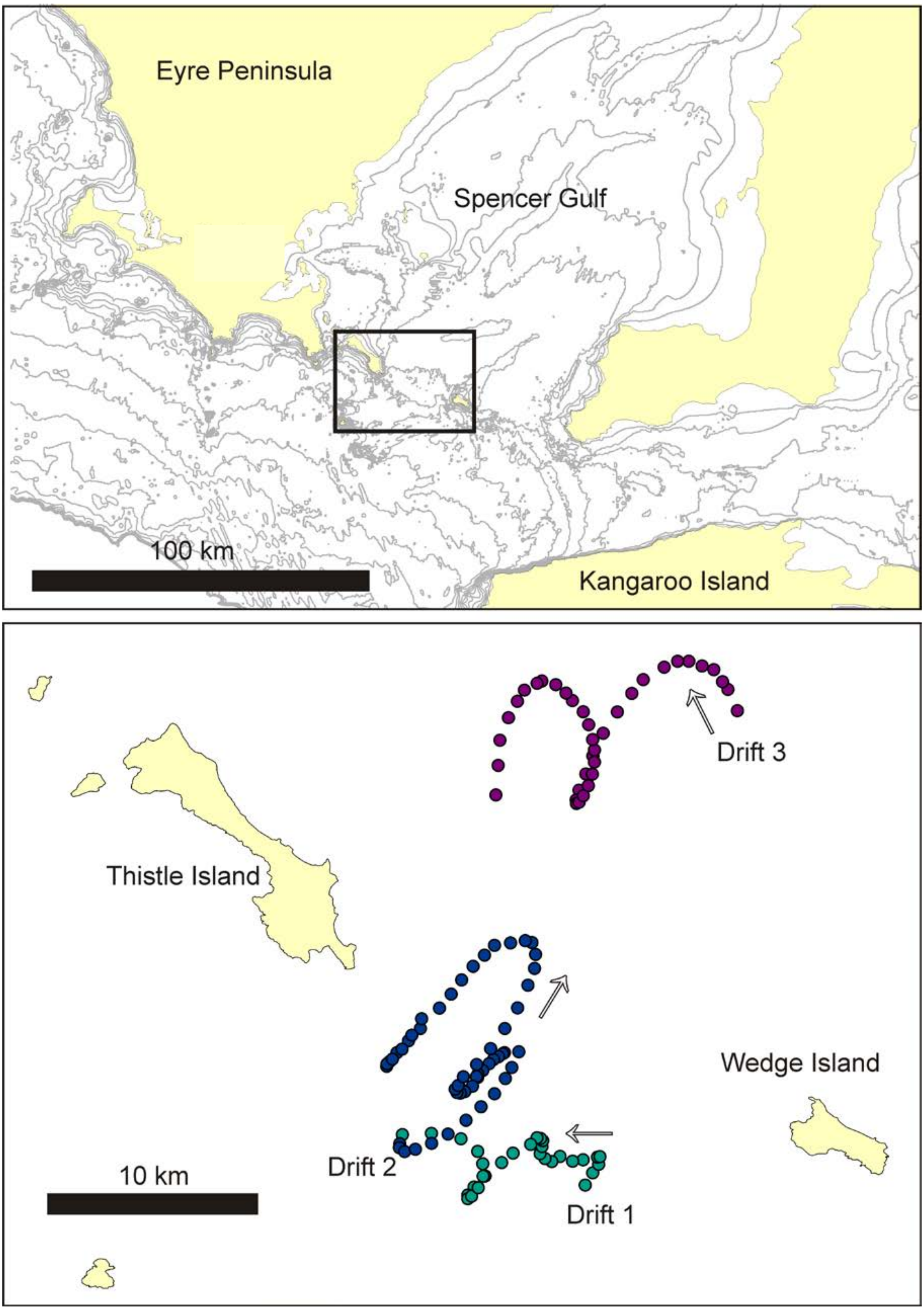

Figure 5. Location of the three sampling drifts in southern Spencer Gulf.

Table 2. Sample summary of the three drifts conducted.

Drift	Start	End	Samples
1	21/02/2018 16:03	22/02/2018 21:48	31
2	23/02/2018 9:30	25/02/2018 16:45	56
3	25/02/2018 18:54	27/02/2018 8:04	38

Egg identification

Australian Sardine eggs were identified, staged and counted according to White and Fletcher (1998) and converted to the 10 stages described in Ward et al. (2018).

Egg ageing and treatment of zero count egg samples

Based on temperature data collected using the CTD, egg samples were allocated to a temperature band that covered the range of temperatures sampled during the survey (18–22°C). Published egg development rates reported for Sardine by Lo et al. (1996) were used to assign a mean age to each egg (Ward et al. 2018).

As samples often included eggs spawned on more than one night, eggs in each sample were aggregated into daily cohorts. The total egg count and average age for each daily cohort were calculated by assigning each live egg stage to a day of spawning (e.g. day 0, day 1, etc.) and summing the number of eggs. The age assigned to each cohort was the weighted average of the number of eggs observed in each stage.

Samples with eggs could contain several possible combinations of daily cohorts depending on the ambient water temperature and sampling time: (i) eggs of age <1 day (most recent cohort) and no eggs from older cohorts; (ii) no eggs of age <1 day and some eggs from older cohorts; or (iii) eggs of age <1 day and eggs from older cohorts. Since spawning occurs each night, zero counts were allocated for daily cohorts where the cohort was expected to occur in the sample, but was not present.

Egg density (P_s and P_t)

The density of eggs under one square metre was estimated for each sample (P_s) and each daily cohort (P_t) (Equation 2, Table 3). The volume of water filtered by each sampler was calculated using flowmeters positioned in the mouth of each sampler, multiplied by the area of the mouth of the net (Table 1). The same depth value from the vertical tows was used to convert the Nackthai volume densities to avoid bias. Paired t-tests were used to examine differences between estimates of egg density obtained from the different samplers.

Table 3. Equations used to estimate egg density, mean daily egg production (P_0) and instantaneous egg mortality rate (z) for Sardine.

Model Name	Equation	Eq. No.	Parameters	Reference
Egg Density (sample)	$P_s = \frac{C\,D}{V}$	(2)	P_s: density of eggs in a sample C: number of eggs of each age in each sample V: volume of water filtered (m^3) D: depth (m) of net cast	Smith and Richardson (1977)
Exponential egg mortality model (P_0)	$P_t = P_0 e^{-z\,t}$	(3a)	P_t: egg density at age t z: the instantaneous rate of daily egg mortality	Lasker (1985)
Non-linear Least Squares regression	$nls(P_t \sim P_0 e^{-z\,t})$	(3b)	P_t: egg density at age t z: the instantaneous rate of daily egg mortality	
Log-Linear				
Negatively biased estimate (P_b)	$\ln P_{i,t} = \ln P_b - z\,t$	(4a)	P_b: negatively biased P_0 $P_{i,t}$: density of eggs of age t at site i z: instantaneous rate of daily egg mortality	Picquelle and Stauffer (1985)
Bias corrected (P_0)	$P_0 = e^{\ln P_b + \sigma^2/2}$	(4b)	P_b: negatively biased estimate of daily egg production σ^2: variance of P_b estimate	
Generalised Linear Models (GLMs) with error structures of: negative binomial, quasi, and quasi-Poisson	$E[P_0] = g^{-1}(-zt + \varepsilon)$	(5)	E[P_0]: expected value of P_0 g^{-1}: inverse-link function zt: the instantaneous rate of daily egg mortality at age t ε: error term	Wood (2006), Ward et al. (2011, 2018a)

Daily egg production (P_0) and egg mortality (z)

Mean daily egg production (P_0) is the mean density of eggs produced per day per unit area (eggs·m^{-2}·day^{-1}) in the spawning area. It is well known that P_0 and instantaneous egg mortality rate (z) are difficult to estimate precisely (e.g. Stratoudakis et al. 2006, Bernal et al. 2012, Dickey-Collas et al. 2012, Ward et al. 2018).

In the present study, P_0 and z were estimated from the egg densities and average ages of daily cohorts. The underlying model used to estimate P_0 and z was the exponential egg mortality model (Equation 3a, Table 2). The model was applied in several ways. Non-linear least squares regression was used to fit Equation 3a and establish Equation 3b (Table 2). A linear version of the exponential egg mortality model (the 'log-linear model', Equation 4a) with a bias correction factor (Equation 4b, Table 2) was also used (Picquelle and Stauffer 1985). Data were fitted using four generalised linear models (GLMs, Equation 5, Table 3) with three different error structures: negative binomial, quasi and quasi-poisson. Instantaneous egg mortality rate was estimated as a free parameter in each of the models (Table 3). The reliability of model fits and confidence intervals for estimates of P_0 were assessed using bootstrap resampling methods. All analyses were done in the R programming environment (R Core Team, 2019).

Results

Egg density

A total of 104,007 Australian Sardine eggs were collected using the three sampling gears during the three drifts. The Nackthai consistently collected more eggs than the Bongo, which collected more than the CalVET (Table 4). Egg counts for each sample were converted to egg densities (eggs per square metre, Equation 2, Table 2, Figure 6).

Table 4. Counts of Australian Sardine eggs by sampler and drift.

Sampler	Total	Drift 1	Drift 3	Drift 2
Bongo	34,819	8,584	3,731	22,504
CalVET	10,107	2,484	1,084	6,539
Nackthai	59,081	16,144	5,037	37,900

Egg densities estimated from the Nackthai were usually lower than those from the CalVET or Bongo (Figure 6). Mean egg densities obtained from the Bongo were usually lower than the CalVET; mean egg densities from the Nackthai were much lower than those from both the vertical samplers (Table 5, Figure 7). For all samplers the mean was higher than the median; this difference was greater for the Nackthai than the vertical samplers (Figure 7, Table 5). The CVs of the estimates of mean egg density from the Bongo and CalVET were similar; the CV for the Nackthai was higher than for the vertical samplers.

Table 5. Mean egg densities and statistics for the three sampler types. (SD = Standard Deviation; CV = Coefficient of Variation; SE = Standard Error; SECV = SE/Mean)

	Bongo egg density (m^2)	CalVET egg density (m^2)	Nackthai egg density (m^2)
Mean	540	627	372
Median (% of mean)	466 (86)	541 (86)	226 (61)
SD	372	437	320
CV	0.688	0.696	0.861
SE	33.5	39.4	28.9
SECV	0.0621	0.0628	0.0776

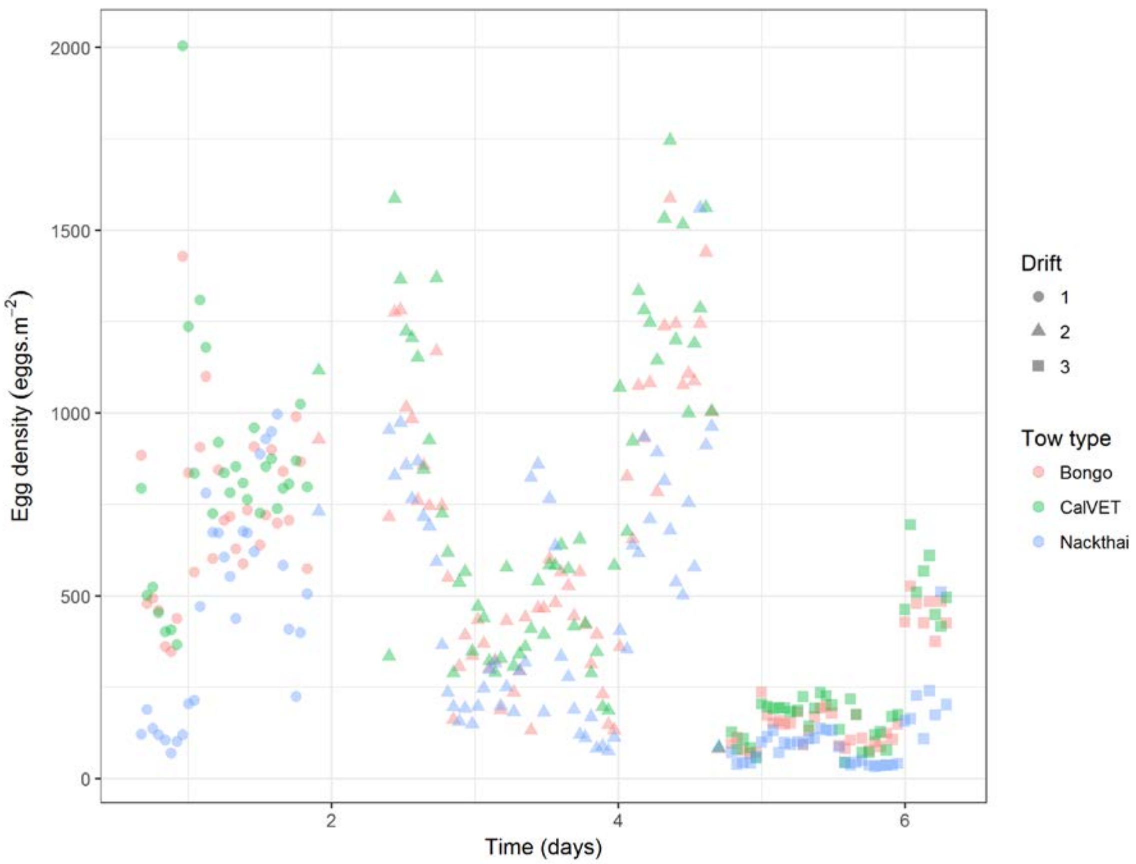

Figure 6. Australian Sardine egg densities by sampler and drift.

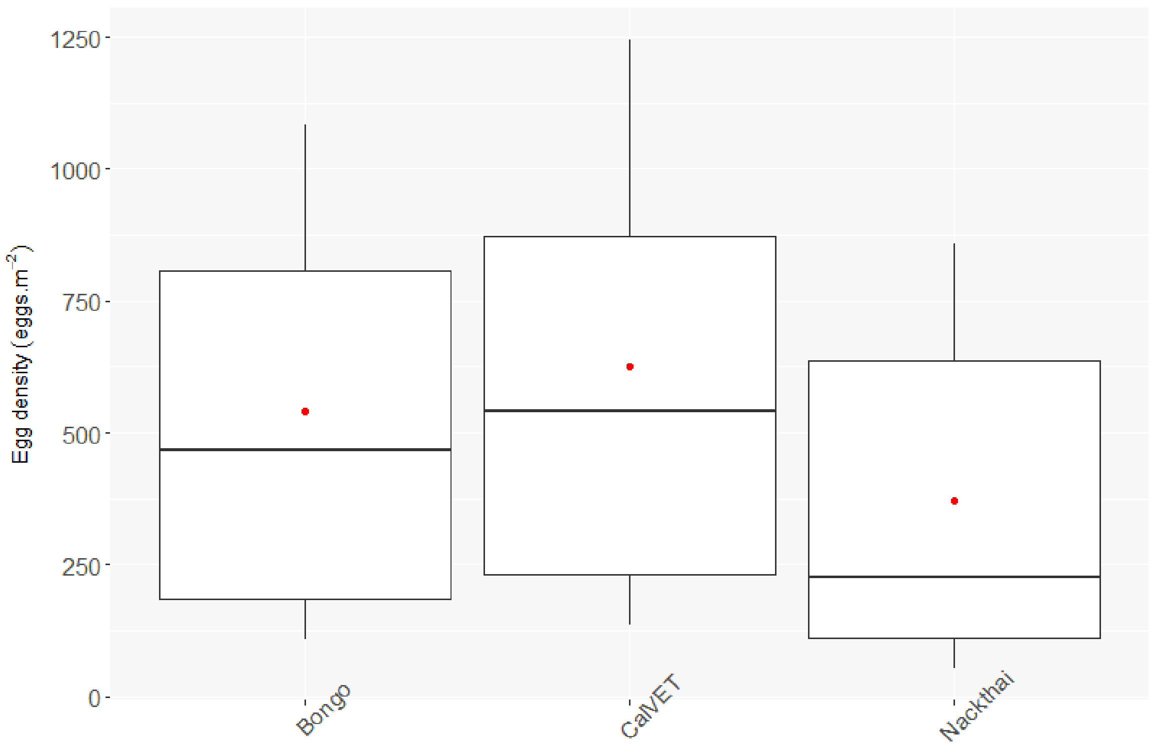

Figure 7. Box plots for all density measurements from the three sampler types. Mean is red dot. Median is the black line.

Paired t-tests were used to detect differences in the means estimated by the three samplers; the Bongo was significantly lower than the CalVET ($t = 6.57$, $df = 122$, p-value < 0.0001) and the Nackthai was significantly lower than the Bongo ($t = 7.22$, $df = 122$, p-value < 0.0001).

The same pattern of mean egg densities was evident when the data were separated by drift (Table 6), with the CalVET measuring a higher mean egg density than the Bongo and the Bongo higher than the Nackthai. These differences were found to be significant by paired t-tests ($p<0.001$) in every comparison (Table 7).

Frequency plots of egg density by drift (Figure 8) show that the oblique sampler produced more low density measures than the two CalVET and Bongo.

Table 6. Mean egg densities and statistics for the three sampler types, separated by drift. (SD = Standard Deviation; CV = Coefficient of Variation; SE = Standard Error; SECV = SE/Mean)

	Drift 1			Drift 2			Drift 3		
	Bongo	CalVET	Nackthai	Bongo	CalVET	Nackthai	Bongo	CalVET	Nackthai
Mean	724	834	464	666	777	498	201	233	107
Median (% of mean)	708 (98)	807 (97)	471 (102)	566 (85)	618 (80)	406 (82)	152 (76)	192 (82)	95 (89)
SD	234	323	293	389	444	330	142	169	88.5
CV	0.323	0.388	0.632	0.584	0.571	0.664	0.705	0.724	0.825
SE	43.4	60	54.4	51.5	58.8	43.7	23.3	27.8	14.5
SECV	0.060	0.072	0.117	0.077	0.076	0.088	0.116	0.119	0.136

Table 7. Paired t-test results comparing data collected by the three samplers over the three separate drifts.

Drift 1	**Drift 2**	**Drift 3**
CalVET versus Bongo	CalVET versus Bongo	CalVET versus Bongo
t = 3.80, df = 28, p-value < 0.001	t = 4.82, df = 56, p-value < 0.001	t = -3.68, df = 36, p-value < 0.001
CalVET versus Nackthai	CalVET versus Nackthai	CalVET versus Nackthai
t = 4.88, df = 28, p-value < 0.001	t = 6.60, df = 56, p-value < 0.001	t = 5.9, df = 36, p-value < 0.001
Nackthai versus Bongo.	Nackthai versus Bongo.	Nackthai versus Bongo.
t = 4.21, df = 28, p-value < 0.001	t = 4.63, df = 56, p-value < 0.001	t = 6.12, df = 36, p-value < 0.001

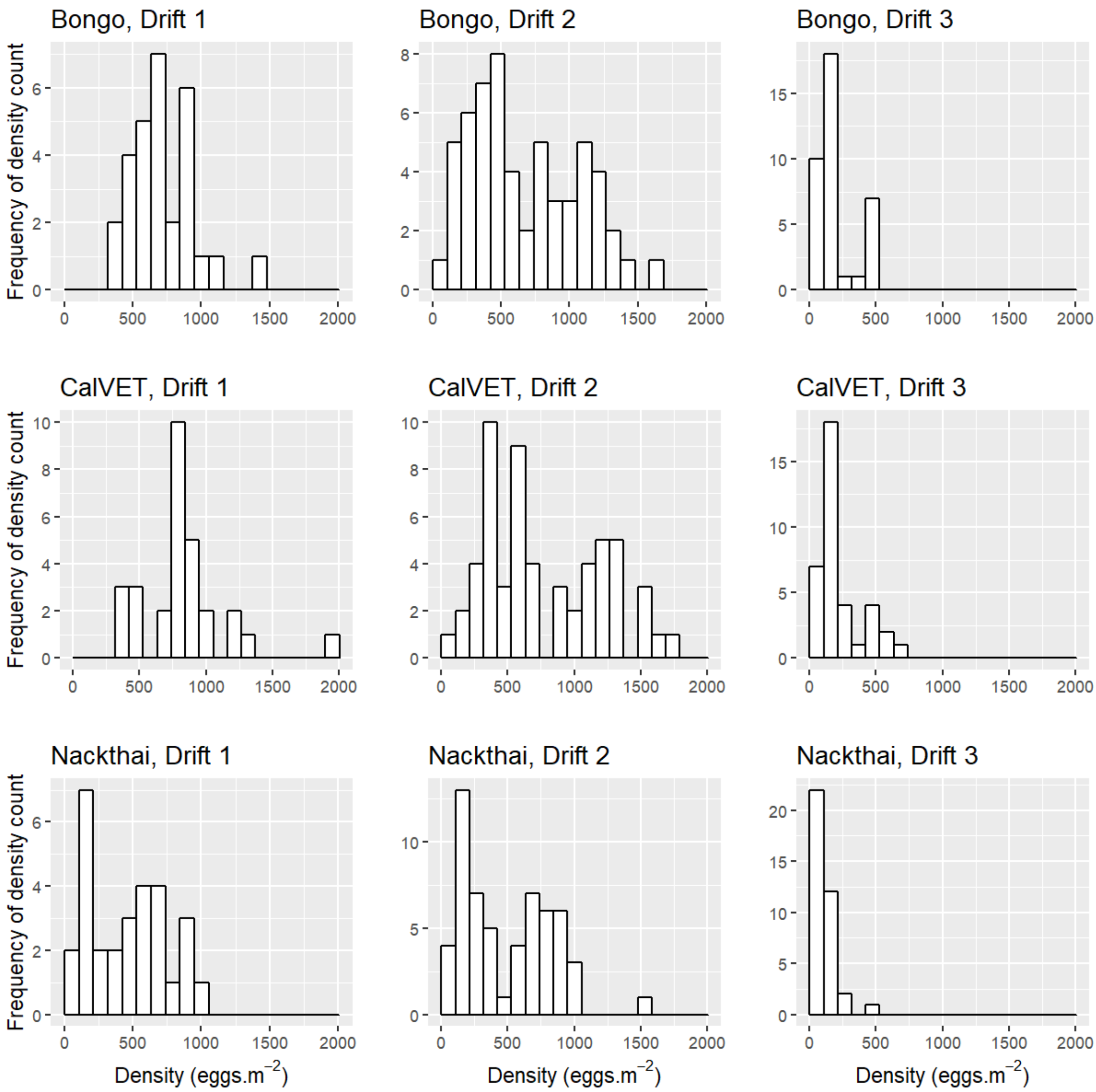

Figure 8. Frequency plots of the egg densities estimated from samples obtained from the three sampler types across the three separate drifts.

Mean Daily Egg Production (P_0)

Estimates of P_0 obtained from the three samplers using the six models are shown in Figure 9, 10, 11 and Table 8. Estimates from the Bongo were consistently similar to, and marginally lower than, those from the CalVET, only varying by +1 to -28% (CalVET/Bongo) across all drifts and models. Estimates of P_0 from the Nackthai were generally lower than those from the CalVET and Bongo. Model estimates of P_0 from the Nackthai were 47–57, 31–36 and 50–59% lower than those derived from the CalVET data for Drifts 1, 2 and 3, respectively.

For Drifts 1 and 2, the log-linear model produced higher estimates of P_0 across all sampling gears, whereas, all models produced more consistent results for each sampler for Drift 3. Estimates of mortality (z) varied more between drifts than between samplers (Table 8, Figure 12). The log-linear model produced the highest estimates of P_0 and mortality (z) for Drifts 1 and 2, where egg density was highest.

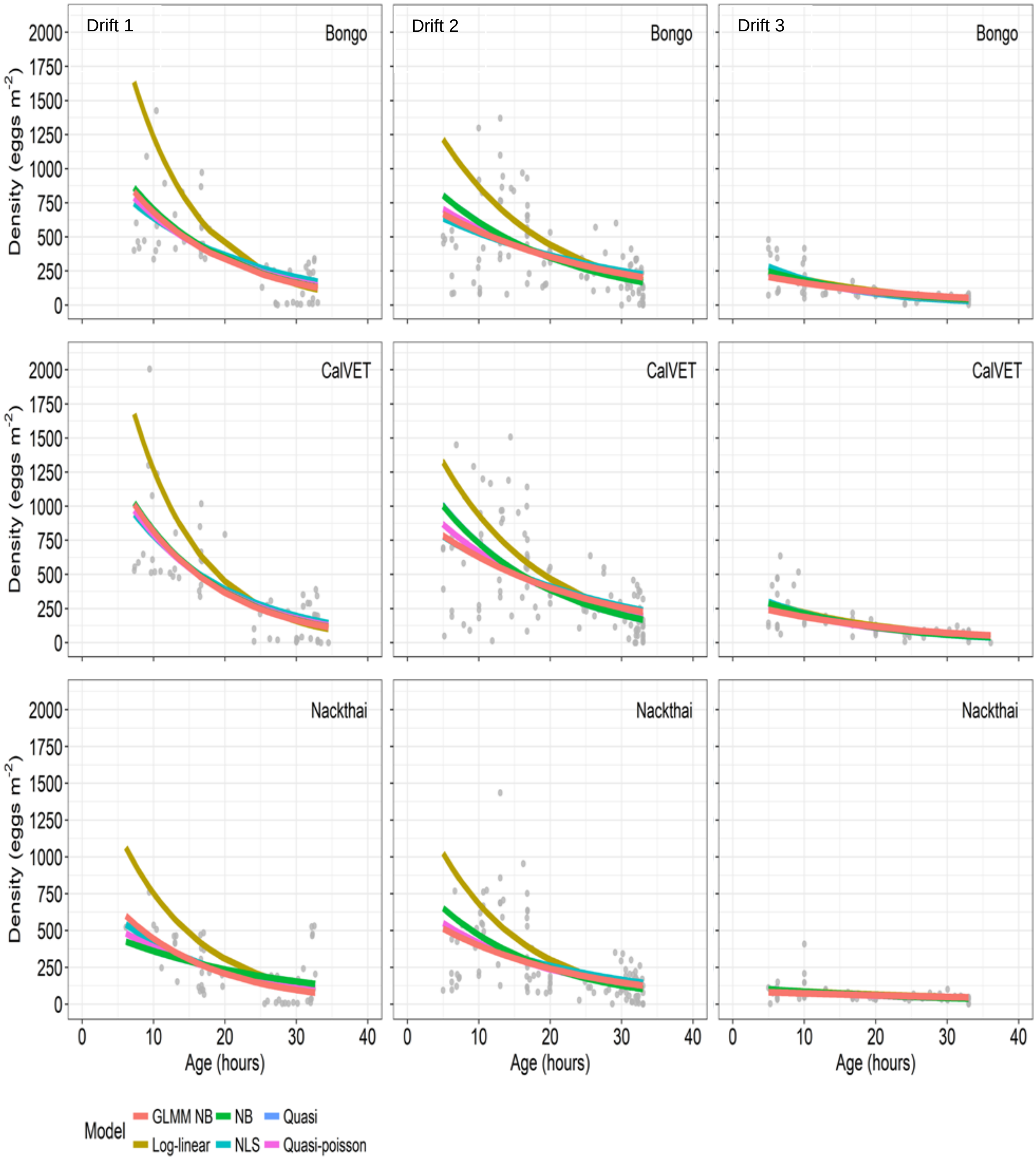

Figure 9. Egg production models (coloured lines) fitted to cohort egg densities (eggs·m^{-2}) and egg age (hours) of Australian Sardine (grey circles) for each of the sampler types and drifts.

Table 8. Estimates of mean daily egg production (P_0, eggs·day^{-1}·m^{-2}) and instantaneous daily mortality (z, day^{-1}) estimates from each of the three samplers and drifts.

	Drift 1		Drift 2		Drift 3	
Sampler			**Bongo**			
Parameter	P_0	z	P_0	z	P_0	z
Log-linear	3,463	0.103	1,701	0.067	347	0.061
NLS	1,128	0.057	767	0.037	420	0.079
Quasi	1,462	0.073	1,064	0.056	335	0.062
Quasi-Poisson	1,272	0.066	888	0.047	323	0.063
Negative Binomial	1,463	0.073	1,063	0.056	336	0.063
GLMM Negative Binomial	1,406	0.072	826	0.042	265	0.049
Sampler			**CalVET**			
Log-linear	3,572	0.104	1,867	0.069	393	0.058
NLS	1,562	0.069	964	0.043	414	0.065
Quasi	1,835	0.080	1,382	0.064	394	0.062
Quasi-Poisson	1,676	0.075	1,130	0.053	359	0.059
Negative Binomial	1,839	0.080	1,373	0.063	394	0.063
GLMM Negative Binomial	1,820	0.080	979	0.044	307	0.048
Sampler			**Nackthai**			
Log-linear	1,839	0.089	1,539	0.081	113	0.028
NLS	799	0.064	646	0.045	117	0.033
Quasi	552	0.042	904	0.066	120	0.034
Quasi-Poisson	679	0.056	728	0.056	110	0.032
Negative Binomial	552	0.043	904	0.066	120	0.034
GLMM Negative Binomial	959	0.077	658	0.050	89	0.020

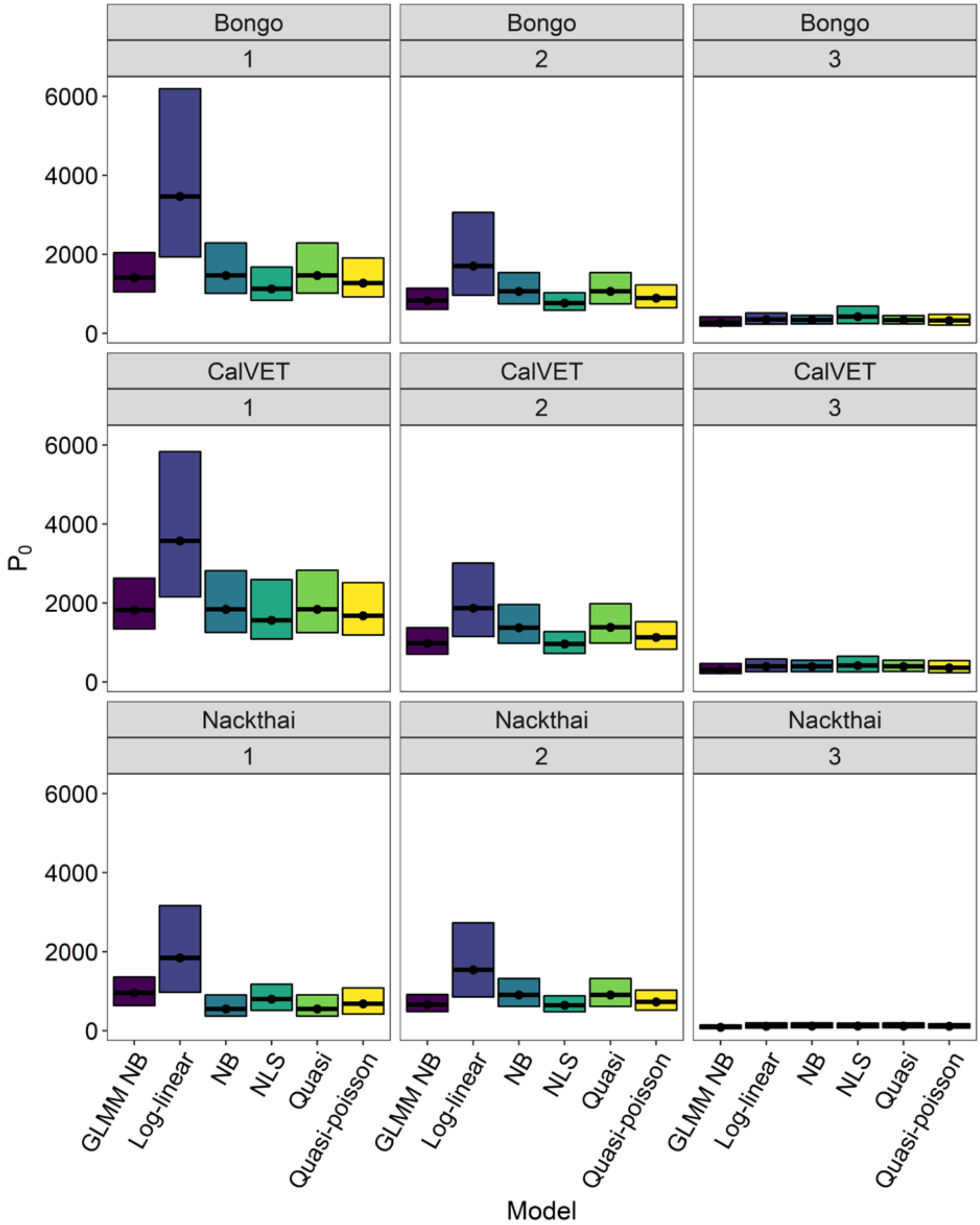

Figure 10. Comparisons of the estimates of mean daily egg production (P_0, eggs·day^{-1}·m^{-2}) from the six egg production models, three sampler types and three drifts (left to right). Boxes show 95% CI: quantiles of 10,000 bootstrap resamples. NLS: Non-linear Least Squares.

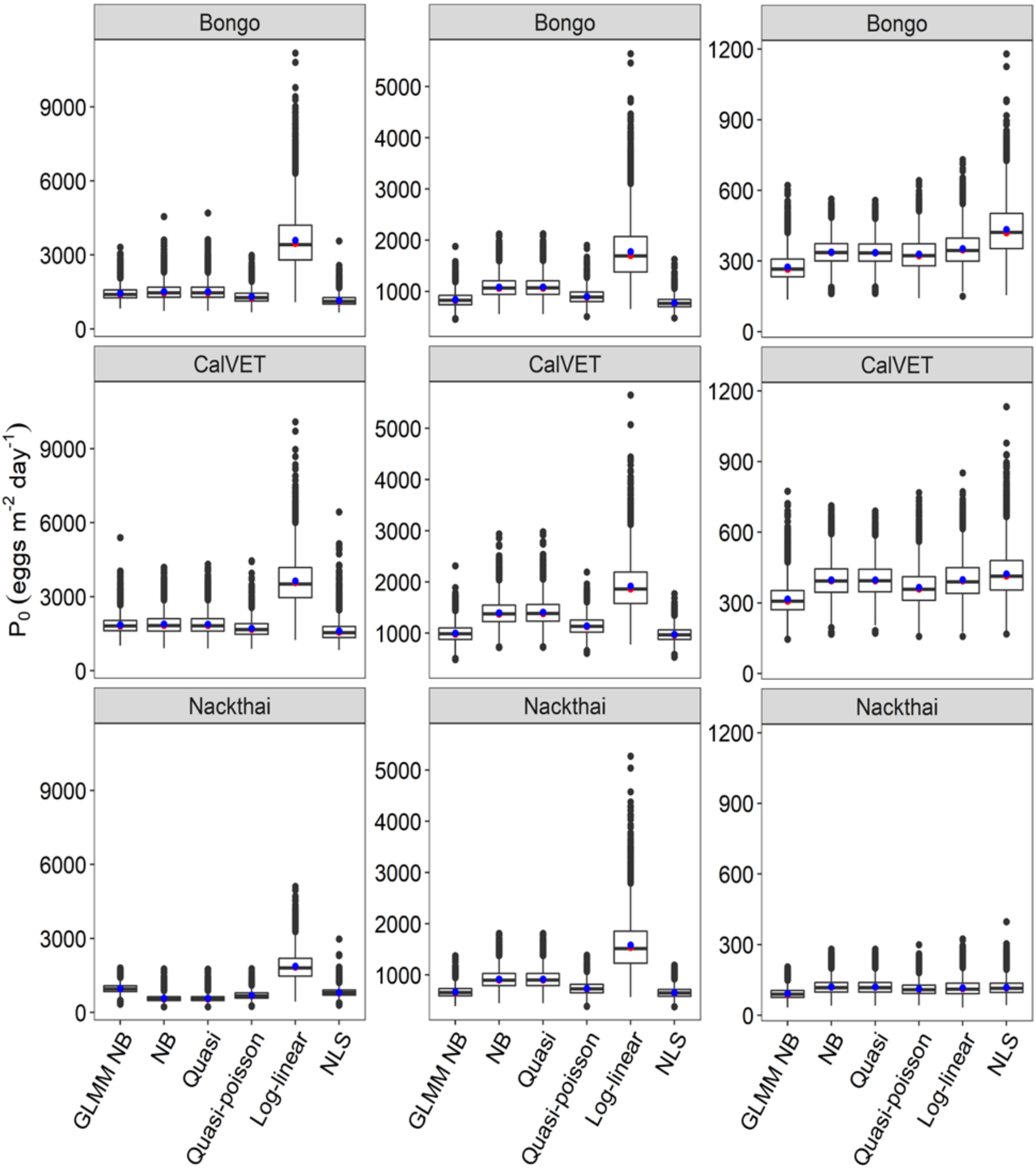

Figure 11. Boxplot of estimates of mean daily egg production (P_0, eggs·day^{-1}·m^{-2}) for Australian Sardine from the six egg production models, sampler types and drifts (1-3, left to right). NLS: Non-linear Least Squares. Red dot: mean estimate from field data; blue dot: mean estimate from bootstrapped data.

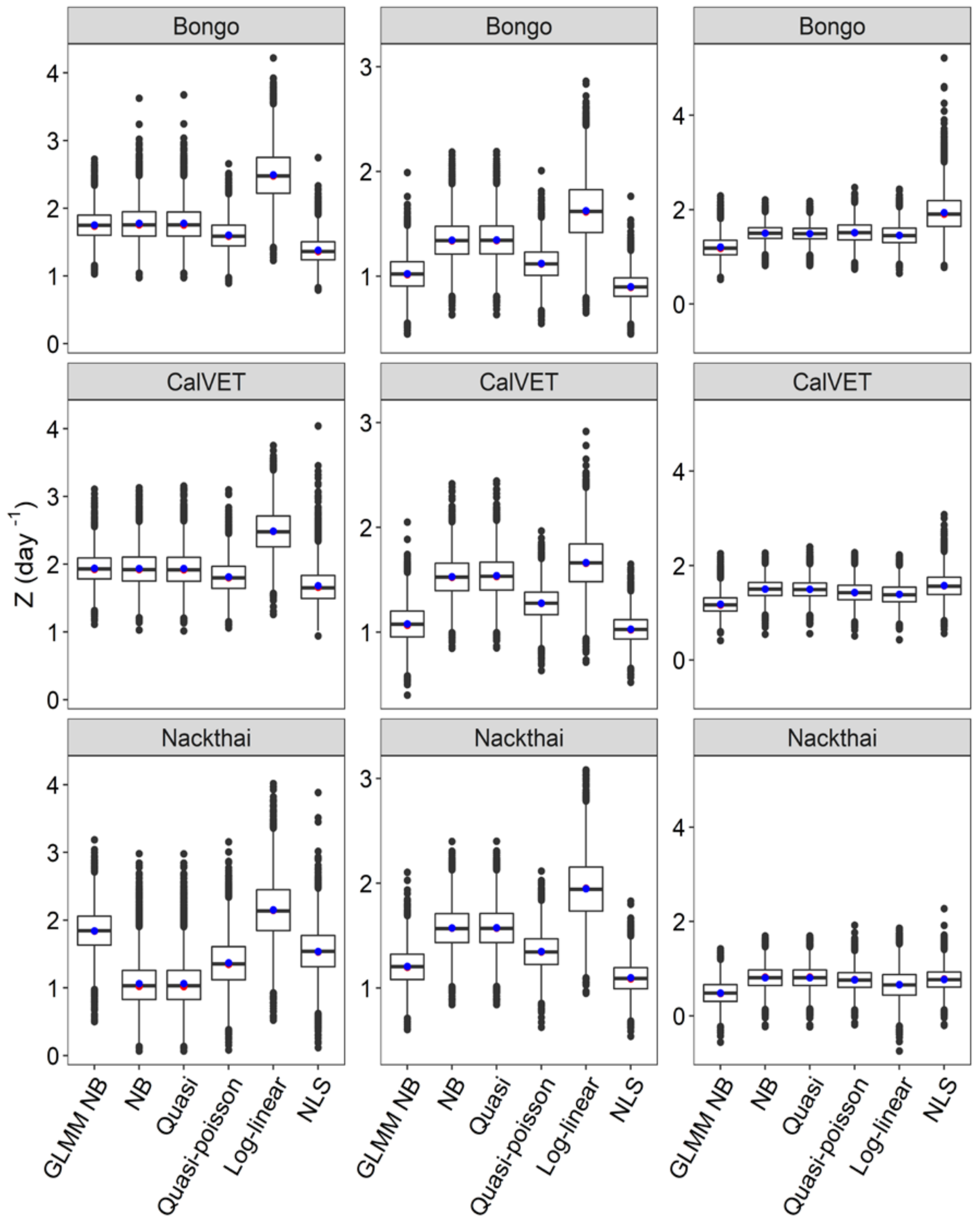

Figure 12. Boxplot of estimates of instantaneous daily mortality (z, day-1) for Australian Sardine from the six egg production models, sampler types and drifts (1-3, left to right). NLS: Non-linear Least Squares. Red dot: mean estimate from field data; blue dot: mean estimate from bootstrapped data.

Egg development

Repeated, multiple egg samples in the same location provided an opportunity to confirm *in situ* egg development rates for Australian Sardine at ~18 – 20°C (Figure 4, 13), with only the bottom 10 m of the water column falling slightly below 18°C.

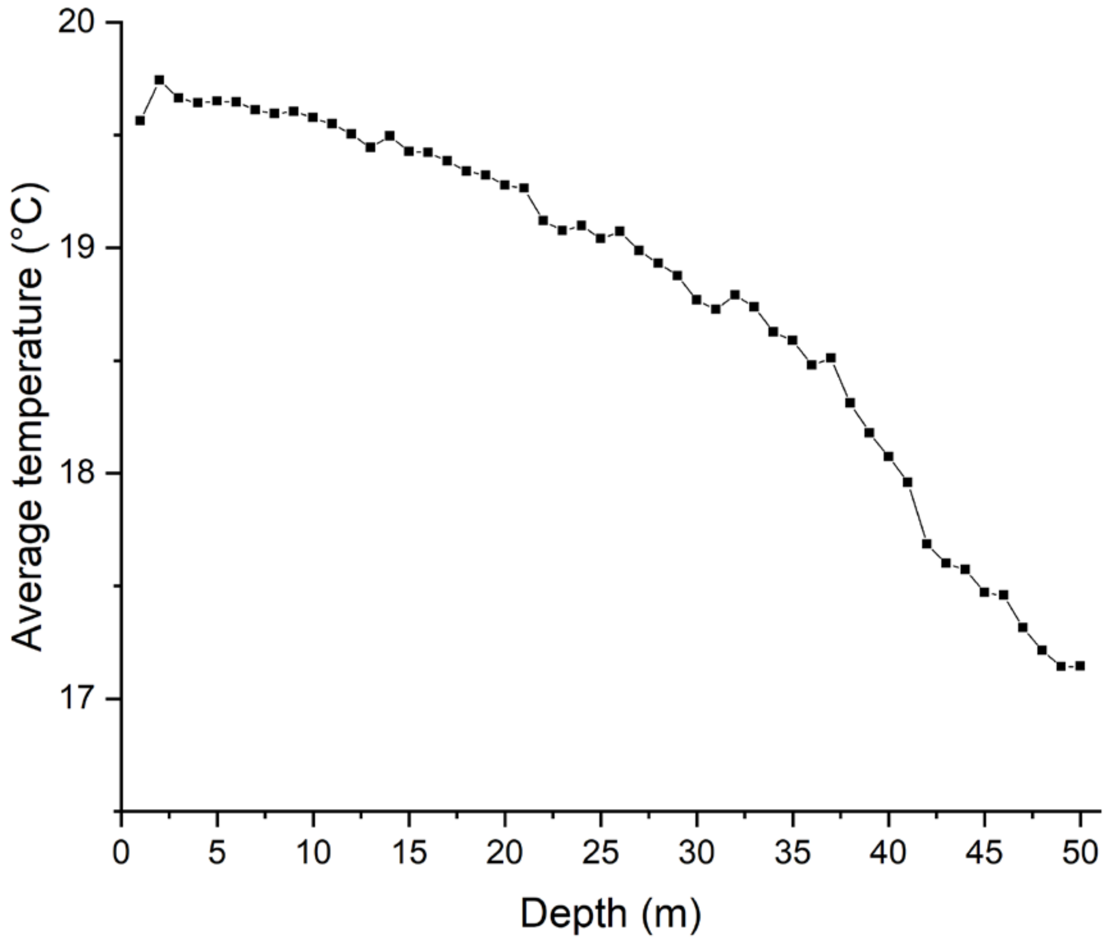

Figure 13. Mean water temperature measured at one metre depth bins for data collected from all Nackthai tows combined.

Early stage 1 eggs were first observed up to two hours before midnight through to 3 am (Figure 14). Mean egg density at stage histograms followed a consistent progression through time. Stage 10 eggs reached a peak density at 35 hours after midnight and were mostly absent after 44 hours.

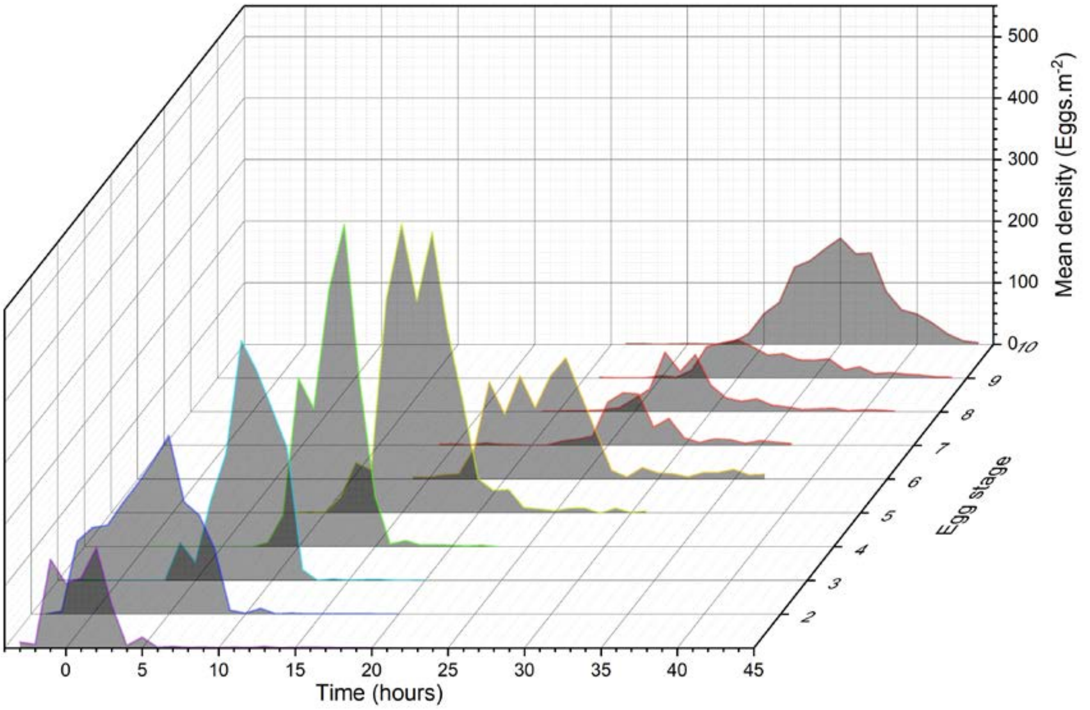

Figure 14. Mean egg density at time for all sampling gears combined. Data has been processed to account for two day development and young eggs observed prior to midnight (midnight = 0).

Discussion and conclusions

Egg density and egg production

The main reason for undertaking this study was to determine if an oblique sampler would provide more precise estimates of mean egg density and egg production than the traditional vertical samplers. The underlying hypothesis, which was generated from simulation modelling (Ward et al. 2018), was that an oblique tow may provide more precise estimates of these parameters because the increased length of the sampling unit may match the size and distribution of egg patches better than a shorter vertical tow (Ward et al. 2018). However, results obtained in the study present study do not support the hypothesis. Estimates of mean egg density and mean daily egg production (P_0) obtained from the Nackthai were less precise than those obtained from the Bongo and CalVET.

Several factors may explain the significant differences in estimates of mean egg density and P_0 obtained from the three samplers. The lower estimates obtained from the Bongo compared to the CalVET suggests that the sampling efficiency of the larger net may have been compromised in the combination Bongo/CalVET arrangement used in this study.

The much lower estimates of egg density obtained from the Nackthai compared to the vertical samplers may reflect the stepped design of the oblique tow. Previous studies have shown that most sardine eggs are found in the upper part of the water column. The shallowest horizontal section of each Nackthai tow was at 10 m. If the highest egg densities occurred in the top 10 m, this would explain the relatively low estimates of egg density obtained from the Nackthai.

Logistical considerations

The ease and safety with which a plankton sampler can be deployed is an important consideration in research surveys conducted in offshore waters where sea conditions be challenging. Due to its relatively small size, light-weight design and straightforward vertical deployment, the CalVET has been the standard sampler for pelagic species such as Australian Sardine, which has relatively high egg densities. For species with lower egg densities (e.g. Jack Mackerel, *Trachurus declivis*), the Bongo has been the preferred method (at least in Australia) as it samples a larger volume of water (~4 times) than the CalVet, and thus catches more eggs. The lower estimates of egg densities obtained with the Bongo compared to the CalVet in the present study may be an artefact of the combination Bongo/CalVET arrangement used in the study. However, this issue warrants further investigation through direct independent comparison of the two vertical samplers.

Due to its large size and cumbersome design (e.g. 22 kg depressor), the Nackthai was more difficult to deploy than the combination Bongo/CalVET used in the present study. It is much more difficult to deploy in rough weather than either of the traditional vertical samplers. Deploying and retrieving the Nackthai net takes at least one extra crew member to deploy and retrieve compared to the CalVET or Bongo.

Samples obtained from the Nackthai also contained more eggs and took longer to process in the laboratory than those from the other nets. There was no evidence to suggest that replacing the traditional samplers with the Nackthai would enhance application of the DEPM to Australian Sardine or to other small pelagic fishes in Australia, where vessels used to conduct egg surveys are typically quite small (less than 25 m in length) and deployment of the Nackthai is logistically challenging.

Implications

The logistical challenges posed by the Nackthai compared to the vertical samplers, combined with the reduced precision of estimates of egg density and P_0, provide compelling reasons for continuing to use CalVET and Bongo nets to sample eggs in applications of the DEPM to offshore pelagic fish species in Australian waters. The results of this study indicate that the Nackthai should not be adopted to replace vertical samplers in applications of the DEPM to Australian Sardine or Jack Mackerel.

Recommendations

Vertical samplers should continue to be used as the primary sampling tool in applications of the DEPM to offshore pelagic fish species such as Australian Sardine and Jack Mackerel. Other opportunities for increasing the precision of estimates of spawning biomass should be evaluated.

Extension and Adoption

The PI will extend results of this project to industry, stakeholders and government during ongoing involvement in SASF Research and Management Committee and SPF Resource Assessment Group and through ongoing engagement with PIRSA and AFMA.

References

Bernal, M., S. Somarakis, P. R. Witthames, C. J. G. van Damme, A. Uriarte, N. C. H. Lo, and M. Dickey-Collas. 2012. Egg production methods in marine fisheries: An introduction. Fisheries Research **117–118**:1-5.

Dickey-Collas, M., S. Somarakis, P. R. Witthames, C. J. G. van Damme, A. Uriarte, N. C. H. Lo, and M. Bernal. 2012. Where do egg production methods for estimating fish biomass go from here? Fisheries Research **117–118**:6-11.

Lasker, R. 1985. An egg production method for estimating spawning biomass of pelagic fish: application to northern anchovy, *Engraulis mordax*. NOAA. Tech. Rep. NMFS **36**:1 - 99.

Lo, N. C. H., Y. A. Green Ruiz, M. J. 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. California Cooperative Oceanic Fisheries Investigations Report.

Picquelle, S. and Stauffer, G. 1985. Parameter estimation for an egg production method of anchovy biomass assessment. In: Lasker, R. “An egg production method for estimating spawning biomass of pelagic fish: application to northern anchovy, Engraulis mordax.” NOAA Tech. Rep. NMFS. **36**: 7-17.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Smith, P. E., and S. L. Richardson. 1977. Standard techniques for pelagic fish egg and larva surveys. Food and Agriculture Organisation. Fisheries Technical Paper No. 175

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

Ward, T. M., P. Burch, L. J. McLeay, and A. R. Ivey. 2011. Use of the Daily Egg Production Method for stock assessment of sardine, Sardinops sagax; lessons learned over a decade of application off southern Australia Reviews in Fisheries Science **19**:1-20.

Ward, T. M., Carroll, J., Grammer, G. L., James, C., McGarvey, R., Smart, J. and Ivey, A. R. 2018. Improving the precision of estimates of egg production and spawning biomass obtained using the Daily Egg Production Method. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. FRDC Project No. 2014/026.

White, K. V., and W. J. Fletcher. 1998. Identifying the developmental stages for eggs of the Australian pilchard, Sardinops sagax. Fisheries Western Australia. Fisheries Research Report No.103.

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

FRDC FINAL REPORT CHECKLIST

Project Title:	Validating a new sampling technique for estimating egg production		
Principal Investigators:	Ward, T.M., McGarvey, R., Ivey, A., and Grammer, G.L. March 2020		
Project Number:	FRDC Project No 2017-027		
Description:	Estimates of spawning biomass obtained using DEPM are the primary biological performance indicator in Australia's largest fishery, the South Australian Sardine Fishery (SASF), as well as the Commonwealth Small Pelagic Fishery (SPF). The objective of this study was to improve the accuracy and precision of estimates of spawning biomass obtained using the DEPM. To address this objective, we evaluated the potential benefits of using a high speed oblique sampler (Gulf 7 or Nackthai net) instead of vertically towed CalVET or Bongo nets to measure egg density and estimate egg production in the application of the DEPM. Field experiments were conducted to compare the precision of estimates of egg density and egg production obtained using the Nackthai and the traditional methods (CalVET or Bongo nets). The Nackthai was more difficult to deploy in rough weather and required more staff than the other samplers. Estimates of egg density derived from the Nackthai were lower and had higher variance than those obtained from the CalVET or Bongo nets. Estimates of egg production derived from Nackthai data were lower and less precise that those obtained from the vertical samplers. Vertical samplers should continue to be used as the primary sampling tool for measuring egg density in applications of the DEPM to offshore pelagic fish such as Australian Sardine and Jack Mackerel.		
Published Date:	March 2020	**Year:**	2020
ISBN:	978-1-876007-25-6	**ISSN:**	
Key Words:	Australian Sardine, *Sardinops sagax*, mean daily egg production, egg mortality, South Australian Sardine Fishery, Small Pelagic Fishery		

	Is it included (Y/N)	Comments
Foreword (optional)	N	
Acknowledgments	Y	
Abbreviations	Y	
Executive Summary	Y	
- **What the report is about**	Y	
- **Background – why project was undertaken**	Y	
- **Aims/objectives – what you wanted to achieve at the beginning**	Y	
- **Methodology – outline how you did the project**	Y	
- **Results/key findings – this should outline what you found or key results**	Y	
- **Implications for relevant stakeholders**	Y	
- **Recommendations**	Y	

Introduction	Y	
Objectives	Y	
Methodology	Y	
Results	Y	
Discussion	Y	Discussion and conclusion combined
Conclusion	Y	
Implications	Y	
Recommendations	Y	
Further development	N	
Extension and Adoption	Y	
Project coverage		No media
Glossary	N	
Project materials developed	N	
Appendices	Y	Reference