Improving the precision of estimates of egg production and spawning biomass obtained using the Daily Egg Production Method

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Abbreviations

AFMA	Australian Fisheries Management Authority
CalVet	Californian Vertical Egg Tow net
CTD	Conductivity Temperature Depth
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
LOO	Leave-One-Out (cross validation)
MCE	Monte-Carlo Ensembles
P_0	Mean daily egg production
PIRSA	Primary Industries and Regions South Australia
RMSE	Root Mean Squared Error
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

This project was undertaken by a team of fisheries biologists, statisticians, modellers and oceanographers from the South Australian Research and Development Institute (SARDI), Aquatic Sciences. Findings have been used to refine the application of the Daily Egg Production Method (DEPM) to Australia's largest fishery, the South Australian Sardine Fishery (SASF), and the Commonwealth Small Pelagic Fishery (SPF). Key findings and outcomes from this study include: 1) a new generalised egg staging method that has several advantages over previous egg staging systems; 2) refinements to methods used to identify samples where a zero count should be allocated to one or more egg cohorts; 3) identification of factors that cause the high levels of uncertainty associated with estimates of mean daily egg production (P_0) and egg mortality (z); 4) confirmation that the log-linear model is the most precise method currently available for estimating P_0 and z for Australian Sardine (*Sardinops sagax*) off South Australia; 5) a simulation model that can be used to evaluate the effects of key processes (e.g. sampling method) on the precision of estimates of P_0 and z; and 6) recommendations to trial a new oblique plankton sampler that may improve the precision of future estimates of P_0. These findings and outcomes will improve the ongoing application of the DEPM to Australian Sardine off South Australia and further enhance the application of the method to Australian Sardine, Jack Mackerel (*Trachurus declivis*), Blue Mackerel (*Scomber australasicus*) and Redbait (*Emmelichthys nitidus*) off south-eastern Australia, as well as other species to which the method is being applied.

Background

The benefits of using fishery-independent methods to estimate indicators of stock status are widely recognised. The DEPM is the primary stock assessment technique in the SASF and SPF. The DEPM estimates the biomass of spawning adults by dividing the mean number of pelagic eggs produced per day throughout the spawning area (total daily egg production) by the mean number of eggs produced each day per unit mass of adult fish (mean daily fecundity). Estimates of spawning biomass obtained using the DEPM are accurate but imprecise; much of this imprecision is caused by uncertainties in estimates of P_0.

Objectives

1. Improve methods used to determine the age of egg cohorts when estimating mean daily egg production.
2. Compare the performance of current and developmental statistical methods for estimating egg production using long-term datasets for several species.
3. Use simulations to formally evaluate the performance of different approaches to sampling and statistical analysis on estimates of egg production.
4. Establish improved methods for estimating daily egg production in applications of the DEPM.

Methodology

Ichthyoplankton sampling

Eggs of Australian Sardine, Jack Mackerel and Blue Mackerel were collected in DEPM surveys previously conducted off southern and eastern Australia. Samples were collected in vertical towed paired ichthyoplankton nets. Location, sampling date/time, sea surface temperature and depth were recorded for each sample.

Improve methods used to determine the age of egg cohorts

Preserved eggs of each species were categorised into 10 'universal' stages. Each developmental stage is based on distinctive morphological characteristics that can be easily distinguished in the laboratory and are common to many pelagic fishes. Samples were grouped into temperature ranges of 14–18°C, 18–22°C and 22–26°C. Eggs were assigned an age based on the development rate of the same or a closely related species in each temperature range. Mean spawning time was calculated by subtracting the age of Stages 1–3 eggs from the collection time for each sample.

Compare performance of current statistical methods on long-term datasets for several species

P_0 is estimated from the densities of daily cohorts and their ages in samples collected from the spawning area. Each sample with eggs contains information about cohorts spawned over several nights. Measured densities of egg stages were aggregated into daily cohorts. The age assigned to each daily cohort was the average age of each stage, weighted by the number of eggs in each stage. We compared the performance of several statistical models for estimating P_0 (non-linear least squares regression; regression of log-transformed data; Gaussian, Negative Binomial and Quasi Generalised Linear Models (GLMs); three General Additive Models (GAMs)). Mortality (z, day^{-1}) was estimated as a free parameter in the regressions and GLM models. This approach assumes egg density declines exponentially with age due to mortality. Other factors that reduce egg density, e.g. diffusion of eggs after spawning, are confounded with mortality and not considered explicitly in these models. The reliability of model fits and confidence intervals for estimates of P_0 were assessed using bootstrap and jackknife resampling methods. Leave-one-out (LOO) cross validations and root mean squared errors (RMSE) were calculated to evaluate the predictive power of the different models.

For Australian Sardine, z was estimated both annually and for all years combined. Raw densities for each year were scaled by correcting for mortality based on the weighted cohort age and the two estimates of mortality. The means of these scaled densities produced two alternative estimates of P_0. GAMs were fitted to estimates of P_0 for each sample to evaluate effects of depth and/or temperature.

Use simulations to evaluate performance of different sampling and statistical analysis

A computer simulation was constructed to test the performance of the DEPM in determining P_0. The model consisted of: 1) the 'egg-space'– a constrained, random, three dimensional distribution of egg concentrations within a fixed volume as a function of time, and 2) a simulated ichthyoplankton survey and sampling strategy based on vertical and oblique tows which calculates the number of eggs captured at each site and their age. Egg concentrations were computed by applying an analytical diffusion formula and a vertical advection formula. The model is continuous in space and time. Spawning occurs once a day; spawning time has a normal distribution. Diffusion was modelled with an analytical equation with horizontal and vertical diffusion. Egg ages at the time of collection were used to assign eggs to a stage; eggs were then assigned the average age of that stage. Offsets of egg age were used to ensure estimates of P_0 were not influenced by knowledge of the true egg age. Simulated surveys were conducted over 300 sites sampled over ~20 days. Travel times between station and transects were allowed to vary randomly to simulate variations in survey conditions due to tide, weather and other factors.

Results and discussion

Improve methods used to determine the age of egg cohorts

The universal egg staging method developed in this project has two advantages over other staging systems. Firstly, stages are based on distinctive morphological characteristics that are easily identified in the laboratory, reducing the likelihood of staging errors. Secondly, all stages have similar durations which simplifies the interpretation of variations in the observed densities of different stages. This second advantage allowed us to clearly demonstrate that young Australian Sardine eggs occur infrequently in plankton samples collected in vertical tows.

We used a combination of two methods to estimate the age and developmental rates of eggs from data collected during the surveys. The estimates of development rates of Australian Sardine eggs obtained from field data in water temperatures (18–22°C) were similar those obtained during incubation experiments conducted in other studies. This finding is important as it justified the use of data from incubation experiments conducted elsewhere to estimate the age of eggs sampled off southern Australia.

Another important development made during this project was the refinement of the method used to identify samples where a zero count should (and should not) be allocated to one or more egg cohorts. This development is important because the presence/absence of zeros in the data can impact significantly on estimates of P_0.

Compare performance of current statistical methods

The most important finding from this study is that for Australian Sardine off South Australia the log-linear model provides more plausible and precise estimates of P_0 and z than all of the other non-GAM models

(e.g. in 2014 all models except the log-linear produced unrealistic values of $P_0 > 420$ eggs·m^{-2}·day^{-1} with very broad 95% CIs). This result occurs because log transforming the data reduces the influence of samples with very high egg densities, which cause the other models to produce unrealistically high estimates of P_0 and z in some years. The 95% CIs of the estimates of P_0 and z obtained using the log-linear model were much narrower than those obtained using the other models. GAMs have several major limitations: z needs to be assumed to allow estimation of P_0 at each site (i.e. z is not estimated from data); GAMs failed to merge in half of the years for which data were available; and the inclusion of environmental data did not appear to significantly improve estimates of P_0.

Conduct simulations to evaluate performance of different approaches to sampling and data analysis

Simulation modelling confirmed that the log-linear approach provides more precise and conservative estimates of P_0 than non-linear least-squares fitting. Two other factors also affect the precision of estimates of P_0 and z: i.e. egg density and the sampling strategy. The Coefficients of Variation of estimates P_0 are highest when mean egg density is low. The simulations showed that oblique tows are likely to provide datasets which produce more precise estimates of P_0 than vertical tows.

Implications for relevant stakeholders

Several significant developments made during this project have already been adopted in the application of the DEPM in the SASF and SPF. For example, eggs are now staged using the universal system developed here and the method used to allocate zero counts to egg cohorts has been adopted as standard practice. Results presented here also confirm previous findings that suggested the log-linear model is the method that should be used to estimate P_0 and z for Australian Sardine off South Australia.

Historical estimates of spawning biomass for the Southern Stock of Australian Sardine have recently been updated using the refined methods for estimating P_0 developed in this project. The key finding of the simulation study, i.e. that oblique tows may produce more precise estimates of P_0 than vertical tows, has resulted in members of the SASF and FRDC funding a trial of a new oblique plankton sampler (the Nackthai) in 2017/18 (FRDC Project 2017-027). Concurrent studies have suggested that alternative indicators of stock status (e.g. spawning area) may also be warranted for species where uncertainty in estimates of P_0 are exacerbated by difficulties associated with the reliable estimation of other key DEPM parameters, especially spawning fraction.

Keywords

Australian Sardine, *Sardinops sagax*, mean daily egg production, egg mortality, simulation modelling, South Australian Sardine Fishery, Small Pelagic Fishery

Introduction

Background

Indicators of stock status, such as spawning biomass, underpin the sustainable management of many exploited fish stocks (Smith et al. 2011, Pikitch et al. 2012). For many species, including small pelagic fishes, there is growing recognition of the benefits of using fishery-independent methods to estimate these indicators (e.g. de Moor et al. 2008, Bernal et al. 2012, Dennis et al. 2015). Egg production methods, which calculate the size of the adult biomass from estimates of the number of eggs spawned and average fecundity, are used to inform the management of many large fisheries (Bernal et al. 2012, Dickey-Collas et al. 2012). For example, the Daily Egg Production Method (DEPM; Lasker 1985, Stratoudakis et al. 2006) has been used to estimate the spawning biomass of sardine off North America, Europe, and Australia (e.g. Lo et al. 1996, Somarakis et al. 2006, Ward et al. 2011) and anchovy in waters off southern Africa, South America, Europe and Australia (Hampton 1996, Somarakis et al. 2002, Cubillos et al. 2007, Dimmlich et al. 2009). In Australia, there are two main fisheries for small pelagic fishes: the South Australian Sardine Fishery (SASF) and Commonwealth Small Pelagic Fishery (SPF). The application of the DEPM to other species, such as Snapper, has also been trialled recently (Steer et al. 2017).

The SASF was established in 1991 to provide fodder for the ranching of Southern Bluefin Tuna. It is now Australia's largest volume fishery with a total allowable catch (TAC) in 2017 of 42,750 t and a gross value of production (GVP) of >$30M (SARDI, unpublished data). Community concerns about potential impacts of the SASF on the ecosystem have been addressed with field-based studies on predatory species and using ecosystem modelling to inform the adaptive development of a precautionary assessment and management framework (e.g. Goldsworthy et al. 2013). This framework includes: a stock assessment program based on fishery-independent surveys (e.g. Ward et al. 2011, 2015b); an evolving series of harvest strategies that have all included decision rules for setting TACs (e.g. Shanks 2005; PIRSA 2014); a Code of Practice for mitigating operational interactions with protected species (Hamer et al. 2008, Ward et al. 2015a); and guidelines for managing the spatial distribution of catches (PIRSA 2014).

The SPF was established in 2002, and the Management Plan and Harvest Strategy for the fishery draw heavily on the approach taken in the SASF (http://www.afma.gov.au/managing-our-fisheries/harvest-strategies/small-pelagic-fishery-harvest-strategy/). For example, the DEPM is the prescribed stock assessment method for both the SASF and the SPF (Ward et al. 2015b, Ward and Grammer 2017). Estimates of spawning biomass are the key biological performance indicators underpinning the harvest strategies for both fisheries (see Smith et al. 2017).

The DEPM estimates the biomass of spawning adults by dividing the mean number of pelagic eggs produced per day throughout the spawning area (total daily egg production) by the mean number of eggs

produced each day per unit mass of adult fish (mean daily fecundity) (Parker 1980, Parker 1985). Total daily egg production is estimated from plankton surveys completed during the main spawning season and across the entire spawning area (e.g. Lasker 1985, Stratoudakis et al. 2006). Mean daily fecundity is estimated from adult surveys undertaken concurrently with plankton surveys (Alheit 1993, Hunter and Lo 1997, Ganias 2012). The DEPM produces unbiased but imprecise estimates of spawning biomass (Stratoudakis et al. 2006), with much of the imprecision attributed to uncertainties in estimates of total daily egg production (Alheit 1993, Ward et al. 2011, Dickey-Collas et al. 2012). The need for improvements in the precision of estimates of egg production has been identified in papers and reports by numerous authors (e.g. Hunter and Lo 1997, McGarvey and Kinloch 2001, Stratoudakis et al. 2006, Bernal et al. 2011a, b, Ward et al. 2011).

Need

A project to refine methods for estimating egg production in applications of the DEPM is needed because: 1) spawning biomass estimates calculated using the DEPM are the key biological performance indicators in the SASF and SPF; 2) the DEPM is recognized as being imprecise and the main source of this imprecision comes from the estimation of mean daily egg production; 3) a range of field and statistical methods are used to estimate total daily egg production, but there is no international consensus about which approach is most appropriate for the range of circumstances that are encountered, with different methods currently used in the Americas, Europe and Australia.

Objectives

1. Improve methods used to determine the age of egg cohorts for estimation of mean daily egg production*.
2. Compare the performance of current and developmental statistical methods for estimating egg production using long-term datasets for several species.
3. Conduct simulations to formally evaluate the performance of different approaches to sampling and statistical analysis on estimates of egg production.
4. Establish improved methods for estimating daily egg production in applications of the DEPM.

*Objective 1 was not an objective of the original proposal. It was added to the work program during the course of the project to: i) overcome the need to establish a species-specific staging system each time the DEPM is applied to a new species; ii) reduce the likelihood of staging errors by establishing stages that are easily identified in the laboratory; iii) simplify the interpretation patterns of egg density versus age by establishing stages with similar durations. The other three objectives (i.e. 2-4) are unchanged from the original proposal.

Methods

Egg samples of Australian Sardine (*Sardinops sagax*), Jack Mackerel (*Trachurus declivis*) and Blue Mackerel (*Scomber australasicus*) collected in surveys off southern and eastern Australia were used as case studies to evaluate and refine methods for estimating egg production in applications of the DEPM (see Ward et al. 2011, 2015 a, b; 2017; Ward and Rogers 2007).

1. Ichthyoplankton sampling

Ichthyoplankton surveys covered the known spawning area of Australian Sardine, Jack Mackerel and Blue Mackerel during the peak spawning seasons off southern and south-eastern Australia (Figure 1, Table 1). Paired ichthyoplankton nets (CalVet or bongo; 330 or 500 µm mesh; plastic cod-ends) were deployed to 10 m above the seabed or a maximum depth of 200 m and retrieved vertically at a speed of ~1 $m \cdot s^{-1}$. A Sea-Bird™ Conductivity-Temperature-Depth (CTD) attached to the net recorded water temperature profiles (°C). Samples from the paired net cod-ends were combined into one sample and fixed in a 5% buffered formalin and seawater solution.

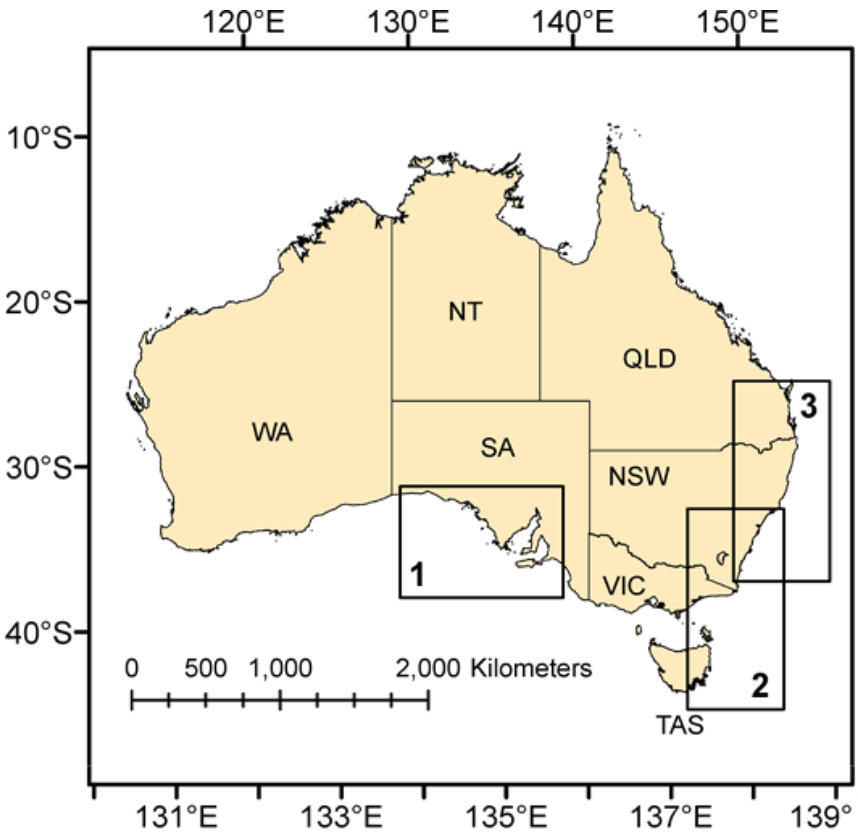

Figure 1: Location of DEPM ichthyoplankton surveys along the Australian coast for Sardine (1 and 3), Jack Mackerel (2), and Blue Mackerel (3).

Table 1: Ichthyoplankton DEPM survey details for the study species. SA: South Australia, QLD/NSW: southern Queensland and New South Wales (eastern Australia); New South Wales and Tasmania (southeastern Australia). CalVET: Californian Vertical Egg Tow net.

Species	Survey location	Survey years	Net type	Max net deployment depth (m)
Australian Sardine	SA	1998-2016	CalVET	70 m or 10 m from seabed
Australian Sardine	QLD/NSW	2014	Bongo	200 m or 10 m from seabed
Jack Mackerel	NSW/TAS	2014	Bongo	200 m or 10 m from seabed
Blue Mackerel	QLD/NSW	2014	Bongo	200 m or 10 m from seabed

2. Improve methods used to determine the age of egg cohorts

Egg staging

Preserved eggs of the each species were categorised into 10 developmental stages (
Table **2**). These 'universal' stages are based on distinctive morphological characteristics common to fish with similarly sized, pelagic eggs. An objective of developing this staging system was to establish stages of similar duration, as stages in other staging systems were of variable duration (e.g. at 17°C in Lo et al. 1996, Stage 1 is 1 hour and Stage 3 is 5 hours) and enable staging to be done more quickly and accurately in the laboratory due to the easily distinguished characteristics. Published egg descriptions for the same or closely related species with experimental data on temperature-development rates were aligned to the 'universal' stages (Tables 3 and 4). This allowed the published temperature-development rates to be directly comparable to the DEPM study species and to be used to assign ages to the survey eggs.

For Blue Mackerel, temperature specific development rates from Lockwood et al. (1981) and Mendiola et al. (2006) for Atlantic Mackerel (*Scomber scombrus*) were combined and used to hindcast the stage durations from the 50% temperature-based hatch times of Hunter and Kimbell (1980) for Pacific Chub Mackerel (*Scomber japonicus*). The regressions of Lockwood et al. (1981) of the time taken for an egg to reach a developmental stage in a given temperature were used to predict egg stage times at temperatures comparable to our survey temperatures (i.e. 18 to 26°C). These regressions were also applied to the incubation times of Mendiola et al. (2006). Predicted age at stage by temperature from Lockwood et al. (1981) and Mendiola et al. (2006) were averaged to produce a mean developmental rate at a given temperature. Since these temperature development rates were for Atlantic Horse Mackerel, we used hatch times for Pacific Chub Mackerel recorded at similar temperatures to our survey temperatures to calibrate expected temperature development rates for Blue Mackerel (Hunter and Kimbell 1980). Pacific Chub Mackerel are most closely related to Blue Mackerel within the *Scomber* genus (Scoles et al. 1998) and had faster temperature development rates (Hunter and Kimbell 1980) than our mean predicted rates for Atlantic Horse Mackerel. These developmental rates are used for Blue Mackerel eggs from the surveys.

Table 2: Description of 'universal' egg stages used to classify eggs of Australian Sardine, Jack Mackerel and Blue Mackerel.

Stage	Description
Stage 1	cells ≤ 64
Stage 2	cells > 64
Stage 3	blastoderm covers > 1/2 of yolk; no blastopore
Stage 4	blastopore present; head distinct; tail undefined; optic vesicles begin to differentiate
Stage 5	blastopore closed; optic cups form; somites appear
Stage 6	embryo ~1/2 around yolk; tail bulbous & just beginning to separate from yolk in late stage
Stage 7	embryo ~2/3 around yolk; tail fully separated from yolk and becomes pointed, tail still straight (no bend ('kink') in tail)
Stage 8	embryo ≤ 3/4 around yolk, head structure and caudal fin fold becoming more defined, tail 'kinked' or bent at angle
Stage 9	embryo ≥ 3/4 around yolk, head structure and caudal fin fold well developed, tail near snout
Stage 10	embryo fully developed, tail near snout (almost touches or past snout), twisted off embryonic axis just prior to hatching

Table 3: References used to align 'universal' stages with closely related species and for species-specific egg temperature-development rates.

DEPM Study Species	Egg staging Reference: Species	Egg temperature-development rates Reference: Species
Australian Sardine	Lo et al. (1996): *Sardinops sagax* White and Fletcher (1998): *S. sagax*	Lo et al. (1996): *S. sagax*
Jack Mackerel	Ahlstrom and Ball (1954): *Trachurus symmetricus* Crossland (1981): *T. declivis* Cunha et al. (2008): *Trachurus trachurus*	Cunha et al. (2008): *T. trachurus*
Blue Mackerel	Kramer (1960): *Scomber japonicus* Ward and Rogers (2007): *S. australasicus* Neira and Keane (2008): *S. australasicus*	Hunter and Kimbell (1980): *S. japonicus* Lockwood et al. (1981): *S. scombrus* Mendiola et al. (2006): *S. scombrus*

Table 4: Published egg descriptions used to stage and age eggs aligned to the 'universal' stages.

	Published egg stages transformed to universal stages			
Universal Stages	Lo et al. (1996) (Sardine)	White and Fletcher (1998) (Sardine)	Cunha et al. (2008) (Pacific Mackerel)	Lockwood et al. (1981), Mendiola et al. (2006) (Atlantic Horse Mackerel)
1	1 + 2	1 + early half 2	I	early half IA
2	early to late 3	late half 2 + 3	II	late half IA
3	very late 3 + 4	4	III + early half IV	IB
4	5	5a	late half IV + V	early to mid II
5	6	5b + 6	VI	late II
6	7	7 + 8	VII	early III
7	8 + early 9	9	VIII	mid III
8	mid 9 + early 10	10	IX	late III
9	mid to late 10	11	X	IV
10	11	12	XI	V

Egg aging

Location, sampling date/time, water temperature (sea surface temperatures measured by CTD), and depth were recorded for each egg sample. Samples were binned into three temperature bands that covered the range of temperatures sampled (14–18°C, 18–22°C, and 22–26°C). These temperature bins made the staged survey eggs comparable to the published temperature development rates. Generally, pelagic marine fish eggs of approximately 1 mm diameter (mean: 1.1 mm; range: 0.6–3.4 mm) hatch in about 48 hours at temperatures of 18-22°C, >48 hours in waters <18°C and <36 hours in waters >22°C (Pauly and Pullin 1988).

Age estimated using a known temperature-development rate

This method requires knowledge of the egg development rate from the same or closely related species at water temperatures similar to those of the surveys. It assumes eggs are spawned synchronously (see 'Estimating Spawning Time' section below). This approach assumes that: i) developmental rates do not vary among individuals, and ii) growth rates between spawning and egg capture are constant. The following approach was used to assign an age in days to each egg cohort.

1. Stages were assigned using the 'universal' egg stages. Species-specific temperature-development rates for each temperature bin (e.g. 14–18°C, 18–22°C, and 22–26°C) were used to assign a mean age to each egg (Table 4).
2. Eggs (*a*) were grouped into 'day classes' (day 0, day 1, or day 2) based on the mean age at stage being: 0 hour $\leq a <$ 24 hour (day 0), 24 hour $\leq a <$ 48 hour (day 1), 48 hour $\geq a$ (day 2).
3. A record was created for each day class at each station, either with the measured count of eggs in that day, or zero eggs.
4. The age of each day class was calculated as the count-weighted mean of ages present in that day class.
5. Where no eggs were present, the age of the day class was calculated by offsetting the adjacent day class age by 24 hours.
6. The hatch time for each temperature bin was the predicted age of a hypothetical stage 11. No zero day class records were assigned (and no eggs were collected), beyond this time period.

Age estimated from field data

The following method combines egg ages estimated from spawning time with ages estimated using multinomial models to produce a survey-specific temperature-egg development rate. Pooled data for Australian Sardine collected during DEPM surveys in South Australia from 1998–2016 were used to demonstrate the method.

Estimating egg age using survey temperature and spawning time

1. Eggs were staged using 'universal' egg stages and split into temperature bins (e.g. 14–18°C, 18–22°C, and 22–26°C).
2. Egg ages were calculated by subtracting the assumed time of spawning (2 am; see 'Estimating Spawning Time' section below) from the time of sampling for each egg.
3. The age distribution for each stage was used to infer the modal age (in hours) of each stage with a normal distribution. Kernel density plots of these distributions were made to show the progression of age at stage.
4. As pelagic, marine fish eggs tend to hatch in about 48 hours in temperatures of 18-22°C (e.g. Pauly and Pullin 1988), adjustments of 24 hours are needed to place some egg stages in the correct day. Sardine eggs hatch within 48 hours at temperatures between 18–22 °C (e.g. Lo et al. 1996, White and Fletcher 1998), so:
    a. 24 hours was added to Stages 8, 9, and 10, as each of these stages are known to be more than 24 hours old.
    b. Since Stages 5, 6, and 7 are not necessarily 24 hours old and can occur at times close to midnight, their age distributions were bimodal when inferred from sampling time. To account for this, individual adjustments of 24 hours were made to each of these stages based on the time of sampling. These adjustments were made when Stage 5 eggs were sampled prior to 8 am, Stage 6 eggs were sampled prior to 3 pm, and Stage 7 eggs were sampled prior to 8 pm. The adjusted times were determined from the original bimodal distribution for the samplings times of each stage.
5. Kernel density distributions were replotted to show the finalised progression of age at stage.

Determining modal ages for egg stages using multinomial models

Eggs were staged using the 'universal' egg stages and split into temperature bins (e.g. 14–18°C, 18–22°C, and 22–26°C). The multinomial model was applied to the field data to estimate egg age at stage. Date were considered as observations from a multinomial distribution, where the classes were the egg developmental stages (i.e. Stages 1–10 and hatched eggs) (Bernal et al. 2008). In this distribution, all sampled eggs were in a class i out of k possible classes ($i = 1, \ldots, k$), with probability, p_i as a function of age. This multinomial model was proposed by Ibaibarriaga et al. (2007) as:

$$f(n|p,N) = \frac{N!}{n_1! \ldots n_k!} p_1^{n_1} \ldots p_k^{n_k}, \quad (1)$$

Where $n = (n_1, \ldots, n_k)$ is the number of randomly sampled eggs of stage i ($i = 1, \ldots, k$), from a population of $N!$ eggs and $p = (p_1, \ldots, p_k)$ is the probability of belonging to stage i. Each probability depends on the age of the egg such that [$p_i = f$(age)].

The modal egg age of that stage was determined by calculating the mode of the resulting probability density functions (pdf) for each stage. As age was applied as a continuous variable that ranged from 0-48 hours, the multinomial pdfs of the first and last stage (Stage 1 and Stage 10, respectively) had exponential rather than normal distributions. To account for this for Stage 10, an eleventh stage was added to represent the probability of eggs hatching. This was done by adding a pre-determined normal distribution for hatching time (*N [43.5, 4.5*]) estimated *a priori* from the standard deviations of the modal egg development times in Stages 3-9 of that temperature range (18–22°C temperature bin). As the modal ages of Stage 1 and 2 were not normally distributed, age was inferred from the empirical mode of those stages.

The multinomial model was fitted using a generalised linear model (GLM) with a logit-link function and a binomial error structure using the 'nnet' package (Venables and Ripley 2002) in the R programming environment (R Core Team 2017).

Combining two egg ageing methods for final temperature development rate

The modal ages from the two field data-based methods were combined to produce the final egg age at stage progression—i.e., temperature development rate.

Limitations of estimating egg age using survey temperature and spawning time

After the 24 hour adjustments were made, egg stages were expected to have a normal distribution. However, the age distributions of some stages had wide variances that caused a large gap in the ages of Stages 5 and 6. This created a developmental lag over their age range, because the 24 hour addition increased the variation of these stage distributions and resulted in a gap of 11 hours between the modes of the two stages.

Limitations of egg ageing using multinomial models

The multinomial model pdfs are expected to be normally distributed with a constant standard deviation between stages, which is interpreted as the duration of those stages (Bernal et al. 2008). This was true for Stages 3-10, and the modal ages were similar to the empirical modes of the 'temperature and spawning time' ageing method, but had a reduced time gap between Stages 5 and 6. The multinomial age estimates offered an improvement over the empirical modes, since the bias introduced through the stage-specific 24 hour age adjustments were removed. However, the pdfs for Stages 1, 2 and 11 (hatched eggs) were exponentially distributed and could not be used to infer the modal ages for those stages. This happened because the pdfs are the probability of a given age being in stages $i - k$ (Bernal et al. 2008). The probability of being in Stage 1 was greatest at 0 hours while the probability of eggs hatching was greatest at 48 hours. Consequently, the pdfs of these stages were not used to determine the modal age.

Estimating spawning time

Mean spawning time was calculated by subtracting the age of Stages 1–3 from the collection time. Spawning times were grouped into 0.5 hour bins (e.g. -24 to -23.5 hour , -12 to -11.5 hour , 0 to 0.5 hour). The number of records were summed for each bin and histograms were plotted. The mean spawning time was the time bin with the highest peak in egg records (e.g. the time bin of 2.5 to 3 hour or 2:30 to 3:00 am).

3. Compare performance of current statistical methods on long-term datasets for several species

In this study, we compared the performance of several statistical models for estimating mean daily egg production and variance using datasets collected for Australian Sardine, Jack Mackerel and Blue Mackerel in surveys off southern and eastern Australia.

Treatment of zero count egg samples

Every egg sample for the species and temperature ranges considered in this study potentially contains egg cohorts spawned on several nights. After eggs were staged and aged, samples where no eggs were observed in any stage were excluded from the analyses (i.e. the sample was considered not to be part of the spawning area). Samples with eggs contain one of the following possible combinations:

(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.

As spawning occurs each night, a count (zero or more eggs) corresponding to each daily cohort should be present, except where cohorts are older than the hatching time.

Estimation of daily egg production

P_0 is the mean daily density of eggs produced per unit area within the spawning area (eggs·m^{-2}·day^{-1}). To estimate spawned egg density (P_0), the measured densities of egg stages in each sample are aggregated into daily cohorts that are treated as statistically independent. Where multiple stages are present in a sample, the total egg density and average age for each daily cohort is calculated by assigning each stage to a day of spawning, summing the number of eggs, and averaging their ages across stages within each daily cohort. These average ages are weighted by the number of eggs observed in each stage. The daily cohort egg densities and their average ages are used to estimate P_0.

Model descriptions

Several models were used to estimate the daily spawned egg density. The underlying model (Lasker 1985) was:

$$P(t) = P_0 e^{-z\,t} + \varepsilon \tag{2}$$

where *P(t)* is the density measured at age *t*, P_0 is the initial spawned density, *z* is the mortality rate, and *t* is the age of the sample (time since spawning), and ε is the measurement error structure. This model assumes that egg density declines exponentially with age under an assumed constant rate of egg mortality, with an additive error. The parameters of this model, P_0 and *z* can be estimated by fitting various models to sampled egg densities versus age, depending on the error structure.

Log-linear model of ln-transformed data

A linear model can be fitted to the log of the observed egg densities by logging Equation (2) (Picquelle and Stauffer 1985):

$$ln(P(t)+ 1) \sim ln(P_0) - zt + \varepsilon \tag{3}$$

where ε is assumed to be normally distributed with constant variance (which assumes the error structure on the original model is lognormally distributed). The densities are offset by 1 egg m^{-2} to prevent the case of ln(0). This offset is removed during back transformation. The regression is performed in R using a linear model fit:

$$lm(ln(P(t)+ 1) \sim ln(P_0) - zt + \varepsilon) \tag{4}$$

Because the data are logged prior to input, the parameters estimated by Equation (4), P_b ($\exp(P_0)$) and *z*, cannot simply be obtained by reversing the log transformation as $\exp(P_b)$ is a biased estimate of $\exp(P_0)$, because it does not take into account the lognormal variance of the error structure. The corrected estimate is:

$$P_0 = \exp\left(P_b + \frac{\sigma^2}{2}\right) - 1 \tag{5}$$

where σ is the standard deviation of the fitted residuals. This model is called the 'log-linear' model in the results section.

Non-linear least-squares regression

Alternatively, Equation (2) can be fit using non-linear least squares in the existing form, as

$$nls\ (P(t) \sim P_0 * exp(-z * t))$$

(6)

for which parameters P_0 and z are obtained. This assumes a normal error distribution for the densities at each age, and requires no log-inverse correction. This model is called 'non-linear least squares'.

Generalised Linear Models (GLMs)

For more complex error distributions, the data are fitted using GLMs which are a form of maximum likelihood fit. We fitted models using three different error structures: Gaussian, negative binomial and quasi. Negative binomial and quasi error structures are considered suitable for over-dispersed data, such DEPM egg density datasets (e.g Ward et al. 2011). The GLMs are written in R as:

$$glm\ (P(t) \sim t,\ family = F,\ link = \text{“}log\text{”}) \tag{7}$$

where *F* specifies either *gaussian*, *negbin* or *quasi* families. We called these models by 'Gaussian GLM', 'negative binomial GLM' and 'quasi GLM', respectively. GLMs permit the use of a link function which generalises the model form to

$$E[P_0] = g^{-1}(-z\,t + \varepsilon) \tag{8}$$

where g^{-1} specifies the inverse-link function, and $E[P_0]$ called the expected value of P_0. The estimate of P_0 is obtained by applying the inverse log link function (with no correction required).

Generalised Additive Models (GAMs)

To allow additional environmental information to be used in the analyses, GAMs were also fitted. GAMs operate differently to GLMs in that they do not fit a prescribed model, rather they generate an empirical model based on an additive smoothing function. A range of model forms for the GAM were run. We tested GAM fits that successively reduced the number of environmental variables. The form of the final chosen model was:

$$Density \sim s(Age) + s(Depth) + s(Temp) + s(Salinity) + s(Depth\ at\ chlorophyll\ maximum) \tag{9}$$

where *s*() is a LOESS smoothing function (Cleveland et al. 1992) applied individually to each data source. We called this model the 'Unconstrained GAM'. The variables are described in (Table 5). The LOESS smoothers for this fit were unconstrained—i.e., they could overfit to data by adding many 'wiggles' to the smoothers. To counteract possible over-parameterisation, a second version of the GAM was fitted where the dimension used to represent the smoothers *k* was reduced to the minimum viable value (2) and increased from this level only where necessary. This model was called the 'Constrained GAM'. We fitted a GAM where only *Age* i was used as a covariate, called 'Age-only GAM'.

Table 5: Variables from DEPM surveys used in the Generalised Additive Models (GAMs).

Variable	Description
Age	Age of egg (calculated) as hours since spawning
Depth	Bathymetric depth of sampling station (m)
Temp	Sea surface temperature (°C, obtained from CTD profile)
Salinity	Measured from CTD, averaged across depth
Depth at chlorophyll maximum	Depth where chlorophyll reaches a maximum observed level in each vertical tow (m)

Five of the above models (Log-linear, Non-linear Least Squares, Gaussian GLM, Negative Binomial GLM, Quasi GLM) were fitted to the egg samples for three species (Blue Mackerel, Jack Mackerel, Australian Sardine) and two regions (east coast and South Australia) in all available survey years. The GAM models were fitted only to data for Australian Sardine in South Australia, and the 'Unconstrained GAM' and 'Constrained GAM' were only applied in years where suitable environmental data were available.

Estimates of Mortality

Instantaneous egg mortality rate (z, day^{-1}) is estimated as free parameter in the Log-linear, Non-linear Least Squares, and all GLM models. In these models, mortality rate (rate of natural egg death through time) is assumed to be an exponential decline of density with age. Other factors that cause egg density to decline, such as diffusion, are not considered. Instantaneous egg mortality rates are known to be difficult to estimate reliably (see McGarvey and Kinloch 2001).

The z parameter was estimated using five non-GAM models for each year and species. For Australian Sardine off South Australia, additional analyses were done using both a yearly z and a common (all years) z. The common z was calculated by combining all available years of data and re-fitting the non-GAM models. The raw densities for each year were then scaled by back-correcting for mortality based on the weighted cohort age and the two estimates of mortality. The means of these scaled densities were computed to produce two mean estimates of spawn (age=0) density called "mean density, common" and "mean density, yearly". These estimates of z were also input into the GAM models to account for dependence on depth and/or temperature. Therefore, the GAM models that were fitted did not include age as a smoothed covariate, since age is accounted for through the rescaling. We refer to these as "non-age GAM" models, with the structure

$$Density * \exp[z * Age] \sim s(Depth) + s(Temp) \quad (10)$$

Computing confidence intervals of P_0 model fits

Bootstrap resampling methods were used to assess the reliability of model fits and compute confidence intervals for the estimates of P_0 (Efron and Tibshirani 1998). Bootstrapping provides a numerical method to estimate confidence intervals that require no assumptions on the distribution of the residuals.

Bootstrapped Errors

Yearly records (each combination of available region/species/year) were resampled with replacement to produce 5,000 replicates for each year. For each of these years and replicates, the models above were re-fitted and parameters re-estimated. The 2.5% and 97.5% quantiles of these parameters, including P_0, defined the 95% confidence interval.

Comparing predictive power of P_0 model estimators

Leave-One-Out (LOO) Cross Validation

The accuracy of the methods used to estimate P_0 were compared using a statistical measure of model predictive power called leave-one-out (LOO) cross validation. In broad terms, cross validation assesses how reliably each model predicts observations when those specific values are omitted from the data set. Because this method uses no information about the maximised likelihood (as AIC methods do) or does not rely on measures of model fit, it can be used to compare the predictive power of any estimators of P_0. Cross validation provides a model-independent method to compare how well models predict successively omitted values of data measurements.

Leave-one-out (LOO) cross validation excludes one data point at a time (one record, both y- and the x-values). With that data point removed from the data set, the model is re-fitted. To compute the overall predictive power of each model, the root mean squared error (RMSE) is summed over the differences of LOO-predicted with actually observed values for all data points. A lower RMSE indicates a better predictive power. We processed data for each combination of LOO-cross validation as follows: i) a single record was excluded from the n records in a given year, ii) the models were fitted to the remaining $(n - 1)$ records, iii) the removed record was predicted from the model, iv) the process was repeated for each of the remaining records, and v) the LOO-RMSE was calculated as the RMSE between the full data set and individually predicted values. This was carried out for all models, with data from each combination of region, species and year.

Jackknife Predictions

The jackknife resampling method, an older but simplified and faster method, was also applied to each data set and model. The jackknife is a linear approximation of the bootstrap. For each jackknife resample, one data point was removed, and the model re-fitted to the remaining $(n - 1)$ data points. This was repeated for

each data point successively. The confidence intervals for P_0 are estimated from the jackknife variance formula (Efron and Stein 1981) for the P_0 estimate, $\hat{P}_0$:

$$Var(\hat{P}_0) = \frac{n-1}{n}\sum_{i=1}^{n}\left(\hat{P}_{0,i} - \hat{P}_{0,jack}\right)$$

where $\hat{P}_{0,i}$ is the estimate of P_0 obtained when the i^{th} data point is omitted, $\hat{P}_{0,jack} = \frac{1}{n}\sum_{i=1}^{n}\hat{P}_{0,i}$ is the jackknife estimate of P_0, and n is the total number of data points in the DEPM egg survey sample. The final jackknife 95% confidence intervals were computed as ±1.96 times $\sqrt{Var(\hat{P}_0)}$. The jackknife will always tend to overestimate the P_0 estimate variance (Efron and Stein 1981).

4. Conduct simulations to evaluate performance of different approaches to sampling and data analysis

A computer simulation of egg production was constructed to test a number of egg distribution and sampling scenarios and the performance of the DEPM in determining the mean daily egg production (P_0). The model consisted of two parts. The first part generated a constrained random three dimensional distribution of egg concentrations within a fixed volume as a function of time; called the 'egg-space'. The second part of the model simulated a typical field survey and sampling strategy based on a vertical tow or oblique tow through the egg-space, which calculated the number of individual eggs captured at each survey site during the tow and the precise age of these eggs. The egg-space was defined as the location and time of the egg spawning locations; the concentrations of eggs were reconstructed for the time of sampling. The egg concentrations were computed by applying an analytical diffusion formula and a vertical advection formula based on the age of the eggs at the time they were sampled during the tow.

The model is continuous in space and time. This approach has the advantages that there are no errors due to binning and the calculation of egg numbers and ages for a particular tow is fast. Simulations of 1,000 surveys consisting of over 300 sites (>300,000 individual tows) were simulated in about five minutes. The speed of the model allowed for Monte-Carlo style simulations of surveys generated randomly but subject to identical parameters. Other survey scenarios could be modelled to determine sensitivity to parameters and highlight statistically significant differences in estimates of P_0.

Physical and biological parameters

The egg-space construction depends on a number of physical and biological parameters that control the random distribution of egg-spawning sites as a function of time (Table 6). The value of P_0 was specified for a particular egg-space. Then the daily number of spawning sites required to satisfy this egg density over the model domain was calculated by dividing the total number of eggs produced each day (P_0 x A) in the model domain by the mean number of eggs produced per spawning site (*EPSS*). The distribution of the spawning sites was determined by the spatial clumping coefficient (*CC*), with *CC* = 1 indicating no clumping and a uniform random distribution of sites. Stronger clumping and a smaller average separation of spawning sites occurred for smaller values of *CC*. The strength of the individual spawning sites were determined by a normal random distribution with an average value of 1 and a standard deviation determined by a coefficient of strength variability (*Sv*).

Table 6: Descriptions and values of physical and biological parameters used in the model to construct the egg space.

Physical/Biological Parameter	Description	Default Value
P_0	Nominal Mean Daily Egg Production (eggs·m^{-2})	100
Female Weight	Mean female fish weight (g)	59.42
Sex Ratio	Sex ratio	0.53
Batch Fecundity	Batch fecundity (eggs/fish)	17,525
Spawning Fraction	Spawning fraction	0.14
Spawn Group Weight	Spawning group weight (kg)	3,000
EPSS	Eggs per Spawn Source (eggs)	65,652,389
K_h	Horizontal Diffusion (m^2·s^{-1})	0.5
K_V	Vertical Diffusion (m^2·s^{-1})	2.0 x 10^{-4}
CC	Clumping Coefficient	1 (uniform distribution)
Sv	Spatial variability of P_0 between sites	0.5
Z	Egg mortality rate (day^{-1})	0.58
t_S	Daily Peak Spawning Time (time)	02:00 am
dt_S	Variability in spawning time (hours)	2.0
A	Spawning Area (km^2)	400
Z_{max}	Egg space depth (m)	70
Z_{spawn}	Mean spawning depth (m)	65
W	Mean Vertical Velocity (m^2·day^{-1})	46.67 (1.5 days to travel 70m)
R	Spawn Source radius	
N_f	Non-integer number of eggs collected per spawning site	

The time of spawning was chosen to occur once a day; the actual spawning time was a normal distribution with an average value equal to t_S and a standard deviation of $dt_S/2$. The diffusion of the egg sites was handled by an analytical diffusion equation with horizontal coefficient of diffusion (K_h) and vertical diffusion (K_h). For egg ages < K_h/R^2, the egg concentration was assumed to be uniform over the area of the spawning site, and for older eggs, the concentration was calculated based on a Gaussian solution to the 3D diffusion equation.

Egg ages were determined during sampling, and because the model set the precise time of spawning, the age is known exactly at the time of collection. In reality, egg ages are estimated from the difference between spawning time and the time of sampling and using egg staging as outlined in Ward et al. (2011) to discriminate between the 2 to 3 cohorts that may co-exist. To simulate this process when applying the DEPM, the exact ages of the eggs were used by the model to stage the eggs, then the eggs were assigned a new age based on the average age of each stage. The eggs were also assigned an age based on the difference between the time of sampling and the spawning time (assumed to be 2 am). Subtracting the offset age from the staged age showed that the errors in the offset age fall into discrete bands from -2 to 2 days (Figure 2, top panel). Correcting these errors gives an estimate of the actual egg age which has a RMSE of less than 1 hour (Figure 2, bottom panel). We use this corrected offset egg age during the DEPM so that our estimates were not influenced by knowledge of the true egg age.

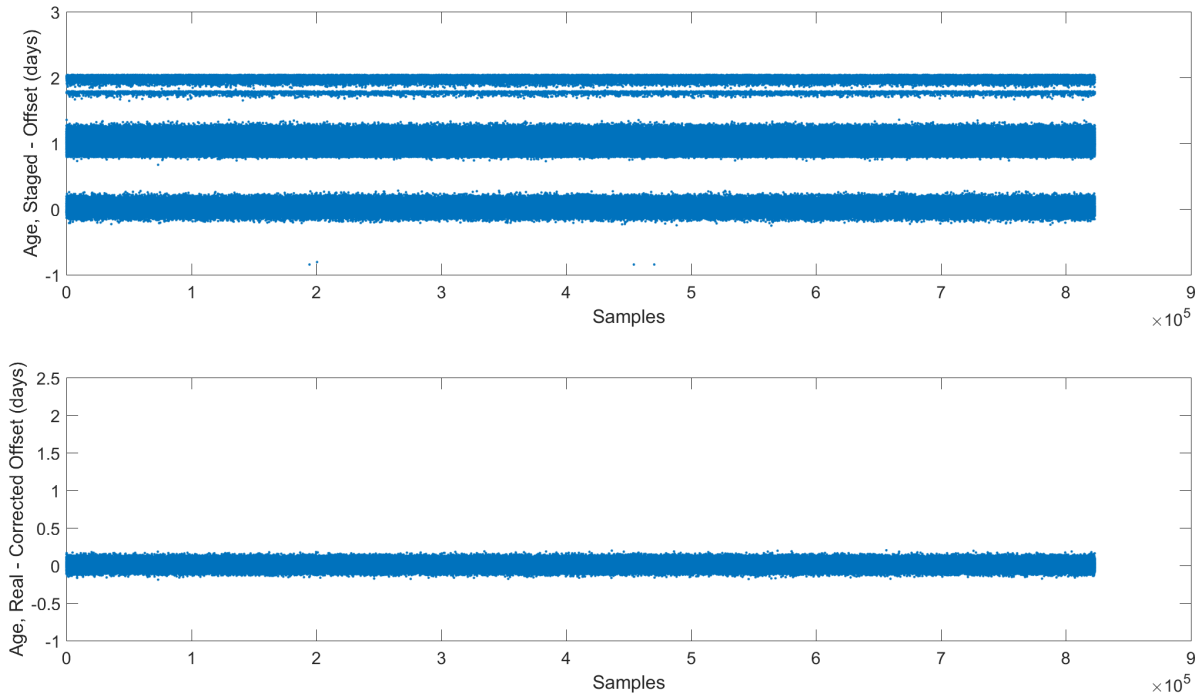

Figure 2: Simulating the DEPM egg ageing process. Top Panel: Staged egg age minus egg age determined by taking the offset between spawning time and sampling time. Bottom Panel: Actual egg age minus offset egg ages corrected with ages based on egg stages.

Egg counts were calculated during sampling by multiplying the eggs per spawning site (*EPSS*) by the diffused concentration at the sampling point to get the local egg density ($eggs \cdot m^{-2}$). The egg density was then multiplied by the actual cross sectional area of the sampling method to calculate the non-integer number of eggs collected from each spawning site (N_f). The integer number of eggs collected was derived using a statistical weighting. For example, the chances of finding an egg for N_f <0.5 was not 0 but proportional to the value of the fractional part of N_f (i.e. 10% for N_f = 0.1). This was done for all values, so for example, N_f = 4.2 eggs resulted in a 20% chance of the sample finding 5 eggs and an 80% chance of finding 4 eggs. *EPSS* was calculated by multiplying the weight of the spawning group by the sex ratio, batch fecundity and spawning fraction and dividing the result by the mean female weight (values for model parameters in Table 6). Approximate values for most of the biological parameters were based on Ward et al. (2011).

Survey Parameters

A typical sardine survey consists of approximately 300 sites sampled over ~20 days along a number of transects across the shelf (see Methods: Section 1 and Table 7). Surveys consist of sites that lie along transects where the travel times between station are relatively short (< 1 hour), and the travel times between transects are longer (> 3 hours). Random variations are allowed for both sets of travel times to simulate realistic variations in survey conditions due to, for example, tide, weather, and mechanical factors.

During the preliminary experiments, 1,000 Monte-Carlo ensembles (MCEs) were run for each scenario. This many runs allowed the sensitivity of the P_0 prediction to the scenario parameters to be statistically analysed. Each member of the ensemble was a survey with unique randomly generated characteristics. The random numbers used to generate the egg-space and survey design were based on the 'Mersenne Twister' algorithm with a unique seed used to shuffle the generator before each simulation. Both uniform and normal distributions were used, depending on the physical situation. For example, spawning times were distributed normally around the peak time, but spawning sites were initialised uniformly over the model domain. The value of P_0 used to initialise each survey was varied between sites to simulate natural spatial variability but the average over all sites was constrained to be equal to the nominal value.

Table 7: Descriptions and values of survey parameters used in model to simulate survey scenarios.

Survey Parameter	Description	Default Value
MCE	Monte-Carlo Ensembles (MCEs)	1000
N_s	Number of sites	308
N_t	Transects	22
Ns/Nt	Sites per transect	14
SSV	Site to site P_0 variability (%)	25
Method	Sample Method	Vertical Tow
D	Tow length (m)	0
NetXS	Net cross-section (m^2)	0.14
Ts	Minimum travel time between sites (hours)	0.8
dTs	Maximum added time between sites (hours)	0.4
Tt	Minimum travel time between transects (hours)	8.0
dTt	Maximum added time between transects (hours)	1.0

Results

1. Ichthyoplankton sampling

Australian Sardine egg samples collected from 14 years of DEPM surveys off South Australia form the basis for many of the analyses (4,075 samples; 33,327 total eggs; Table 8). Egg samples from Jack Mackerel (292 samples; 3,530 eggs), Blue Mackerel (261 samples; 2,330 eggs) and Australian Sardine (261 samples; 3,461 eggs) were collected during DEPM surveys off the Australian east coast in 2014 are used as companion case studies (Table 8).

Table 8: Temperature range (°C) and total number of eggs collected during DEPM surveys for South Australia (SA), southern Queensland and New South Wales (QLD/NSW:); New South Wales to Tasmania (NSW/TAS:).

Year	Species	Region	Min Temp	Max Temp	Mean temp with eggs	Total samples	Samples with eggs	Total eggs
1998	Australian Sardine	SA	16.10	23.80	19.36	164	109	2562
1999	Australian Sardine	SA	15.20	22.20	18.89	213	50	384
2000	Australian Sardine	SA	16.30	23.30	20.30	289	100	992
2001	Australian Sardine	SA	16.55	22.10	19.45	290	95	1084
2003	Australian Sardine	SA	14.45	21.89	18.76	320	97	1260
2004	Australian Sardine	SA	15.59	25.99	19.51	280	103	2570
2005	Australian Sardine	SA	17.13	21.33	19.44	323	110	1343
2006	Australian Sardine	SA	15.45	21.92	19.64	334	134	2864
2007	Australian Sardine	SA	17.15	22.98	19.97	341	141	3450
2009	Australian Sardine	SA	16.94	22.38	19.90	317	142	2266
2011	Australian Sardine	SA	15.58	24.73	19.34	339	120	2484
2013	Australian Sardine	SA	19.11	22.79	20.62	327	105	2142
2014	Australian Sardine	SA	14.69	22.13	18.89	355	196	7955
2016	Australian Sardine	SA	14.11	22.47	19.59	347	137	1971
2014	Blue Mackerel	QLD/NSW	16.18	22.32	20.18	261	70	2330
2014	Australian Sardine	QLD/NSW	16.18	22.32	19.44	261	89	3461
2014	Jack Mackerel	NSW/TAS	14.32	25.75	17.81	292	117	3530

2. Improve methods used to determine the age of egg cohorts

Egg staging

Representative images of the 'universal' egg stages of Blue Mackerel, Jack Mackerel and Australian Sardine are shown in Figure 3. The 'universal' egg stages were also applied to species with published egg descriptions and experimental data on temperature egg-development rates (Table 4). Egg development was approximately linear over time (i.e. eggs remained in each stage for a similar period; Figure 4). The 'universal' egg stages do not have the mix of short and long durations which characterise some of the egg stages described in the original publications (e.g. the original versus the 'universal' *Scomber* spp. proxy for Blue Mackerel, Figure 4).

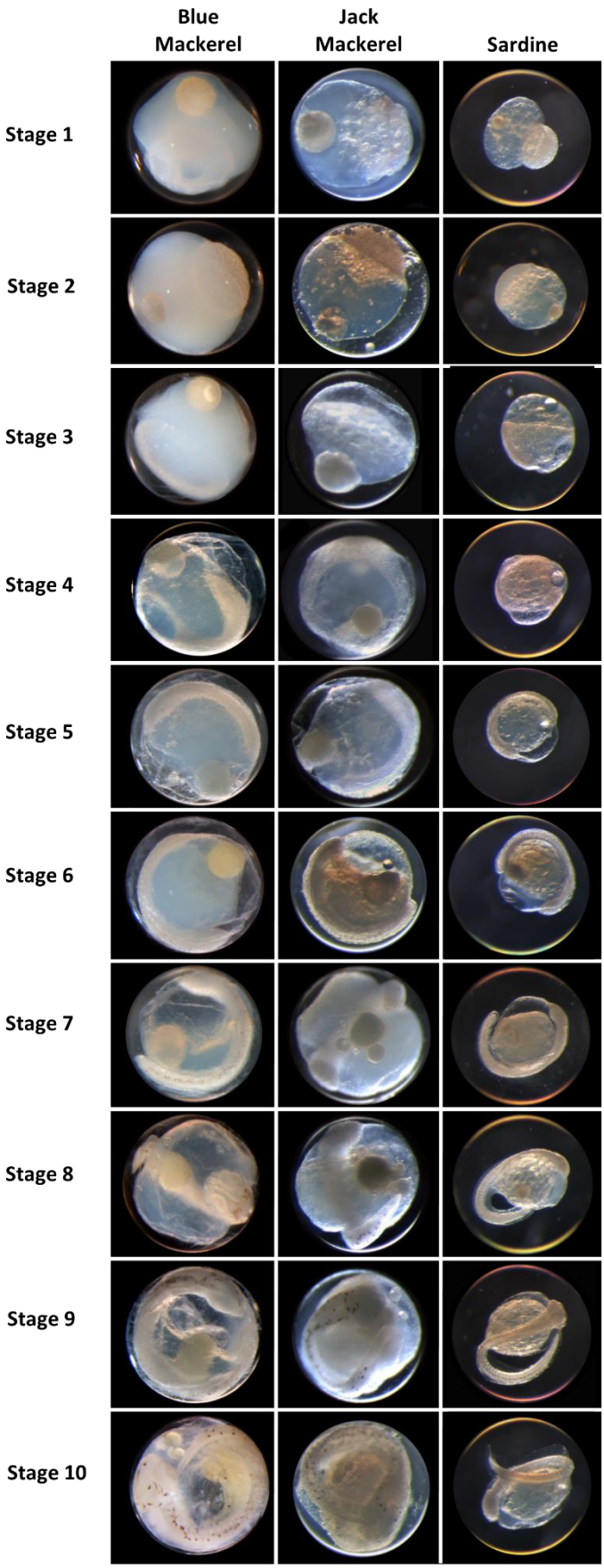

Figure 3: Eggs of Blue Mackerel, Jack Mackerel and Australian Sardine partitioned into 'universal' egg stages. See Table 2 for generic descriptions of each stage.

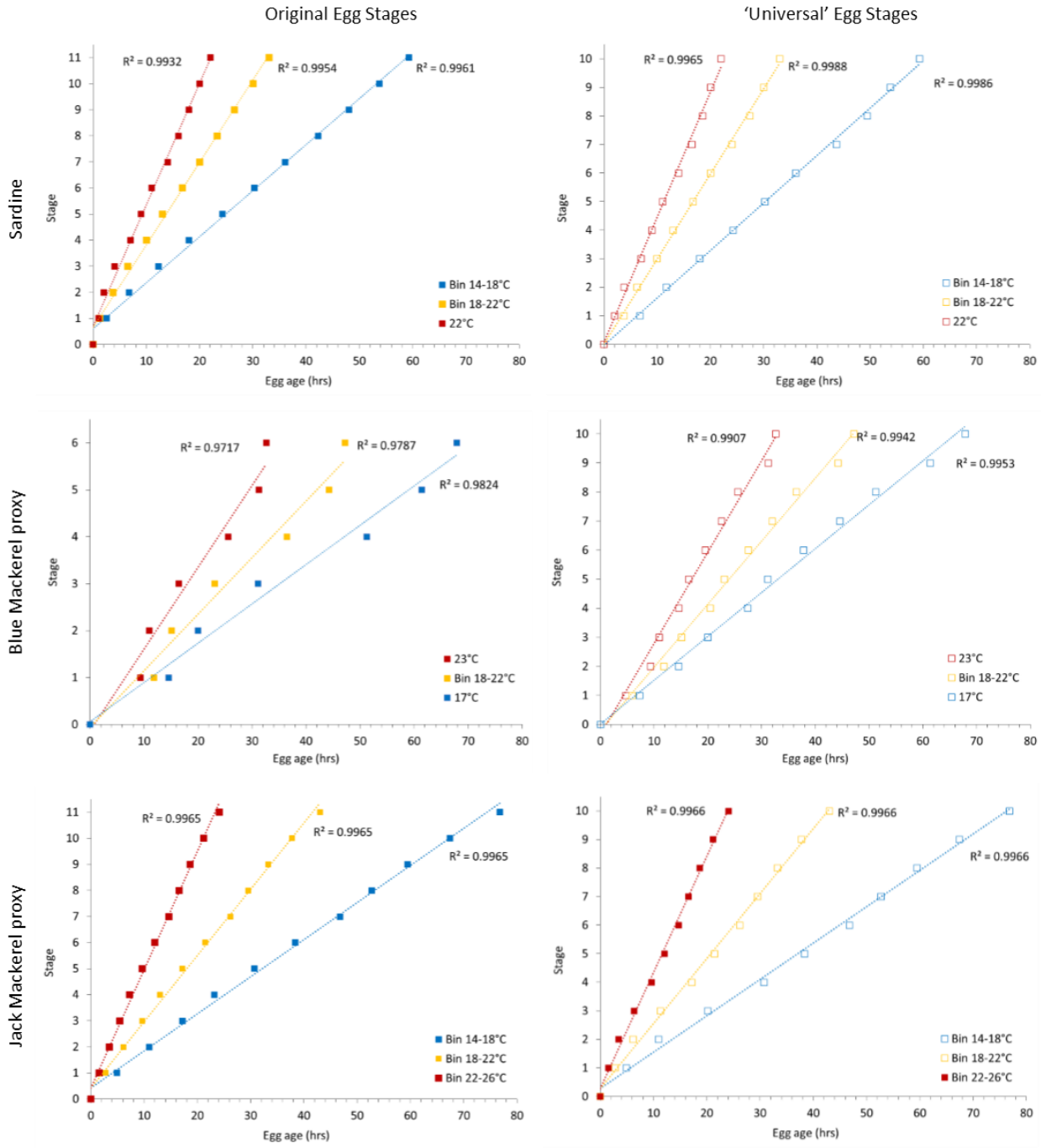

Figure 4: Published temperature egg-development rates from laboratory experiments with varying egg staging schemes (left column) converted to 'universal' egg stages (right column). Experimental temperatures have been binned to correspond with DEPM temperature bins of the current study (14–18°C, 18–22°C, and 22–26°C). Original data sources: Sardine (Lo et al. 1996); Blue Mackerel proxy are combined data (Atlantic Mackerel: Lockwood et al. (1981) and Mendiola et al. (2006); Pacific Chub Mackerel: Hunter and Kimbell (1980)); Jack Mackerel proxy (Atlantic Horse Mackerel: Cunha et al. (2008)). R^2 values show the linear relationships of the egg stages at a certain temperature or bin.

Egg ageing

Mean temperatures where eggs were collected during a survey differed for the three fish species (Table 8). Pooling egg samples into the temperature bins (14–18°C, 18–22°C, and 22–26°C) revealed there were relatively few samples in the warm band (22–26°C) for any survey.

Age using a known temperature-development rate

Temperature egg development rates based on published data for Australian Sardine (Lo et al. 1996), Blue Mackerel (Hunter and Kimbell 1980, Lockwood et al. 1981, Mendiola et al. 2006) and Jack Mackerel (Cunha et al. 2008) are shown in Figure 4 and Tables 3 and 4.

Most Australian Sardine eggs from South Australia were collected from the moderate (18–22°C) and cool (14–18°C) temperature water (overall mean: 19.5°C; Figure 5, Table 8). Australian Sardine eggs from the east coast were mainly collected in moderate waters (18–22°C; survey mean: 19.4°C). The majority of Blue Mackerel eggs were collected in moderate water temperatures (18–22°C, survey mean: 20.2 °C), while most Jack Mackerel eggs were collected in cooler water temperatures (14–18°C, survey mean: 17.8 °C). Cooler water temperatures (14–18°C) cause egg development rates to slow, and eggs to take over 60 hours to hatch (e.g. Australian Sardine, Figure 5 top). Conversely, eggs hatch in <24 hours in warm temperatures (22–26°C, Figure 5 bottom).

Inter-annual variation of water temperature was reflected in year to year variation of Australian Sardine egg densities in the different temperature bins, which translates into annual variation of egg age (Figure 6). Years where higher densities of eggs were collected in cooler water had higher proportions of older egg ages (e.g. 2014; 43% eggs > 36 hours old) than years with more eggs in warmer temperatures (e.g. 2013; 0% eggs > 36 hours old) (Figure 6).

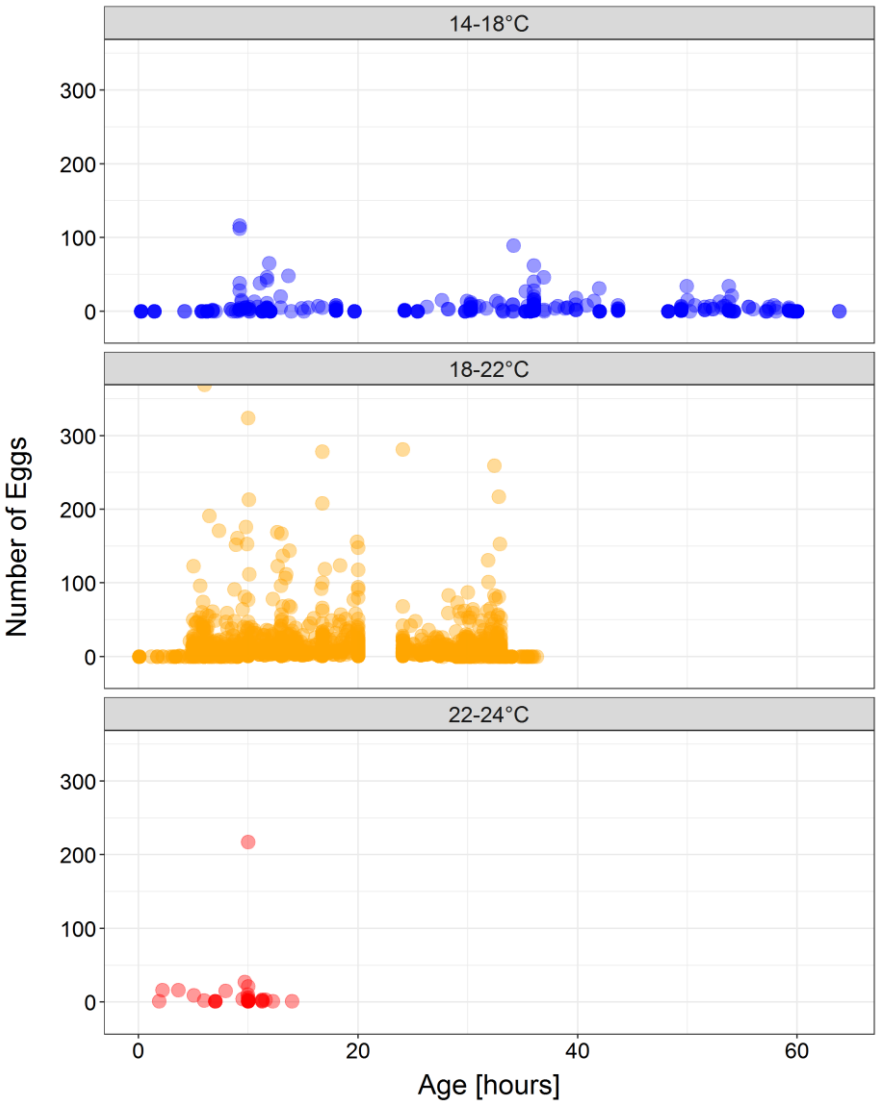

Figure 5: Total counts of Australian Sardine eggs by age (hours) in each of the temperature bins collected during DEPM surveys along the coast of South Australia (all years combined: 1998–2016). Sample sizes are shown in Table 8.

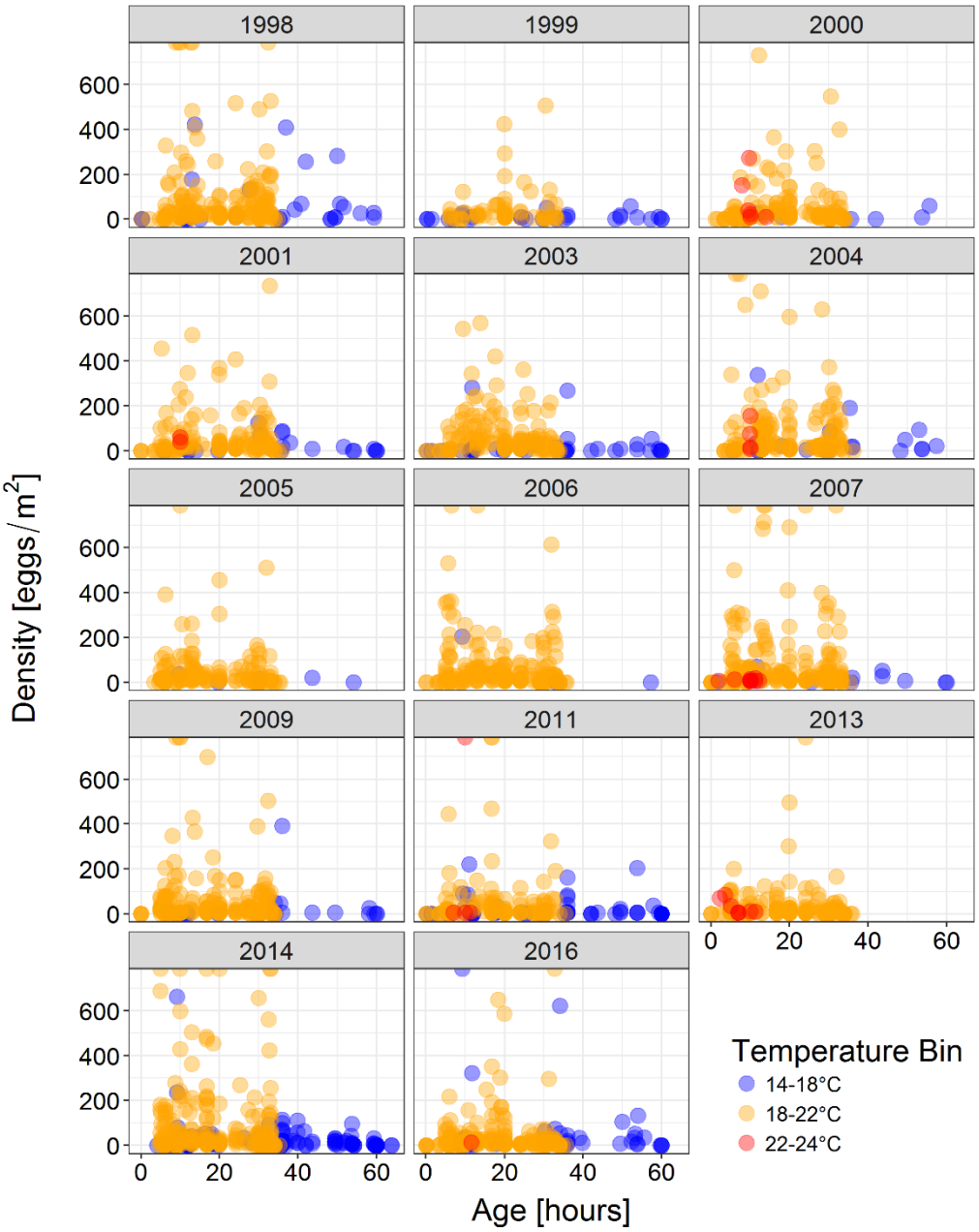

Figure 6: Densities of Australian Sardine eggs (eggs·m^{-2}) by age (hours) in each of the temperature bins from DEPM surveys along the coast of South Australia from 1998–2016. Densities points not shown fully at top of plots are high values between 700 and 11,700 eggs·m^{-2}. Sample sizes are shown in Table 8.

Egg age from field data

Pooled data for Australian Sardine eggs collected in moderate water temperatures (18–22°C) during DEPM surveys in South Australia from 1998–2016 were used to produce a survey specific temperature development rate to age the eggs. This temperature development rate combines estimates of modal age at stage from two different analyses. The final stage at age estimates for Australian Sardine were best described by combining the empirical age at stage modes from Stages 1, 2 and 11 (hatch time) with the multinomial modal age at stage estimates from Stages 3-10 (Table 9).

Table 9: Estimates of age at stage (hours) for Australian Sardine at temperatures between 18–22 °C produced from field data collected during DEPM surveys South Australia from 1998–2016 and incubation experiments by Lo et al. (1996). * Indicates estimates that are included as the final age at stage estimates for South Australian Sardines.

Stage	Empirical estimates (hours)	Multinomial estimates (hours)	Lo et al. (1996) estimates (hours)
1	2.7*	0.0	3.8
2	4.7*	1.3	6.2
3	10.0	11.1*	10
4	14.6	14.0*	13
5	15.3	17.4*	16.8
6	26.5	23.1*	20
7	31.1	28.5*	24.1
8	33.3	32.5*	27.4
9	34.1	36.7*	30
10	39.6	38.3*	33
Hatch time	43.6*	48.0	36.3

Estimating egg age using survey temperature and spawning time

After calculating egg age from spawning time, the stage-specific egg density data for Australian Sardine showed a clear modal progression across the 48 hour age range for the empirical distributions (Figure 7). Once 24 hour adjustments had been made to Stages 5–10, each stage was normally distributed (Figure 7). However, the wide distribution of some stages caused a large gap between the empirical modal ages of Stages 5 and 6, creating a developmental lag over their age range (Figure 7). This happened because the 24 hour addition increased the variation of these stage distributions, resulting in a gap of 11 hours between the modes of the two stages (Table 9).

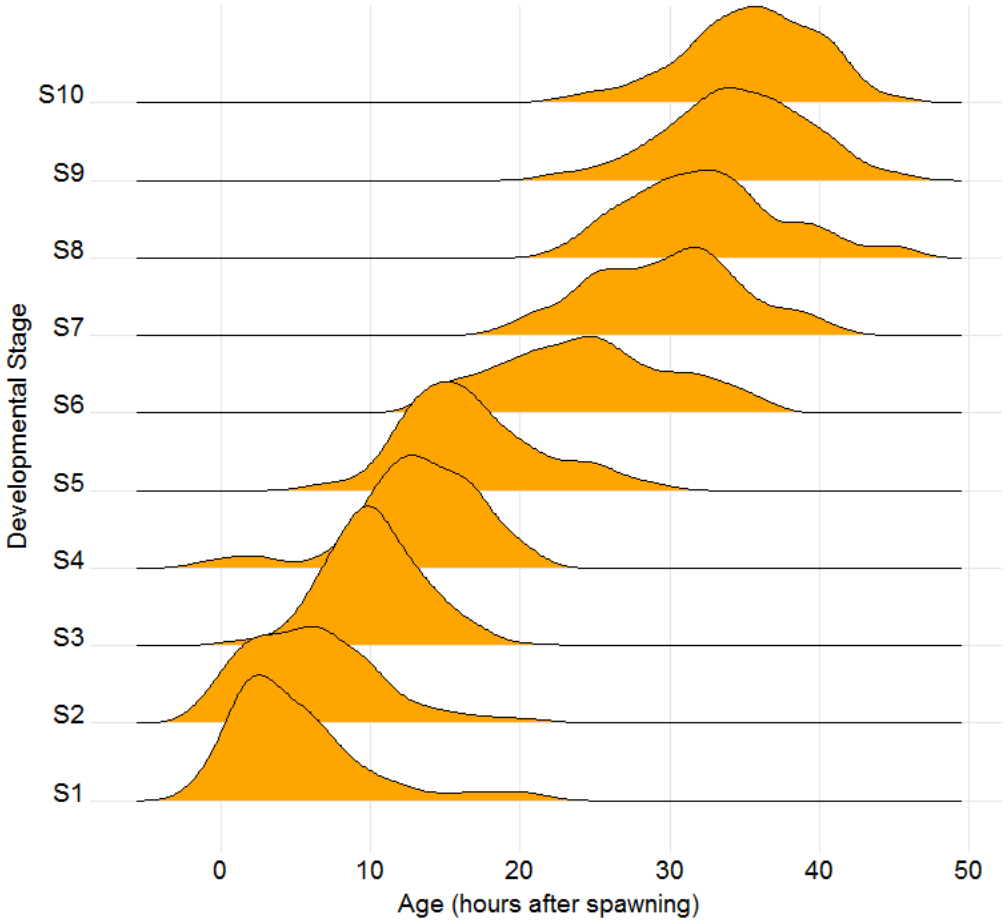

Figure 7: Kernel density distributions of age at stage for Australian Sardines estimated from South Australian field data. Data are pooled for all sampling years from 1998–2016 and only includes eggs collected in moderate water temperatures (18–22 °C).

Determining modal ages for egg stages using multinomial models

Using a multinomial model to age Australian Sardine eggs also produced a clear modal progression across the 48 hour age range when the stage-specific egg density data were plotted (Figure 8). The multinomial model pdfs for Stages 3–10 were normally distributed with a consistent standard deviation of 4.5–5 hours between stages, which represented stage duration (Figure 8). The modal ages produced by this model had a reduced gap between Stages 5 and 6 compared to the age estimates of the empirical modes. The multinomial model removed the bias introduced by the stage-specific 24 hour age adjustments and provides a better age estimate relative to the empirical modes. However, the pdfs for Stages 1, 2 and 11 (hatched eggs) were exponentially distributed and could not be used to infer modal ages for those stages. This was because the probability of being in Stage 1 was greatest at age 0 while the probability of eggs hatching was greatest at 48 hours. Consequently, the pdfs of these stages could not be used to determine their modal age. The final age at stage estimates for Australian Sardine eggs were best described by a combination of the empirical age at stage modes and the estimates from the multinomial model (Table 8).

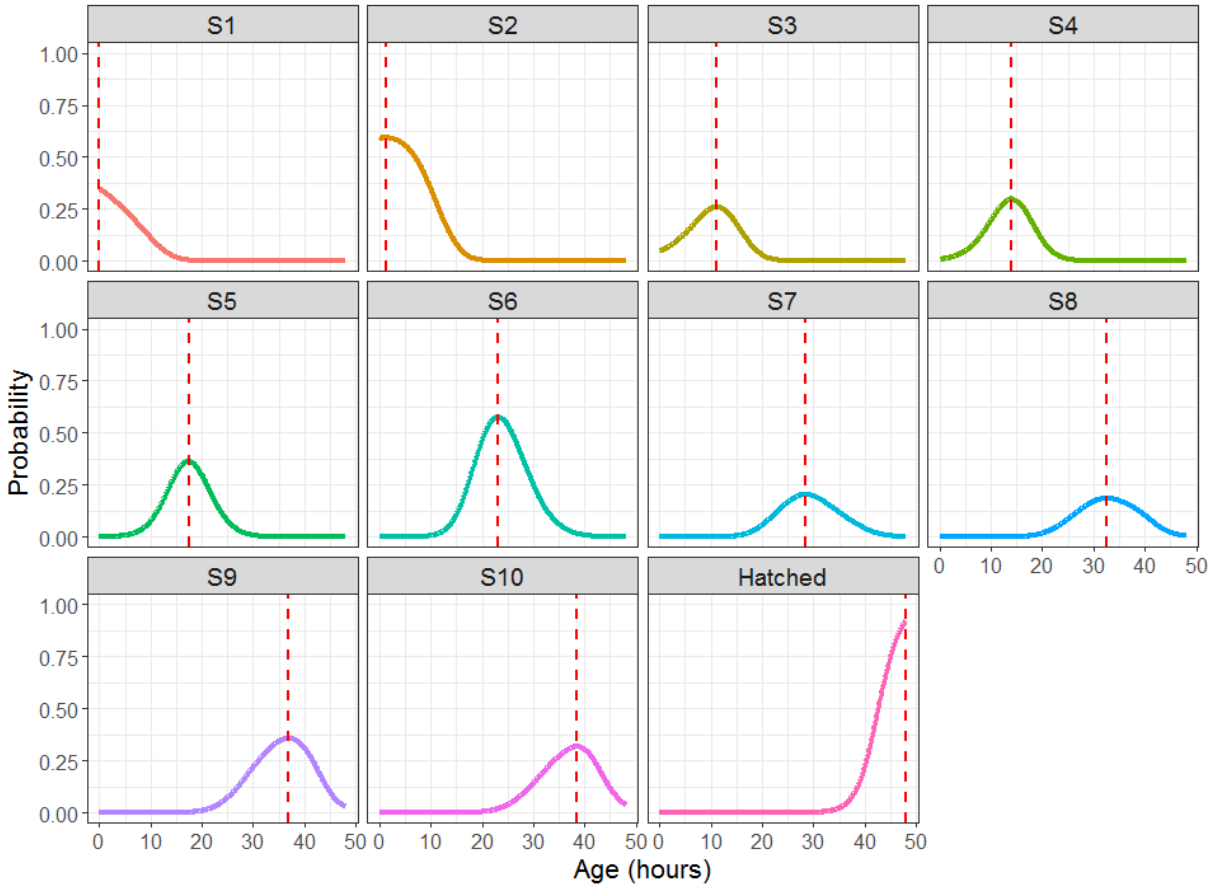

Figure 8: Probability density functions for egg development stages of Australian Sardines from South Australia produced using a multinomial model. Data were pooled for all sampling years from 1998–2016 and only includes eggs collected in moderate water temperatures (18–22 °C). Red dashed lines are the modal age of each stage.

The final stage at age estimates for Australian Sardine that combined the empirical age at stage modes from Stages 1, 2 and 11 (hatch time) with the multinomial modal age at stage estimates from Stages 3-10 produced a temperature development rate that was similar to the laboratory-based rates reported in Lo et al. (1996) (Figure 9). There was some variation between the rate estimated from field data and that of Lo et al. (1996) for the older stages (5–10). However, these results demonstrate that the field data contain reliable and useful information on egg age that can be used in assessments for South Australian Sardine. These results also highlight the potential for local data to be used for other fish stocks to provide regional egg age at stage estimates that may be preferable to surrogate information from other sources.

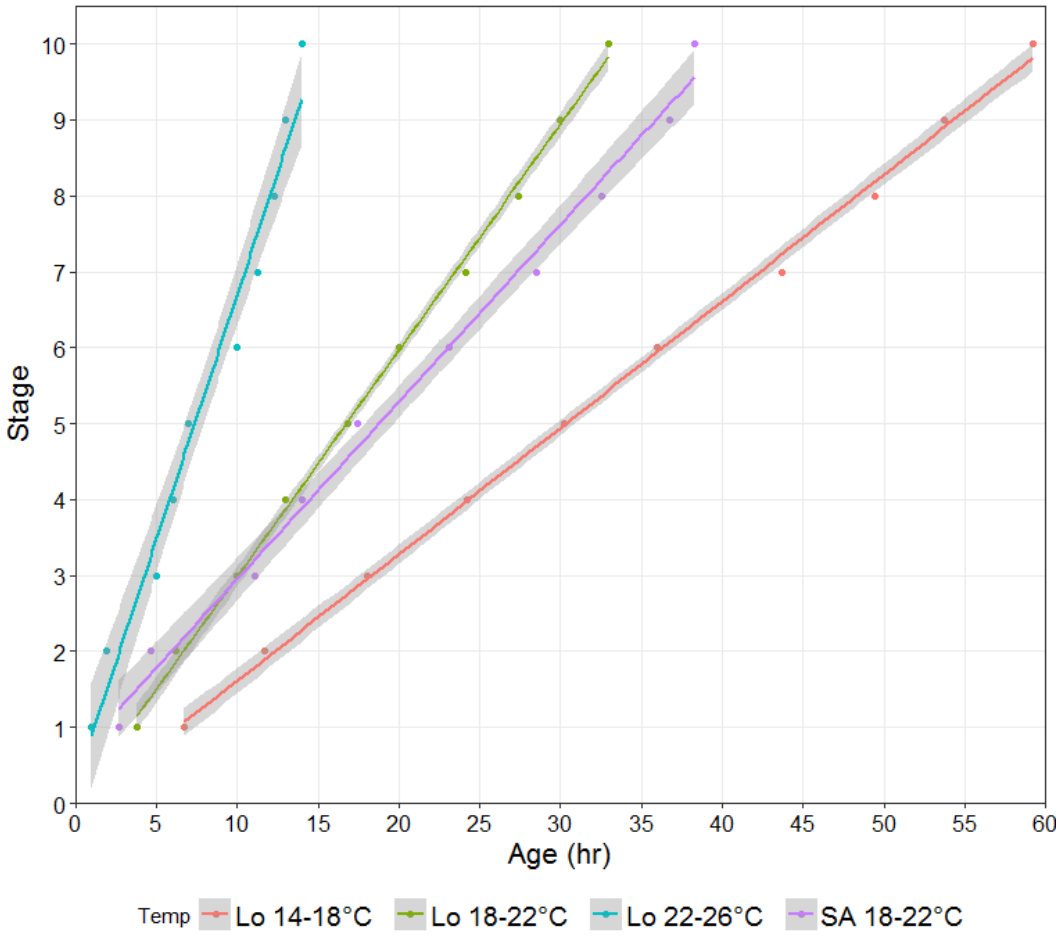

Figure 9: Comparison of the final egg age at stage estimates, i.e. temperature development rate, for Australian Sardine from South Australia in moderate water temperatures (18–22 °C) estimated from field data versus laboratory-based rates for Sardine reported by Lo et al. (1996).

Estimating spawning time

Temperature-based, peak spawning times for Australian Sardine off South Australia were estimated from 747 records of eggs in Stages 1–3 collected during 14 surveys from 1998–2016 (Figure 10). The peak spawning time for all years combine was around 2:30 am (Figure 10). The peak spawning time for Blue Mackerel collected off the Australian east coast during a DEPM survey in 2014 was between 2:00 pm and 9:00 pm (n = 16 egg records; Figure 11). Jack Mackerel collected during a DEPM survey in south-eastern Australian waters in 2014 spawned between 8:00 pm and 2 am (n = 78 egg records; Figure 12). Australian Sardine collected in a DEPM survey off eastern Australia in 2014 spawned at ~ 6:00 pm (n = 39 egg records; Figure 13). Estimates of peak spawning time for Blue Mackerel, Jack Mackerel and Australian Sardine off eastern and south-eastern Australia should be used cautiously, since they are inferred from limited data.

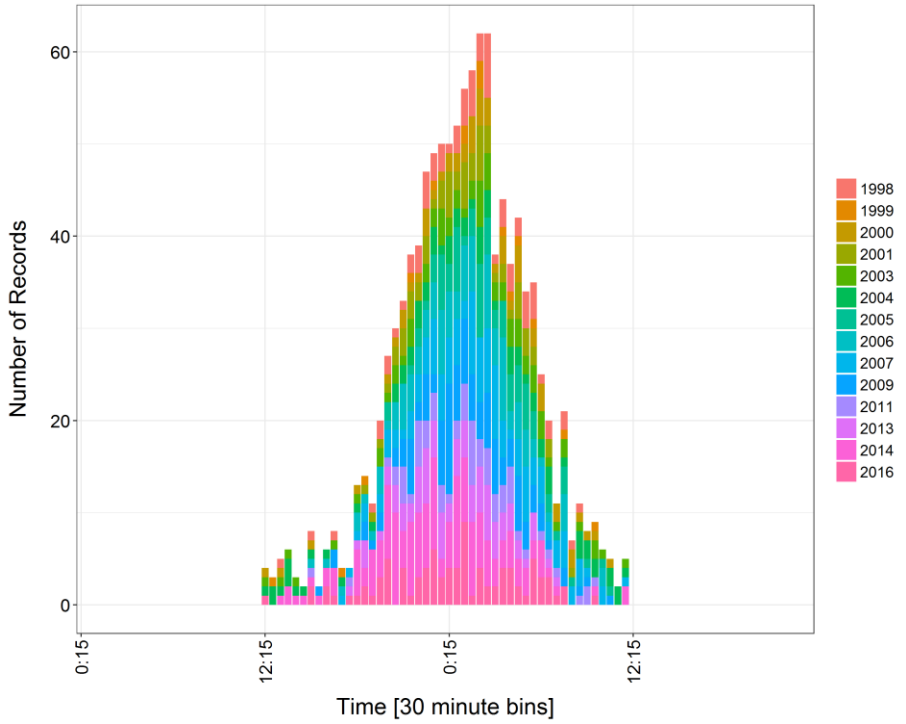

Figure 10: Spawning times estimated from eggs of Australian Sardine (South Australia) in Stages 1-3 binned into 30 minute increments. The histogram peak is the estimated mean spawning time. Sardine egg data came from DEPM surveys along the South Australian coast from 1998–2016.

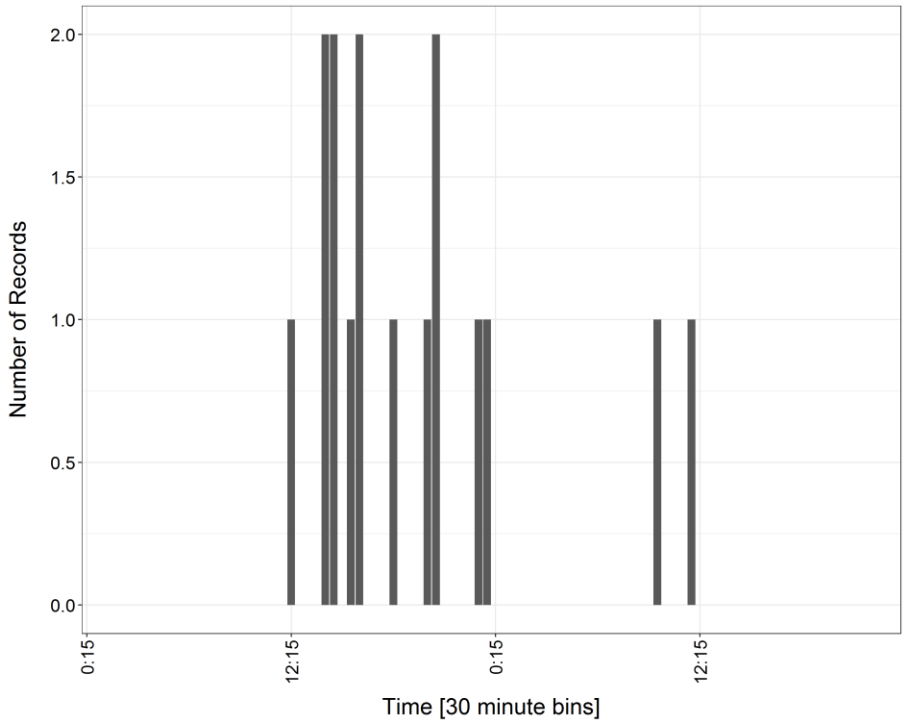

Figure 11: Plots of total records with eggs in Stage 1–3 binned into 30 minute increments of sampling time to infer peak spawning time of Blue Mackerel. Egg data were collected during a 2014 DEPM survey along the east coast of Australia.

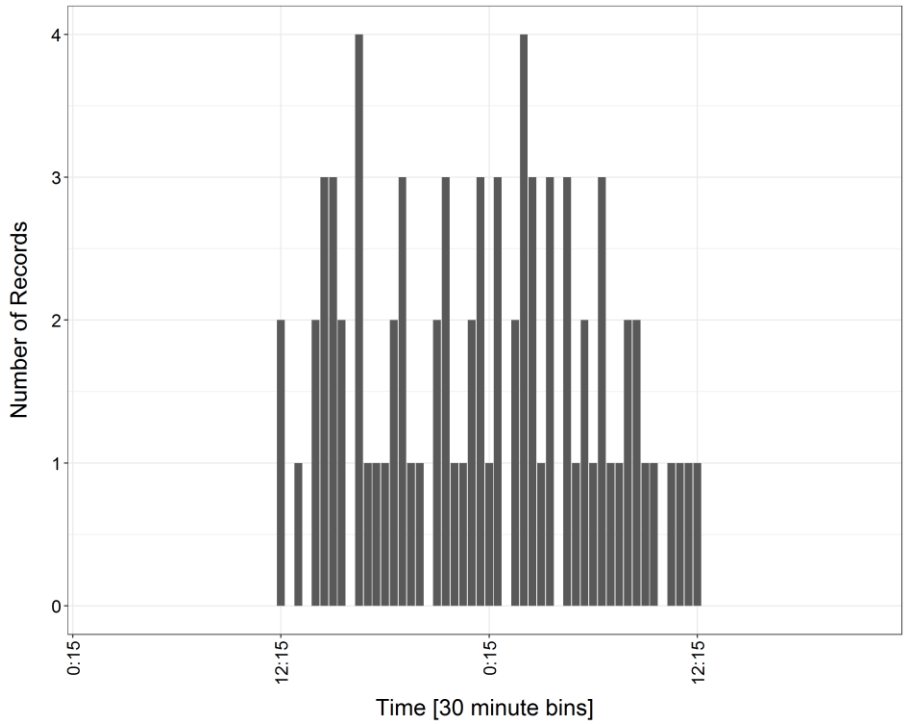

Figure 12: Plots of total records with eggs in Stage 1–3 binned into 30 minute increments of sampling time to infer peak spawning time of Jack Mackerel. Egg data were collected during a 2014 DEPM survey along the southeast coast of Australia.

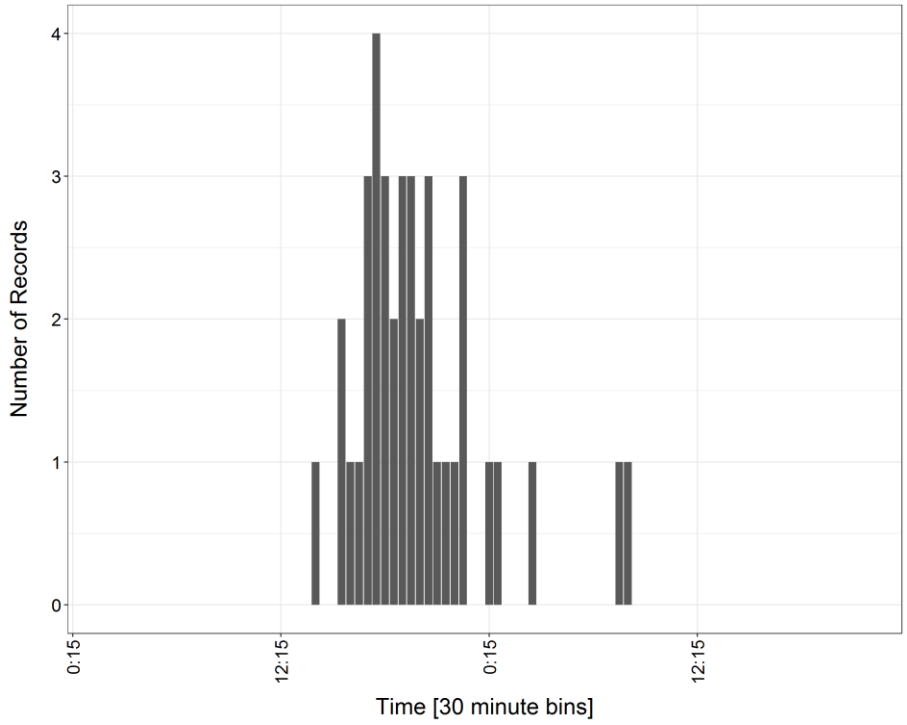

Figure 13: Plots of total records with eggs in Stage 1–3 binned into 30 minute increments of sampling time to infer peak spawning time of Australian Sardine (east coast). Egg data were collected during a 2014 DEPM survey along the east coast of Australia.

3. Compare performance of current statistical methods on long-term datasets for several species

All statistical analyses in this section use egg ages estimated with known temperature-development rates. Egg densities and estimates of egg production calculated using egg ages estimated directly from the field data are shown in Appendix A.

Data Exploration

Mean and median egg densities by age (four hour bins) for Australian Sardine collected during DEPM surveys off South Australia varied among and within years (Figure 14). Mean annual egg densities ranged from 41.6 (95% CI: 25.9–57.4) to 194.1 eggs·m^{-2} (95% CI:-8.0–380.3). Median densities of eggs <8 hours old were generally lower than other groups, suggesting that young eggs are under-represented in the samples (Figure 14). Densities of older eggs mostly decline over time as expected under the assumed exponential mortality model. Where mean density (Figure 14, blue dots) exceeds the median, one or a few high egg density values skewed the distribution upward. For example, the 4-8 hour age bin in 2014 has 6 samples with egg densities >500 eggs·m^{-2} that resulted in a mean density of 257.1 eggs·m^{-2} versus the median density of 24.6 eggs·m^{-2}.

Egg production: non-GAM models

Australian Sardine (South Australia)

Estimates of egg production (P_0, eggs·m^{-2}·day^{-1}) varied among years and models (Figures 15 and 16). Based on yearly estimates of *z*, the log-linear model produced estimates of P_0 that were (in most years) lower (i.e. 35.4 to 107.3) than the four other non-GAM models. All models, other than the log-linear model, produced unrealistically high estimates of egg production (i.e. > 420 eggs·m^{-2}·day^{-1}) in some years. The negative binomial and quasi GLM gave more plausible estimates of P_0 than the non-linear least squares model and the Gaussian GLM in most years. However, both the negative binomial and quasi GLMs produced implausible estimates of P_0 in 2013 and 2014.

All of the models produced implausible (negative) estimates of *z* in at least one of the 14 years (Figure 15). Using a common *z* usually produced higher estimates of P_0 than were obtained using the yearly estimates of *z* obtained from the log-linear model. The estimate of P_0 obtained using a common *z* were higher in 2014 (i.e. ~200 eggs·m^{-2}·day^{-1}) than what is usually considered plausible (i.e. up to ~120 eggs·m^{-2}·day^{-1}) and considerably higher than obtained by applying the log-linear model to the data. These inflated values are due in part to the strong influence that samples with large numbers of eggs have on the estimates of P_0 obtained using this approach.

The 95% CI of the estimates of P_0 are shown in Figure 17. The 95% CI of the estimates of P_0 obtained using the linear model are lower than or similar to the other models in all years (Figure 16). In some years,

such as 2014, the 95% CIs for all models, except the log-linear model were much greater than the mean of the estimate of P_0 (Figure 17).

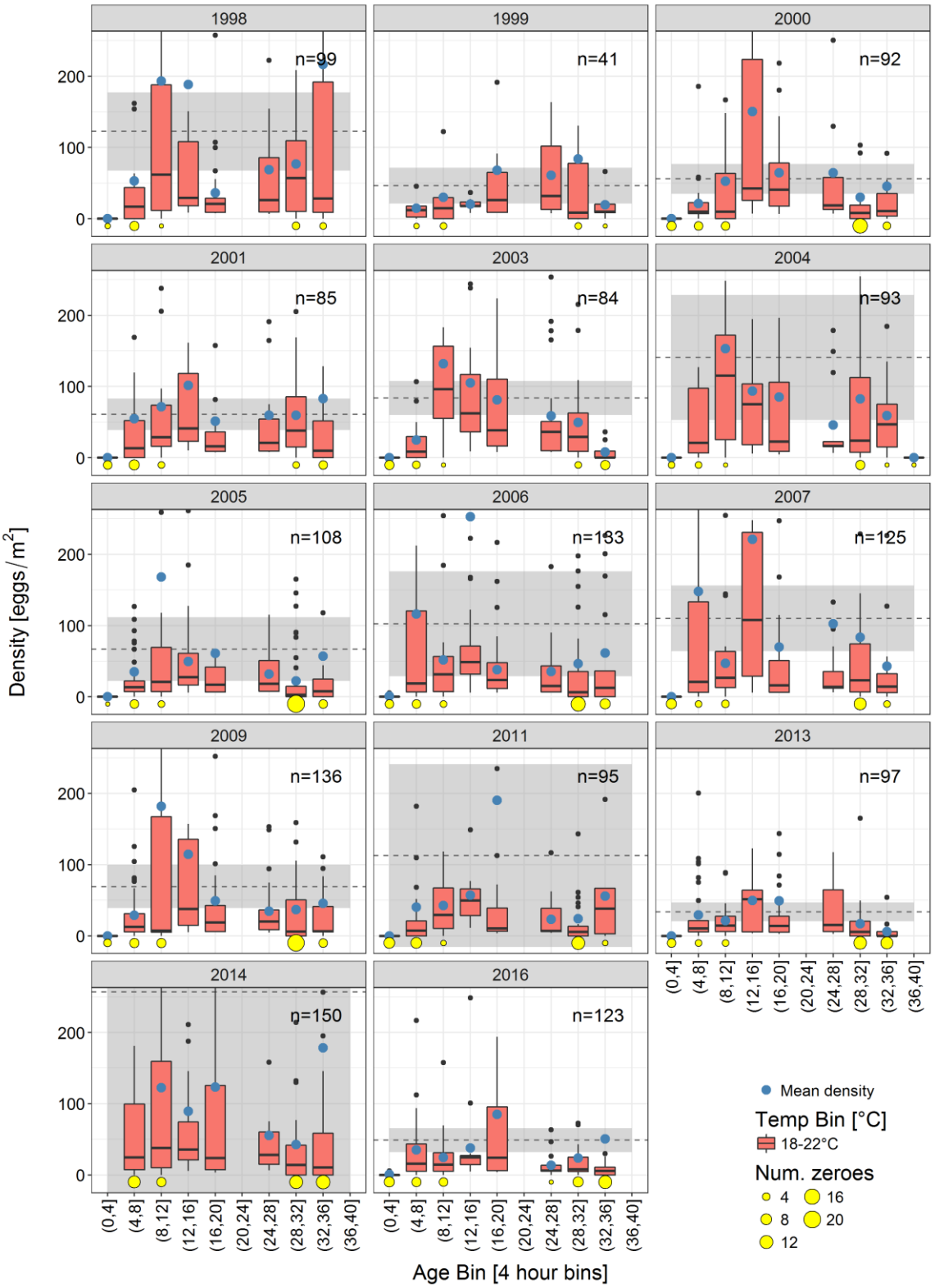

Figure 14: Egg densities by age (4 hour bins) of Australian Sardines (South Australia) collected during DEPM surveys from 1998 to 2016. Yellow dots: number of zero counts in daily cohort; *n*: number of density points per year; dashed line and shading: mean egg density for all values with 95% CI.

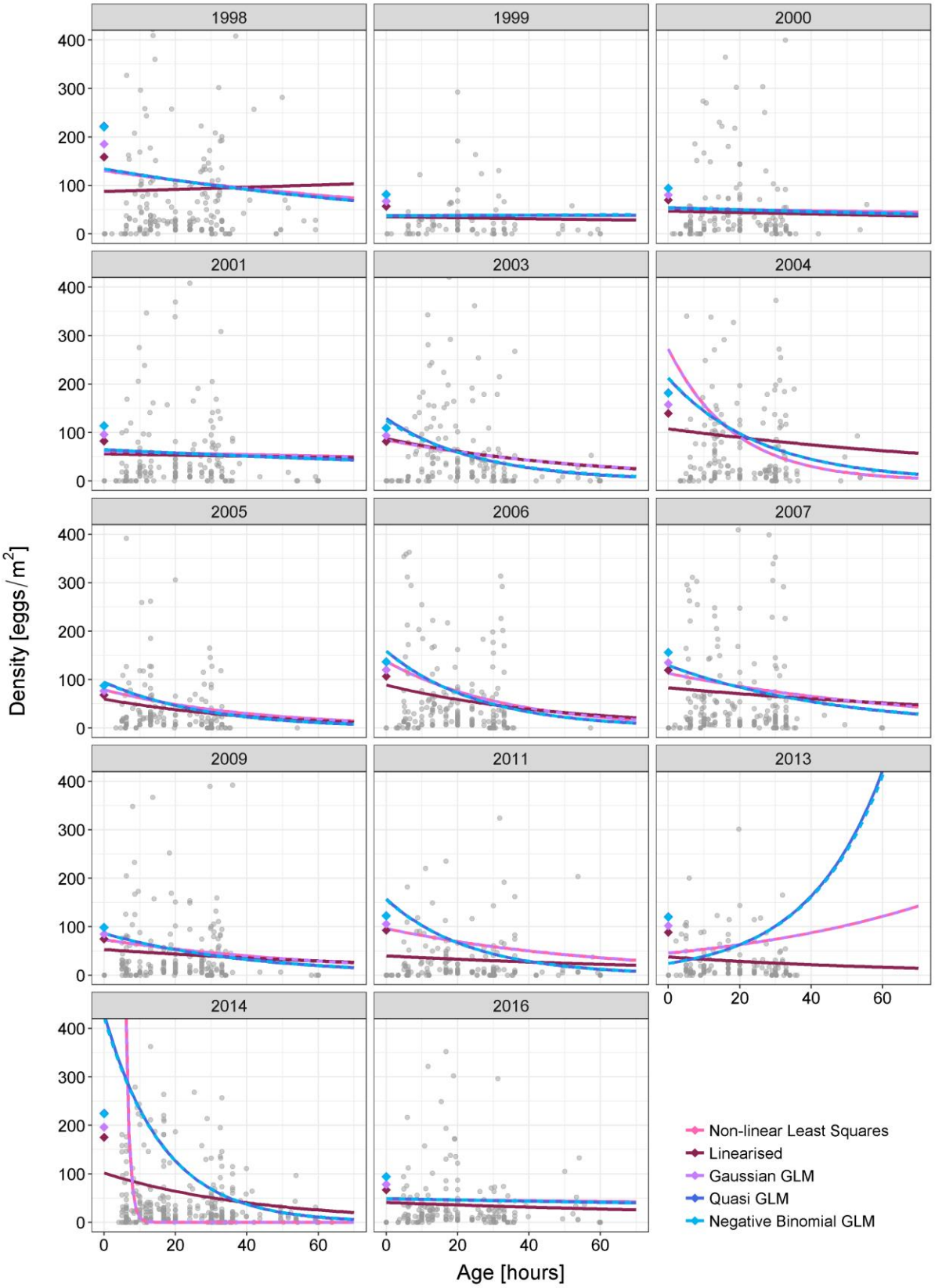

Figure 15: Fits of non-GAM models by year to data of measured DEPM survey egg densities for Australian Sardine (South Australia) by cohort and age. Diamonds: values of P_0 predicted using an all-years mortality obtained by fitting each non-GAM model to an all-years combined data set. Linearised: log-linear model.

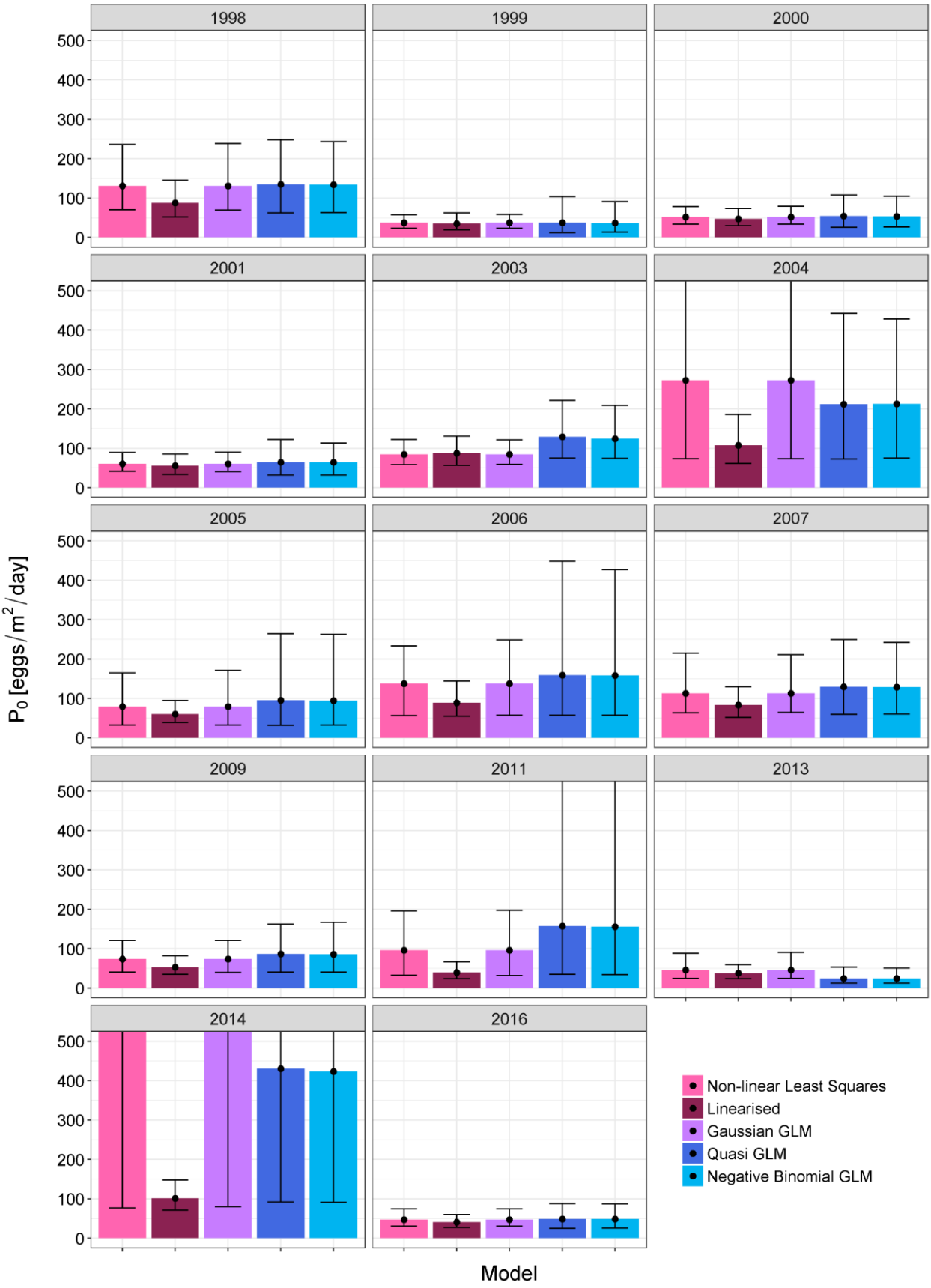

Figure 16: Estimates of P_0 using non-GAM models for Australian Sardine (South Australia) by year. 95% CI: quantiles of 5,000 bootstrap resamples. Linearised: log-linear model.

Blue Mackerel, Jack Mackerel and Australian Sardine (eastern and south-eastern Australia)

The fits of the five non-GAM models varied among species, regions and models (Figure 17). Estimates of P_0 using a yearly mortality differed between species and regions and models within a survey (Figure 18). Estimates of P_0 ranged from 7.1 to 21.6 for Blue Mackerel, 15.8 to 21.8 for Jack Mackerel, and 37.1 to 42.9 for Australian Sardine. An all-years mortality for each species could not be estimated, since only one year's worth of data have been collected to date.

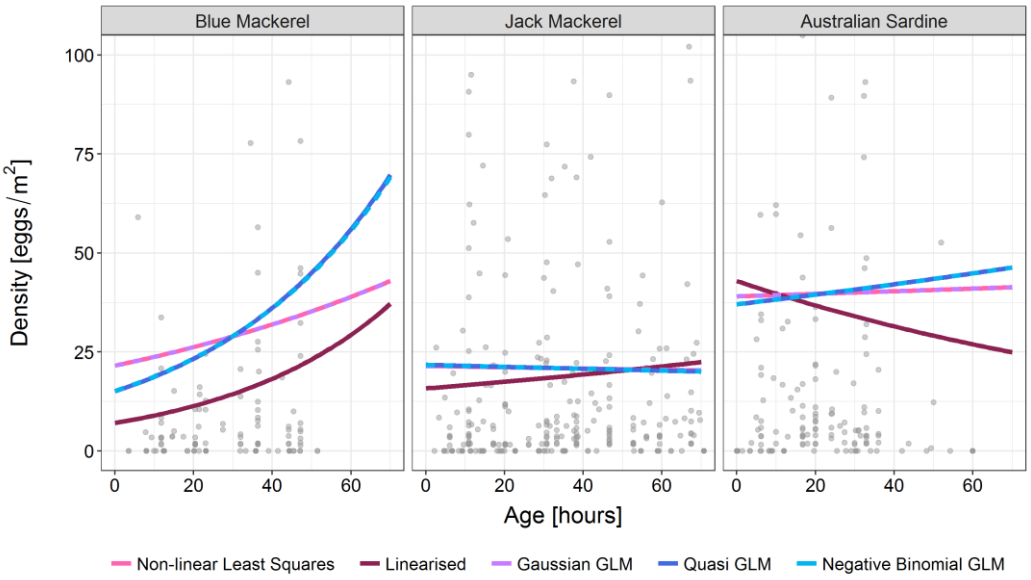

Figure 17: Fits of non-GAM models to data of measured DEPM survey egg densities for Blue Mackerel, Jack Mackerel and Australian Sardine off eastern and south-eastern Australia by cohort and age. Linearised: log-linear model.

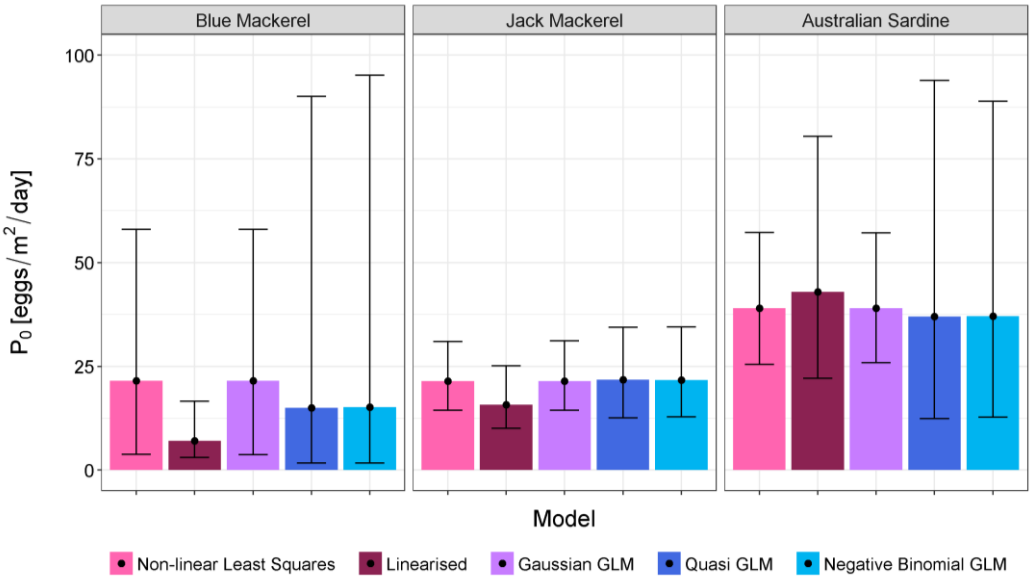

Figure 18: Estimates of P_0 using non-GAM models for Blue Mackerel, Jack Mackerel and Australian Sardine off eastern and south-eastern Australia. 95% CI: quantiles of 5,000 bootstrap resamples. Linearised: log-linear model.

Estimates of Mortality

Australian Sardine (South Australia)

Estimates of instantaneous egg mortality rate (z, day^{-1}) were variable between years and among models within each year (Figure 19). All of the models produced implausible (negative) estimates of z in at least one of the 14 years. The log-linear model produced yearly z estimates that ranged from -0.06 to 0.55. The non-linear least squares model gave z estimates ranging from -0.39 to 23.51. The Gaussian GLM estimates of z ranged from -0.39 to 23.52. The negative binomial GLM returned z values between -1.13 and 1.45. Estimates of z from the quasi GLM ranged from -1.14 to 1.47.

The 95% CIs for the estimates of z obtained using the log-linear model were similar to or narrower than those for the other models (Figure 19). The 95% CIs for all models except the log-linear model were much larger than the mean estimate of z in some years (e.g. 2004, 2013, 2014). Differences among z estimates (i.e. Figure 19) can cause substantial differences in the mortality correction back to the inferred density of eggs spawned (P_0). For example, z=0.3 gives a correction over one day of exp(0.3)=1.35, i.e. 35%, while z=1.5 produces a 350% correction. Very large densities of young eggs cause some models to produce unrealistically high values of z, and therefore unrealistic P_0 values (e.g. 2014, Figures 15 and 19). High densities of older eggs relative to younger eggs cause negative z values in some models, resulting in very low estimates of P_0 (e.g. 2013, Figures 15 and 19).

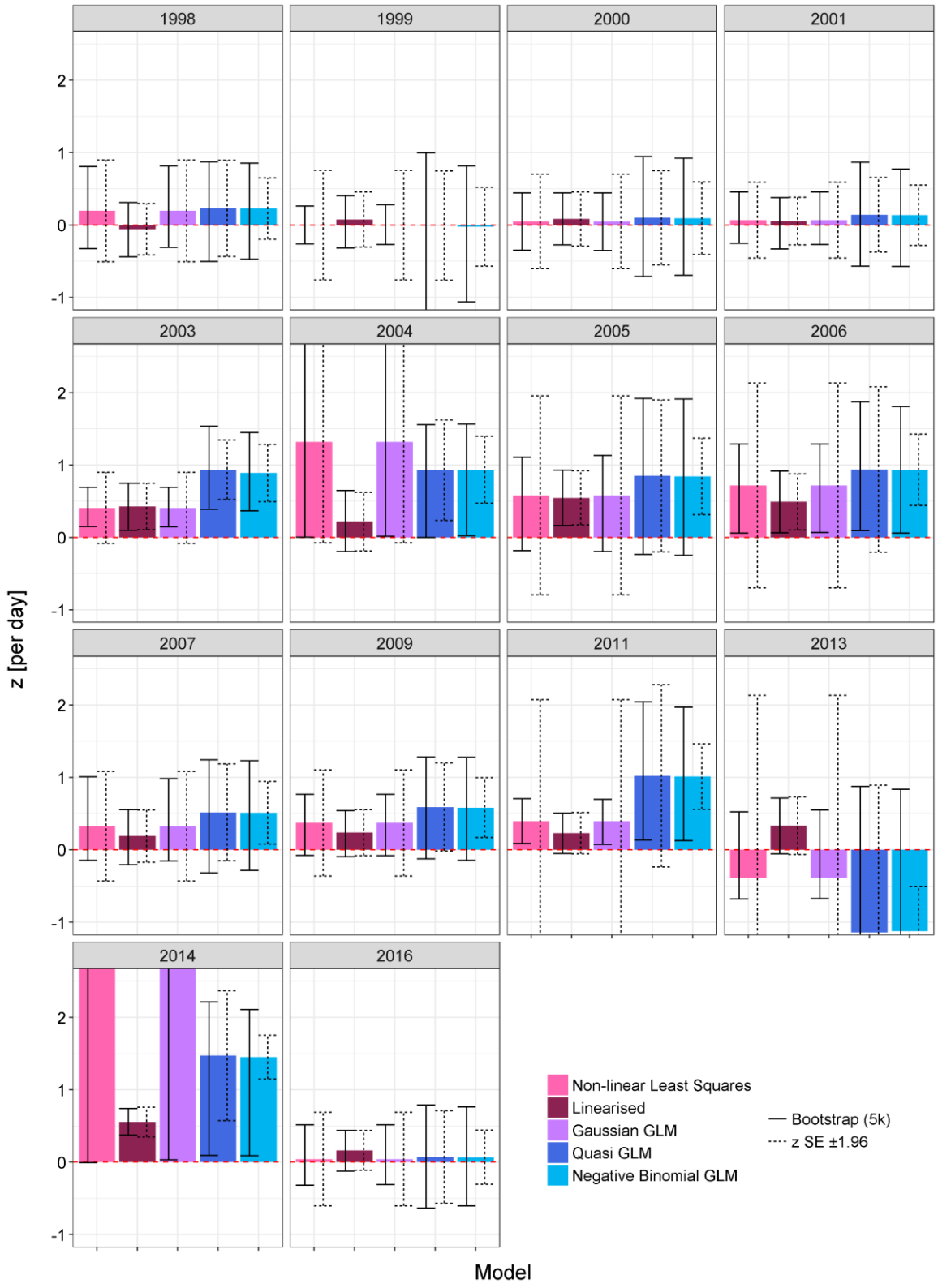

Figure 19: Estimates of instantaneous egg mortality rate (z; day^{-1}) for Australian Sardine (South Australia) for each non-GAM model by year. 95% CI: same quantiles of 5,000 bootstrap resamples used for P_0 in Figure 16. Linearised: log-linear model.

Blue Mackerel, Jack Mackerel and Australian Sardine (eastern and southeastern Australia)

Estimates of *z* were variable between species, regions and among models within a survey (Figure 20). The bootstrap confidence intervals for these were also quite wide. Negative estimates of mortality occurred with all models for Blue Mackerel and the log-linear model for Jack Mackerel (Figure 20). The log-linear model was the only one that provided a plausible estimate of mortality for Australian Sardine, confirming its suitability for this species. Estimates of *z* ranged from -0.57 to -0.24 for Blue Mackerel, -0.12 to 0.03 for Jack Mackerel, and -0.08 to 0.19 for Australian Sardine.

The wide confidence intervals for P_0 (Figure 18) were associated with the wide confidence intervals for *z* (Figure 20). Estimates of *z* centred on the axis of $z = 0$ implying that these estimates were not significantly different from zero, and indicates that a mortality signal was not detected. High densities of older eggs relative to younger eggs cause negative *z* values, resulting in low estimates of P_0 (e.g. Blue Mackerel, Figures 17 and 20).

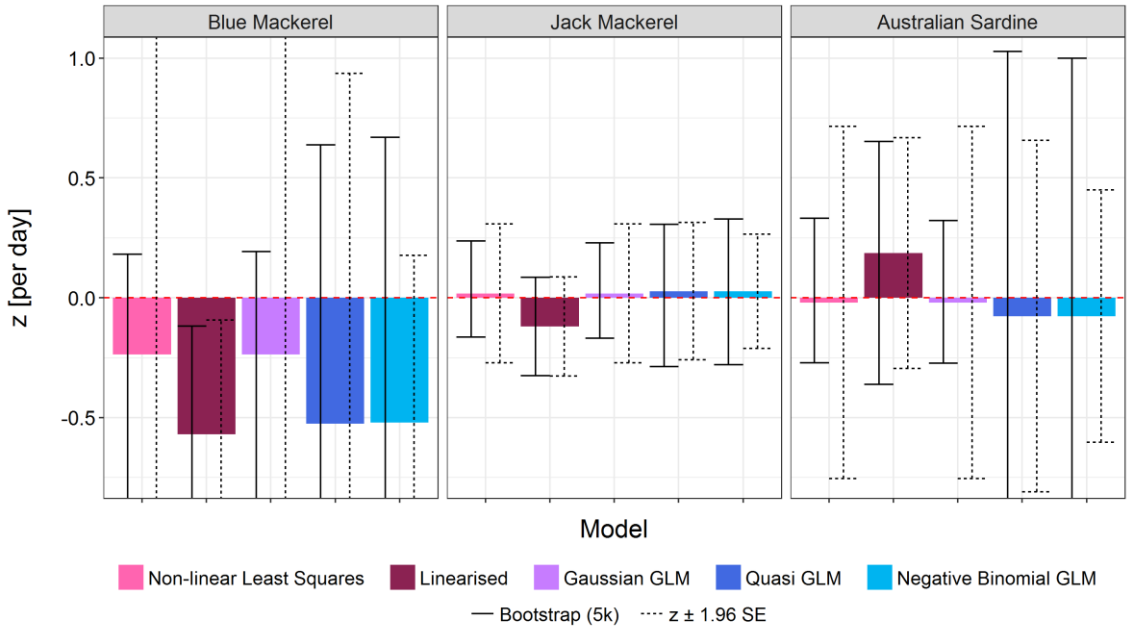

Figure 20: Estimates of instantaneous egg mortality rate (*z*; day^{-1}) for Blue Mackerel, Jack Mackerel and Australian Sardine off eastern and southeastern Australia using non-GAM models. 95% CI: same quantiles of 5,000 bootstrap resamples used for P_0 in Figure 18. Linearised: log-linear model.

Comparing the predictive power of non-GAM models

Cross validation

The LOO cross validations shows that all models have similar predictive power for individual data points of measured egg density (Figure 21). The RMSE values (Figure 21) indicate the average deviation in predicted values of each model when the model is fit to a subset excluding each point sequentially; a lower RMSE indicates a better predictive power (see Methods, Section 2). Within years, model performance is very similar among all models. The RMSE values for all models were high in 2014; this is likely caused by the high densities of early stage eggs recorded in a few samples resulting in exceptionally poor model fits. The RMSE for the log-linear model tended to be slightly higher than the other models, perhaps reflecting the slight negative bias thought to be associated with this approach.

Jackknife

The jackknife analysis shows that estimates of P_0 from all models vary substantially when one estimate of age and density is sequentially removed from the data set and P_0 is re-estimated (Figure 22). However, estimates of P_0 obtained using the log-linear model generally have much narrower jackknifed confidence intervals than all other models.

Figure 21: Cross validation (leave-one-out root mean square error) predictive power comparison among five non-GAM models for estimating P_0 for Australian Sardine (South Australia) by year. Linearised: log-linear model.

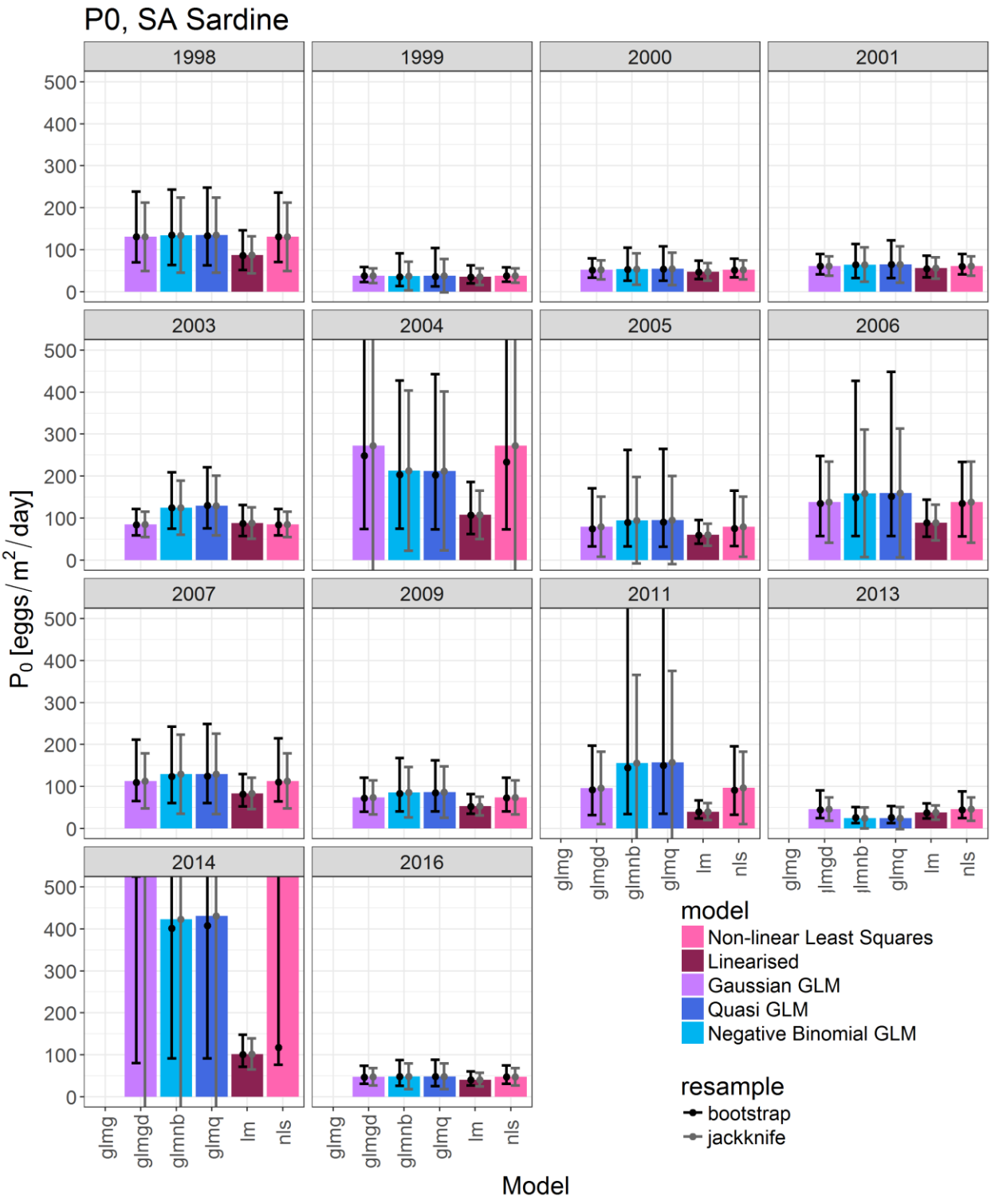

Figure 22: Jackknife estimates of uncertainty of five non-GAM models for estimating P_0 for Australian Sardine (South Australia) by year. Linearised: log-linear model.

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Egg production: GAM models and mean density

Australian Sardine (South Australia)

Estimates of P_0 calculated using the non-age GAM (model structure: Equation 10) varied depending on the non-GAM model used to estimate *z*. P_0 estimates also varied between yearly and common (all years) estimates of *z* (Figures 23 to 28). The GAMs did not converge in seven of the 14 years and are not presented in the figures (i.e. 1998-2003, 2014, 2016, Figures 23 to 28). Mean egg densities scaled by yearly and common estimates of *z* generally gave similar estimates of P_0 to the other models (GAM and non-GAM). Estimates of P_0 obtained from the GAMs based on estimates of *z* from the log-linear model were generally more plausible than those based on estimates of z from other models. Confidence intervals of estimates of P_0 obtained from the GAMs based on estimates of *z* from the log-linear model were generally narrower than those based on estimates of z from other models.

The log-linear model produced yearly *z* estimates ranging from -0.06 to 0.55 and a common *z* of 0.28 (Figure 23). When applied to the GAM, the yearly *z* values produced P_0 estimates ranging from 47.9 to 136.0 and from 49.8 to 126.4 with the common *z*. Mean egg densities scaled by a yearly *z* gave P_0 values between 41.0 and 195.2, while scaling with the common *z* returned values between 49.9 and 161.2. Annual estimates of P_0 from the log-linear model (yearly *z*) varied between 35.9 and 110.9 (Figure 23).

The non-linear least squares model gave yearly *z* estimates ranging from -0.39 to 23.51 with a common *z* of 0.57 (Figure 24). When applied to the GAM, the yearly *z* values gave P_0 estimates varying from 43.1 to 303.8 and from 64.2 to 157.9 with the common *z*. Mean egg densities scaled by a yearly *z* returned P_0 values between 38.0 and 2.1×10^{24}, while scaling with the common *z* returned values between 67.5 and 197.4. Annual estimates of P_0 from the non-linear least squares model (yearly *z*) ranged from 38.0 to 171,764.3 (Figure 24).

Estimates of yearly *z* using the Gaussian GLM ranged from -0.39 to 23.52 with a common *z* of 0.75 (Figure 25). When applied to the GAM, the yearly *z* values gave P_0 estimates varying from 43.1 to 303.8 and from 64.2 to 157.9 with the common *z*. Mean egg densities scaled by a yearly *z* returned P_0 values between 38.0 and 2.2×10^{24}, while scaling with the common *z* gave values between 67.5 and 197.4. Annual estimates of P_0 from the non-linear least squares model (yearly *z*) varied between 38.0 and 172,280 (Figure 25).

The negative binomial GLM returned yearly *z* values between -1.13 and 1.45 and a common *z* of 0.74 (Figure 26). When applied to the GAM, the yearly *z* values gave P_0 estimates varying from 24.0 to 213.4 and from 75.5 to 181.6 with the common *z*. Mean egg densities scaled by a yearly *z* returned P_0 values between 24.7 and 423.4, while scaling with the common *z* returned values between 81.2 and 224.9.

Annual estimates of P_0 from the non-linear least squares model (yearly z) were between 24.7 and 423.2 (Figure 26).

Yearly estimates of z from the quasi GLM ranged from -1.14 to 1.47 with a common z of 0.57 (Figure 27). When applied to the GAM, the yearly z values gave P_0 estimates varying from 23.8 to 212.6 and from 76.1 to 182.7 with the common z. Mean egg densities scaled by a yearly z returned P_0 values between 24.4 and 432.4, while scaling with the common z gave values between 81.9 and 226.3. Annual estimates of P_0 from the non-linear least squares model (yearly z) ranged from 24.4 to 430.9 (Figure 27).

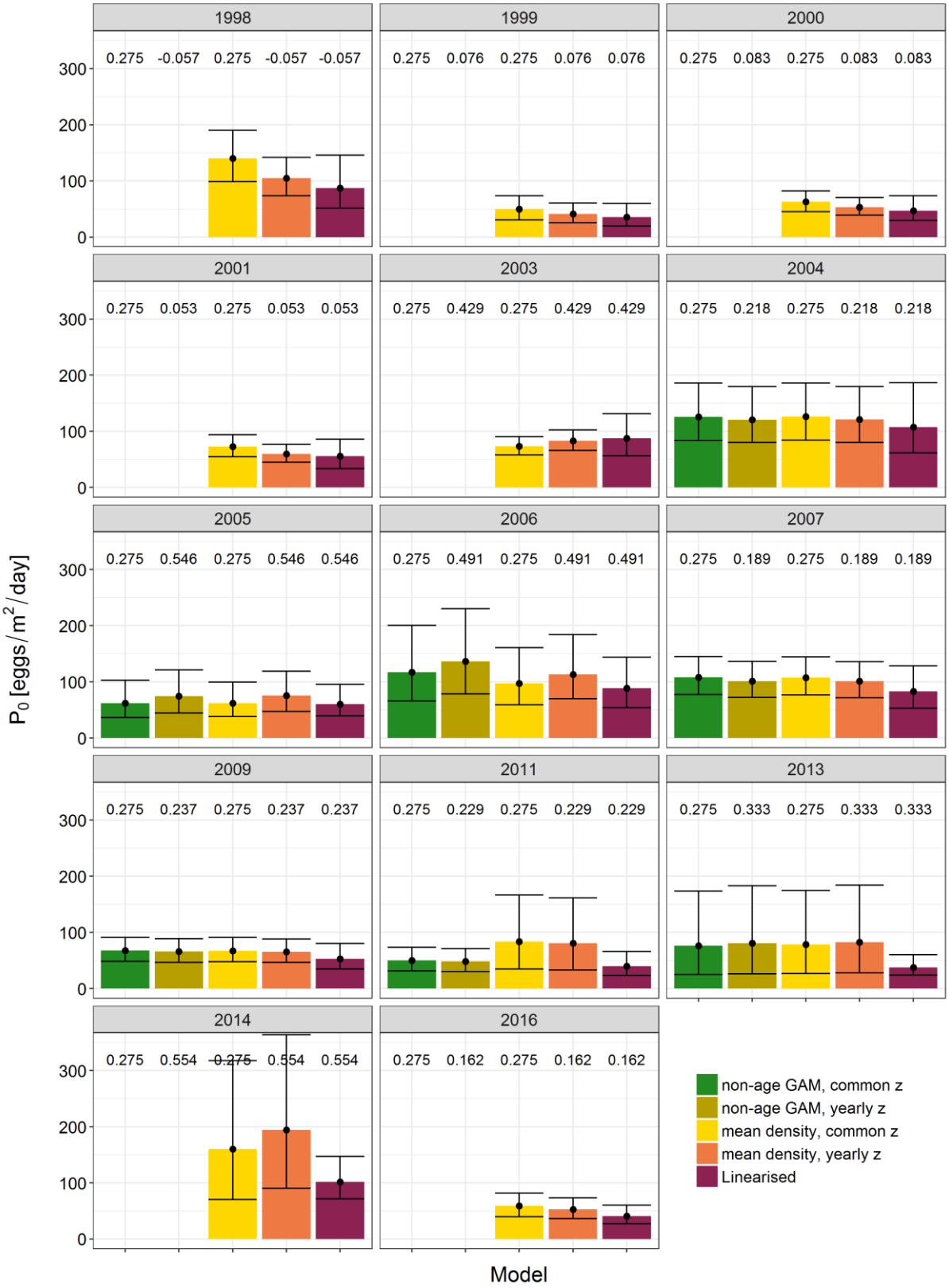

Figure 23: Estimates of P_0 for Australian Sardine (South Australia) by two GAMs using egg densities scaled by an overall mortality rate (common z) or the mortality rate from that year (yearly z) predicted by the log-linear (Linearised) model. Also shown are mean density estimates: back-corrected measured densities of each daily cohort in a sample to age 0 using the two prior z estimates and taking their mean. Estimates of z are listed above each model in each year. 95% CI: quantiles of 5,000 bootstrap resamples.

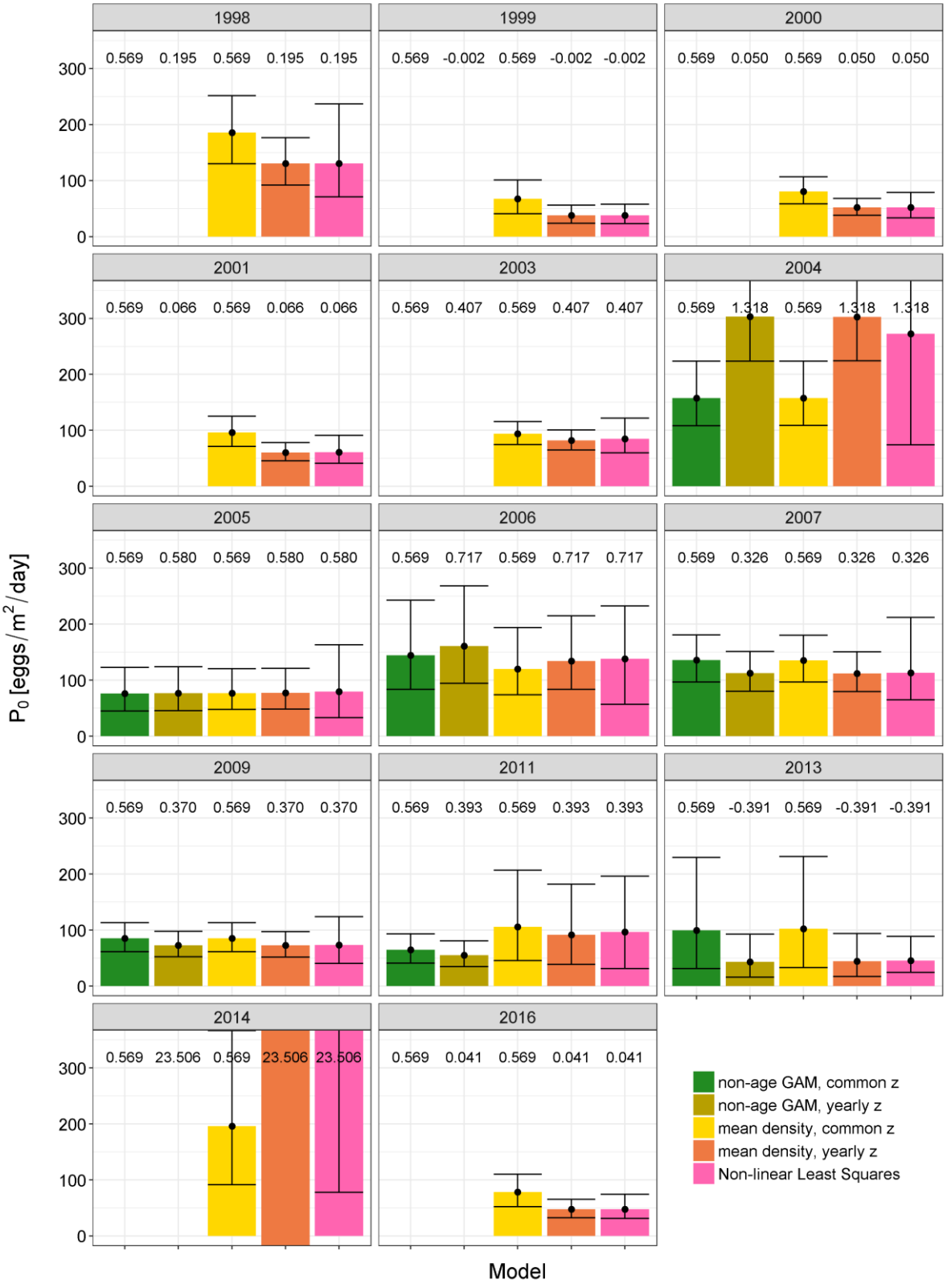

Figure 24: Estimates of P_0 for Australian Sardine (South Australia) by two GAMs using egg densities scaled by an overall mortality rate (common *z*) or the mortality rate from that year (yearly *z*) predicted by the non-linear least squares model. Also shown are mean density estimates: back-corrected measured densities of each daily cohort in a sample to age 0 using the two prior *z* estimates and taking their mean. Estimates of *z* are listed above each model in each year. 95% CI: quantiles of 5,000 bootstrap resamples.

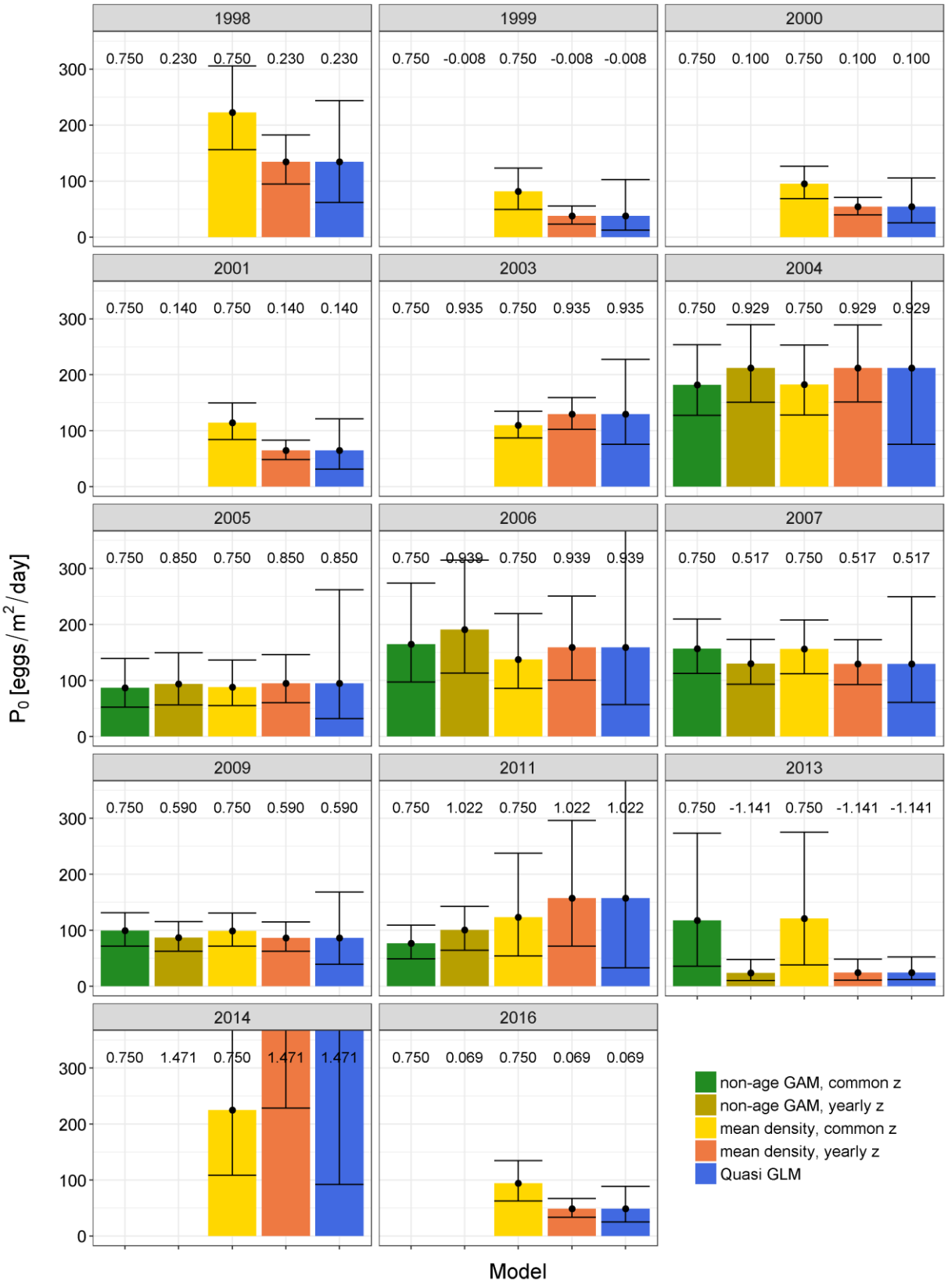

Figure 25: Estimates of P_0 for Australian Sardine (South Australia) by two GAMs using egg densities scaled by an overall mortality rate (common *z*) or the mortality rate from that year (yearly *z*) predicted by the quasi GLM. Also shown are mean density estimates: back-corrected measured densities of each daily cohort in a sample to age 0 using the two prior *z* estimates and taking their mean. Estimates of *z* are listed above each model in each year. 95% CI: quantiles of 5,000 bootstrap resamples.

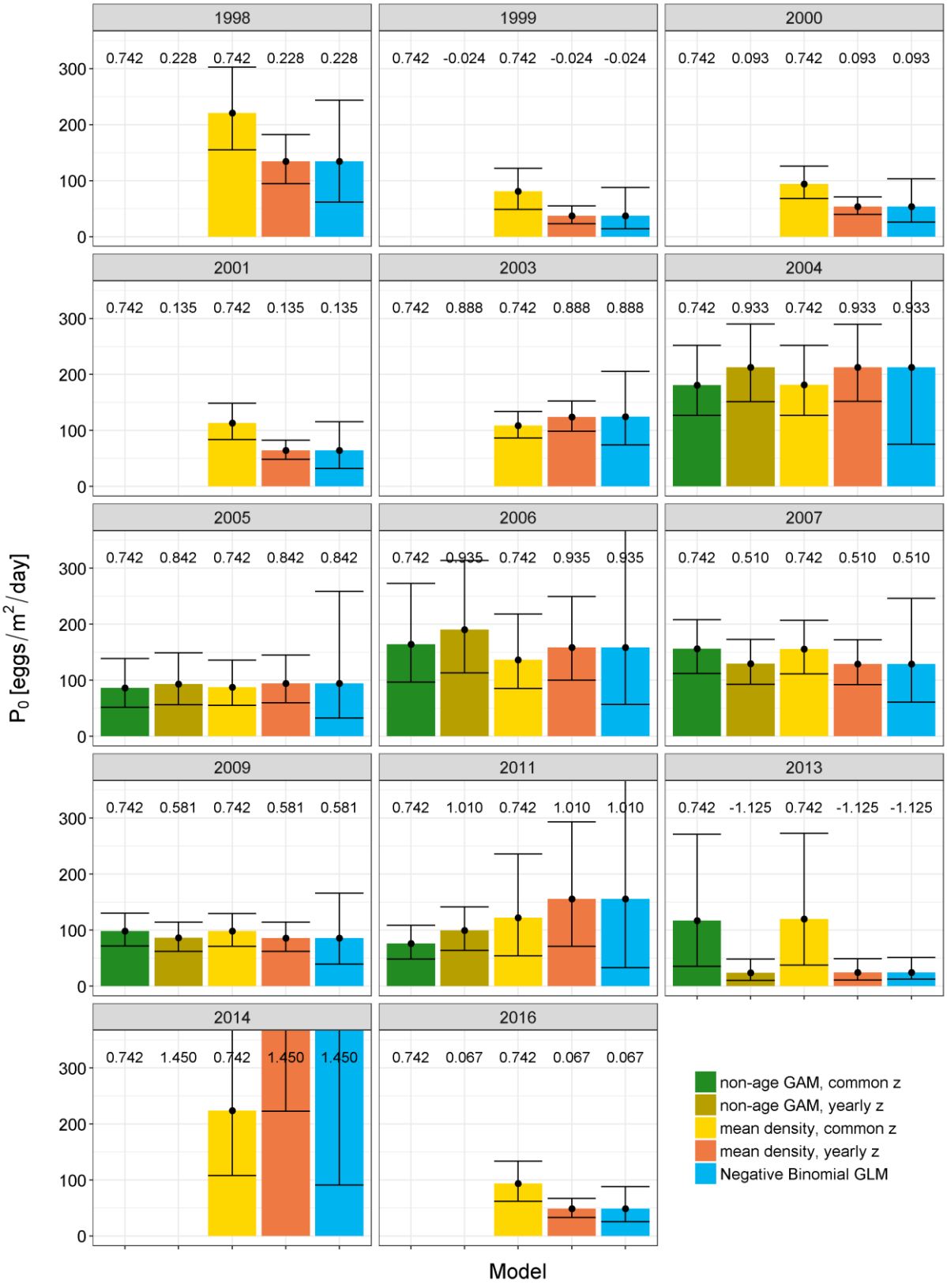

Figure 26: Estimates of P_0 for Australian Sardine (South Australia) by two GAMs using egg densities scaled by an overall mortality rate (common *z*) or the mortality rate from that year (yearly *z*) predicted by the binomial GLM. Also shown are mean density estimates: back-corrected measured densities of each daily cohort in a sample to age 0 using the two prior *z* estimates and taking their mean. Estimates of *z* are listed above each model in each year. 95% CI: quantiles of 5,000 bootstrap resamples.

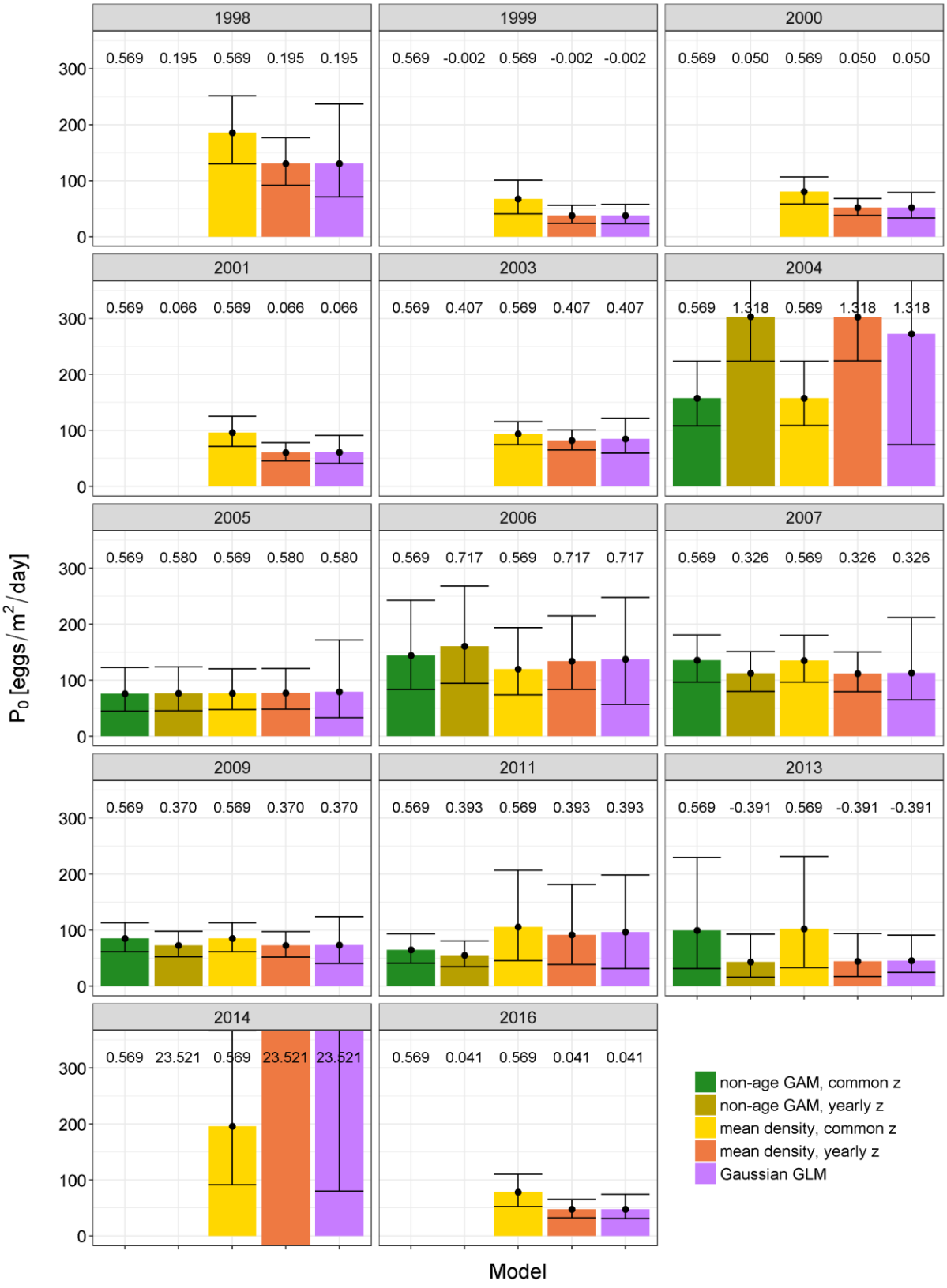

Figure 27: Estimates of P_0 for Australian Sardine (South Australia) by two GAMs using egg densities scaled by an overall mortality rate (common *z*) or the mortality rate from that year (yearly *z*) predicted by the Gaussian GLM. Also shown are mean density estimates: back-corrected measured densities of each daily cohort in a sample to age 0 using the two prior *z* estimates and taking their mean. Estimates of *z* are listed above each model in each year. 95% CI: quantiles of 5,000 bootstrap resamples.

The Unconstrained, Constrained and Age-only GAMs, based on Equation 9, produced different smoothers describing changes of egg density with age (Figure 28). The Constrained and Age-only GAMs produced the most similar smoothers while the Unconstrained GAM overfit these data. The resulting annual smoothers from all three GAMs showed a consistent dome shape for density versus age (Figure 28). Raw egg densities also show this dome pattern (Figure 14). This suggests that young eggs (Stages 1 and 2, and Stage 3 at higher water temperatures) are under-represented in samples. A generally flat trend of density across the middle stages of egg development (taking into account error bars and with considerable variation, Figure 14) suggests low mortality, or limited information about mortality rate at these stages. The tow off in observed density for older eggs mostly reflects the higher number of zeros inferred for these stages (shown as yellow circles in Figure 14). The lack of zeros in mid-range ages is caused by eggs taking under two full days to hatch, which results in a period when there is only a single cohort present in the water, and records being removed that contain only zero counts.

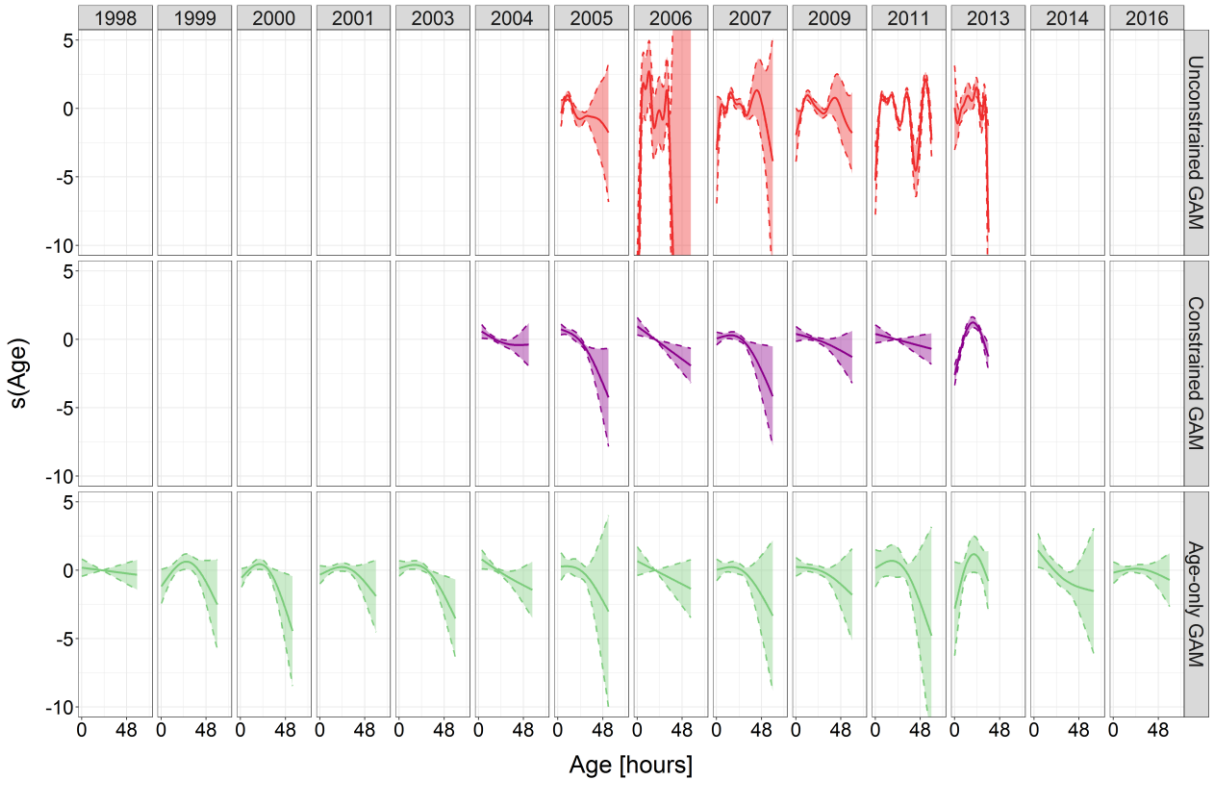

Figure 28: Plotted GAM function smoothers (three GAM variants: see Methods Section 2 for details) versus egg cohort age for Australian Sardine (South Australia) by year. Filled plots show years when required input data were available and the model converged. Shading: ± S.E.

4. Conduct simulations to evaluate performance of different approaches to sampling and data analysis

Survey Design

The design of the survey is critical to the performance of the DEPM. It is important to sample evenly across different times of the day to get the best data on egg density versus egg age. For example, sampling at the same time every day would repeatedly collect eggs of the same two age cohorts, which does not provide enough range in egg ages to fit a regression with any confidence. Sampling should be conducted throughout the entire day, or some age cohorts will be under-represented in the final binning. To show the impact of survey design on the DEPM, we modelled an unrealistic scenario with infinite diffusion and zero mortality. The daily egg production for these simulations was set to 100 eggs·m^{-2}. By using these assumptions, the eggs from every spawning are instantly spread homogenously over the whole region, and the concentrations are constant in time. Every sample gives an accurate egg density of each cohort and the distribution of ages throughout the survey simulation should be uniform. Any deviation from a flat distribution of egg ages is due to aliasing and under-counting in the survey.

The first ("Ideal") survey experiment (MCE=1,000) consisted of 288 sites sampled over exactly 12 days with an interval between sites of 1 hour (Figure 29). There are no random variations in travel time between sites. The resulting histogram of egg ages from all ensembles in bins of width 1 hour is relatively flat with no indication of over or under sampling of any age cohorts.

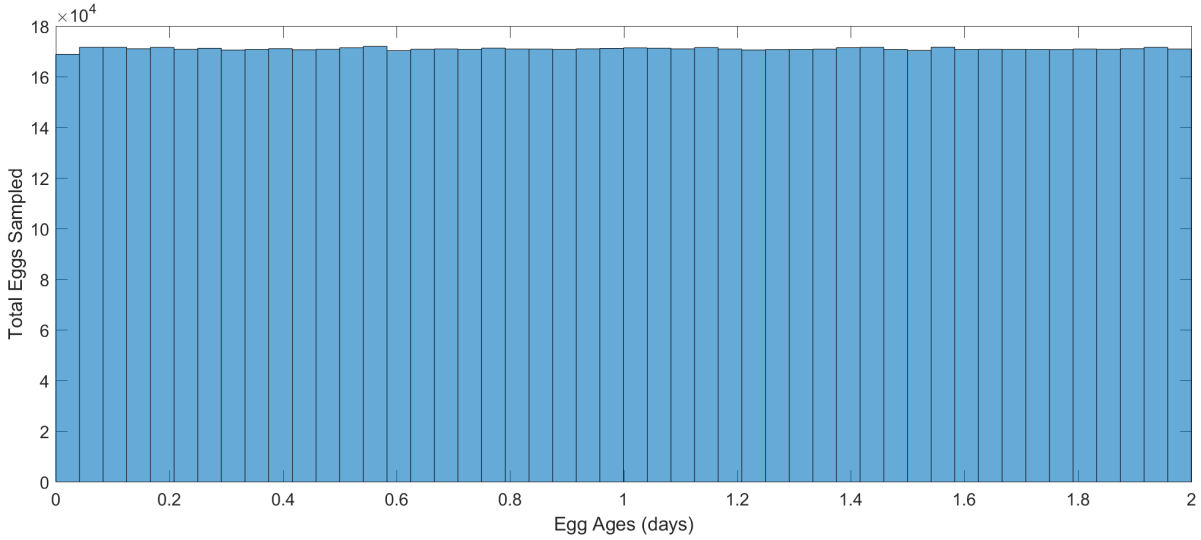

Figure 29: Distribution of egg ages for Ideal Survey that is exactly 12 days long with 1 hour between sites. MCE = 1000.

For a more realistic survey, we devised a survey of 308 stations with 22 transects that was completed in about 20 days. Each transect had 14 stations, and the travel time between stations was randomly set

between 48 and 72 minutes. The travel time between transects was randomly set to be about 6 hours with a standard deviation of 1 hour. The first station was sampled at 0800 hours. The resulting histogram (Figure 30) shows some unevenness but appears to represent a reasonably uniform survey of all age cohorts.

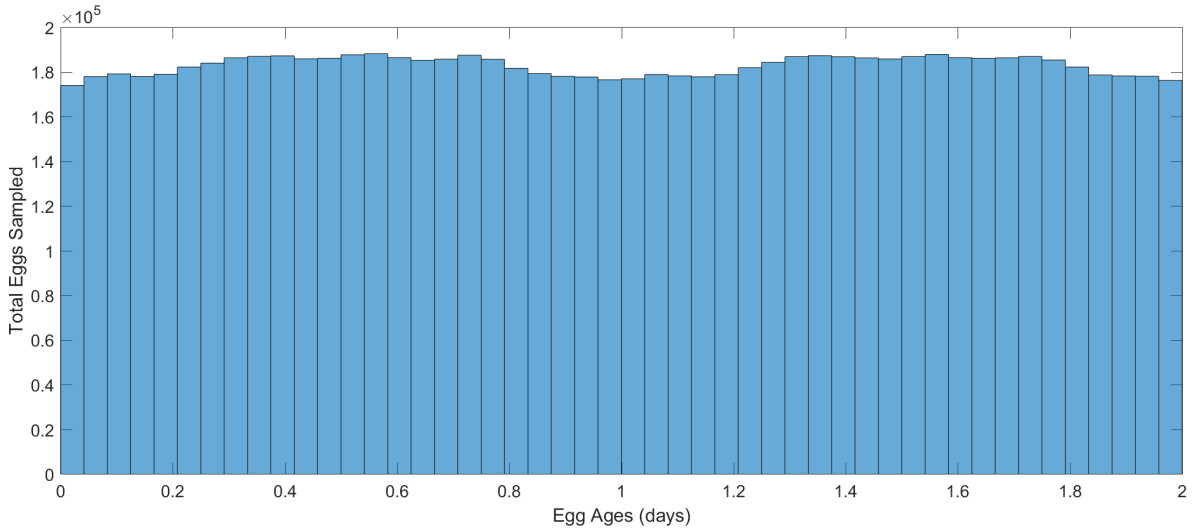

Figure 30: Realistic Survey of 308 stations with 22 transects. MCE = 1000.

The distribution is very sensitive to the time taken between transects. If the travel time between transects is increased to 11 hours, an extremely uneven sampling pattern emerges (Figure 31) which will give rise to a poor estimate of daily egg production.

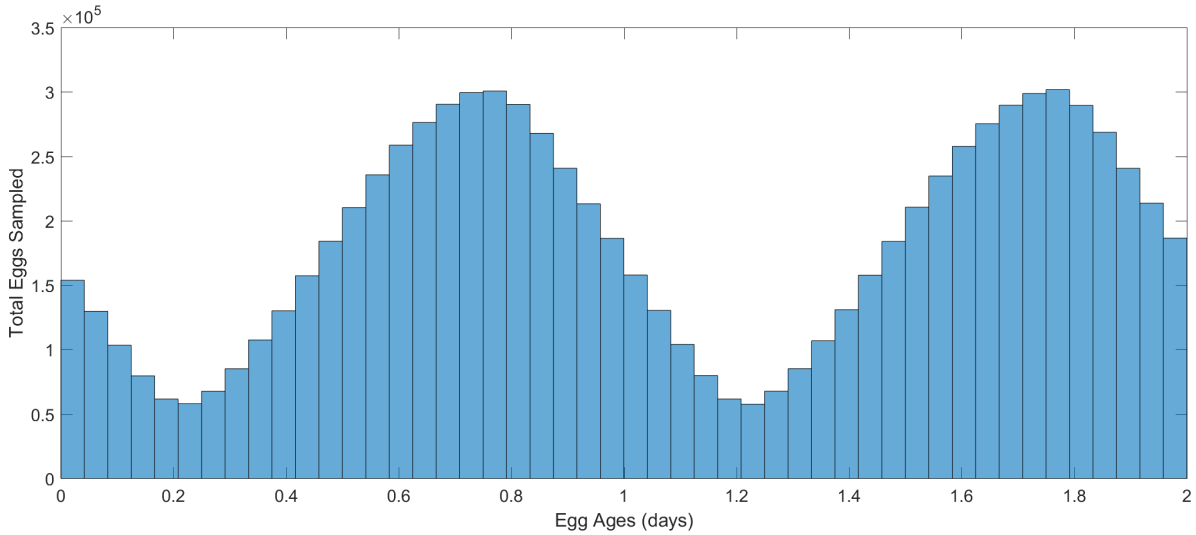

Figure 31: Realistic Survey as in Figure 30 but with 11 hour travel times between transects. MCE = 1000.

The main reason for this bias in the sampling is aliasing of the sites surveyed (bottom panel, Figure 32). For this particular design of survey, the 11 hour transect interval happens to result in too few sites sampled

between 0700 and 0800 hours and too many between 2000 and 2100 hours. Note that because there are always two cohorts present at any time, this pattern is repeated in Figure 31 for egg ages from 1 to 2 days.

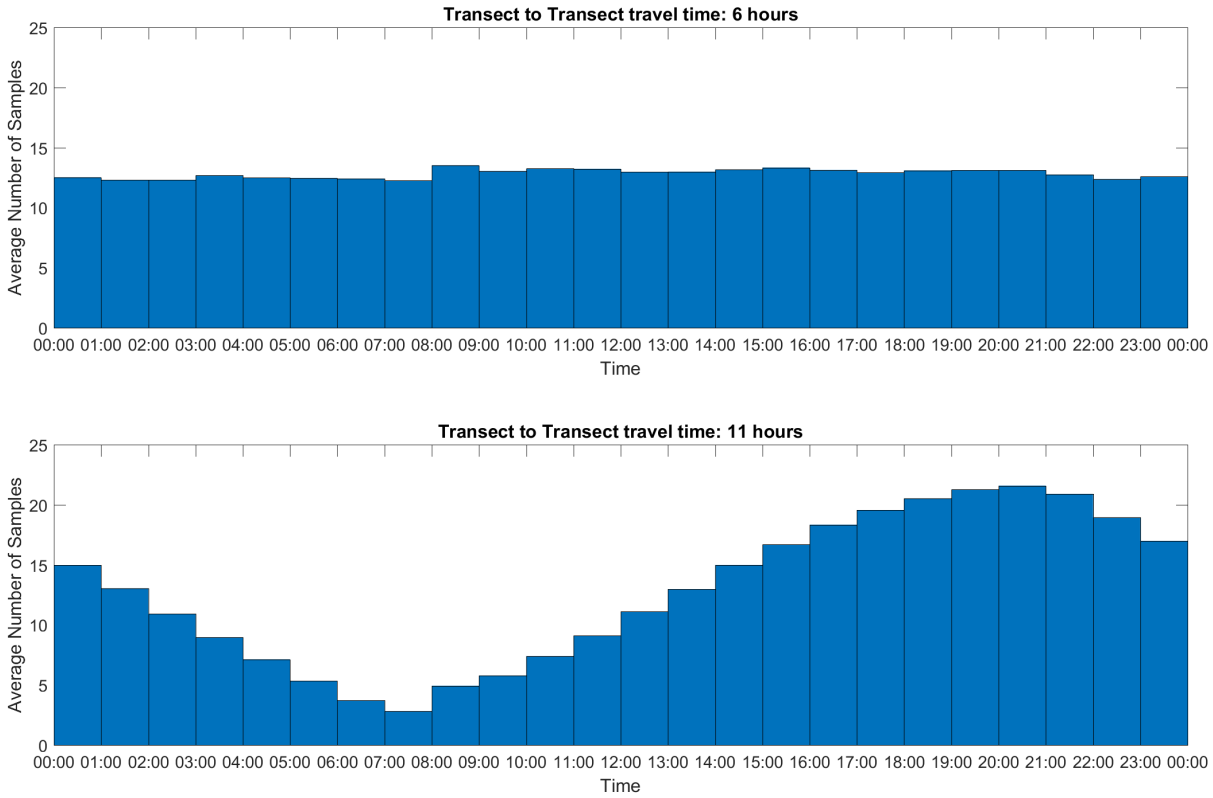

Figure 32: Comparison of the number of sites sampled at different times of day. Top panel shows a realistic survey with time between transects of 6 hours, the bottom panel shows a realistic survey with time between transects of 11 hours. MCE=1000.

For the realistic model simulations in this report, we use the survey design outlined in Figure 30 with a six hour transit time between transects, which has what we consider to be an acceptably small degree of aliasing.

Model Testing

To test the model, we needed to show that the model would produce expected behaviour and that the DEPM would work for different simulations. We used two different regression methods to implement the DEPM: the log transformed linear regression of Picquelle and Stauffer (1985) outlined in Ward et al. (2011) and a least-squares-fit to the binned egg-densities. The least-squares fit produced a consistent estimate of the mortality rate (Figure 33, bottom) but was sensitive to high densities in the youngest eggs and produced some extremely high and unrealistic estimates of P_0. The log-linear regression was more sensitive to low values of older eggs but was usually within a factor of two of the actual P_0 (Figure 33, top). For example, 4% of P_0 values calculated in the bottom panel of Figure 33 exceeded 1,000 eggs·m^{-2} (outside scale of plot) with a maximum value of over 14,000 eggs·m^{-2}, while the maximum value from the log-linear regression (top panel) was only 262 eggs·m^{-2}. One of the key metrics we used to evaluate

scenarios was the coefficient of variation (CV) which is the ratio of the standard deviation to the mean value. This metric is negatively affected by extreme values of P_0. In the remaining plots (Figures 34, 35, 37 and 39), only the log-linear regression curves (light green line) are shown to illustrate the range of regressions within a scenario.

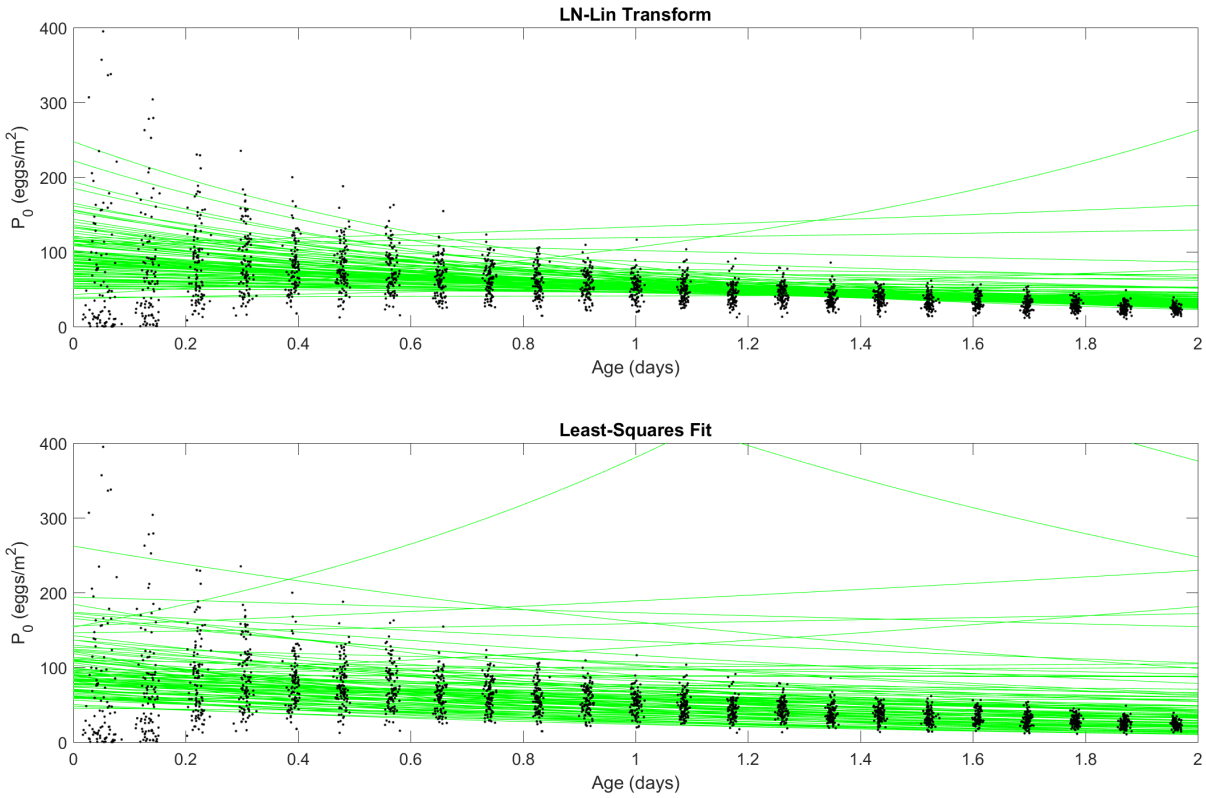

Figure 33: Comparison of log transformed linear regression (top) and least-squares fit (bottom) estimates of mean daily egg production (P_0). MCE=1000.

To test the effect of egg mortality, the horizontal and vertical diffusion, K_h and K_v, were set to infinity as done in the survey design, but this time an egg mortality rate was included with the default value of Z=0.58 day^{-1}. The survey design was the Realistic Survey with reduced aliasing. A simulation with MCE=1000 and the resulting estimates of P_0 are shown in Figure 34. For the regression, the egg counts were binned at two hour intervals, and the egg density was calculated on the basis of a vertical net drop with the default net diameter of 1.4 m^2. The egg-space models were generated for each site with a nominal average P_0 of 100 eggs·m^{-2} and with a site to site variation of about 25%. The actual average P_0 across all 308 sites was 100 eggs·m^{-2}. The sampling times and variability across the 1000 MCEs are plotted in the top panel of Figure 34.

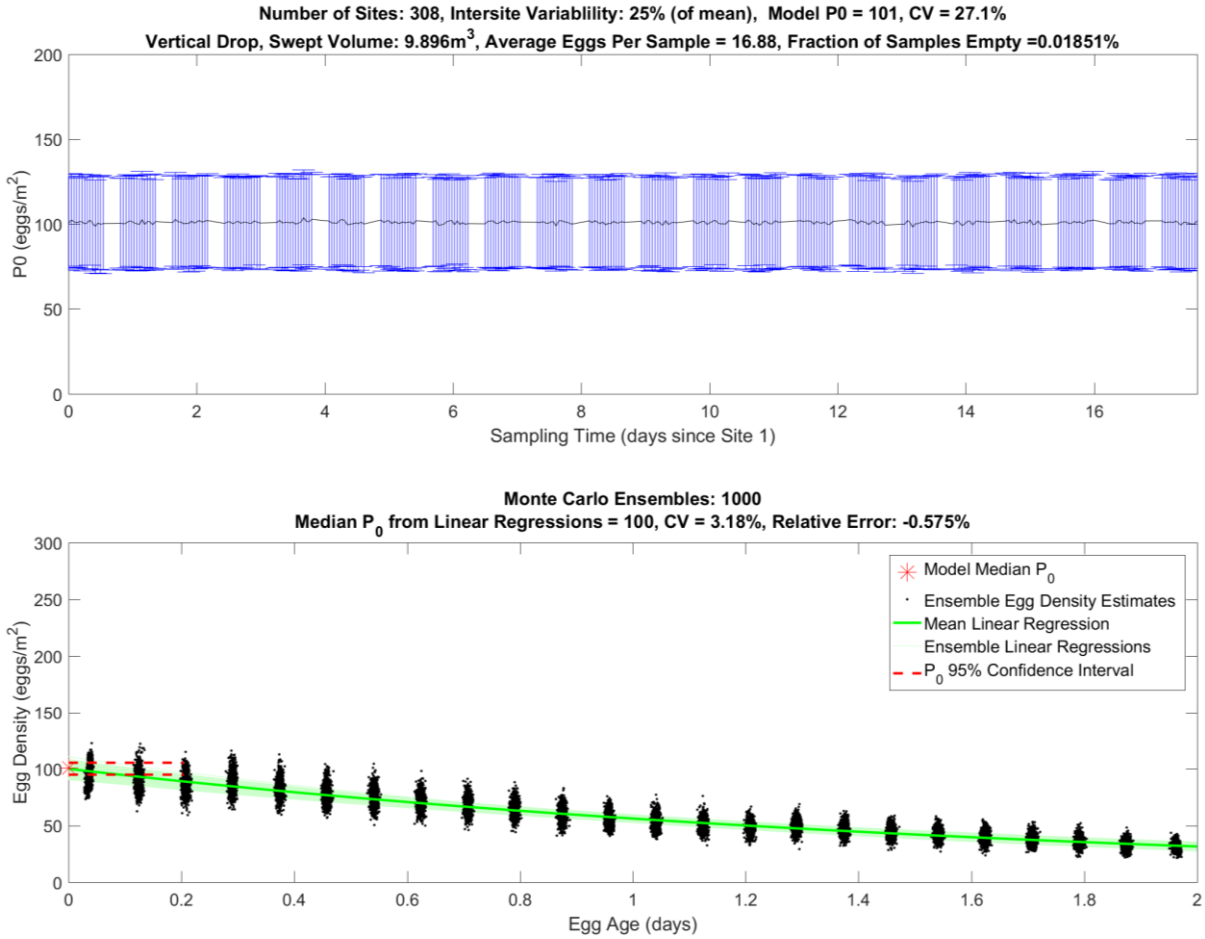

Figure 34: Top panel shows the survey site intervals and error-bars to indicate the standard deviation of eggs (MCE=1000). The average P_0 is nominally 100 eggs·m^{-2} with H_z, H_v=∞ and Z=0.58 day^{-1} and varies by about 25% from site to site. The bottom panel shows the results of a linear regression of log transformed data following the DEPM outlined in Ward et. al. 2011.

Figure 34 shows a linear regression of log transformed egg-density data versus egg-age for each MCE. The mean CV across all MCEs was small (~3%) and reflected the tight fit of the regression model. A mean across all regressions gave a P_0 value of 100 eggs·m^{-2} which was close to the actual value of 101 eggs·m^{-2}. The value of P_0 for the model run was raised to 150 eggs·m^{-2}, and resulted in another very tight fit (Figure 35) and accurate estimate of P_0 with a relative error of less than 0.5%.

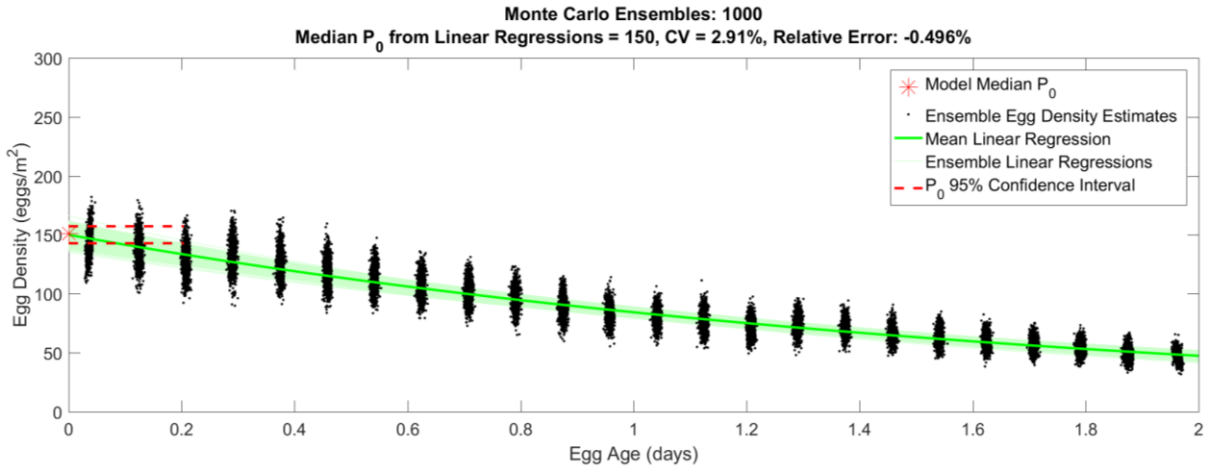

Figure 35: DEPM for nominal P_0 of 150 eggs·m^{-2} with H_z, H_v=∞ and Z=0.58 day^{-1}. MCE=1000.

We also explored the model behaviour by examining histograms of total eggs sampled as a function of age. With a realistic egg-mortality rate and no diffusive effects, the number of eggs decreases with age. A histogram for the data plotted in Figure 34 shows the effect of this across all the MCEs (Figure 36).

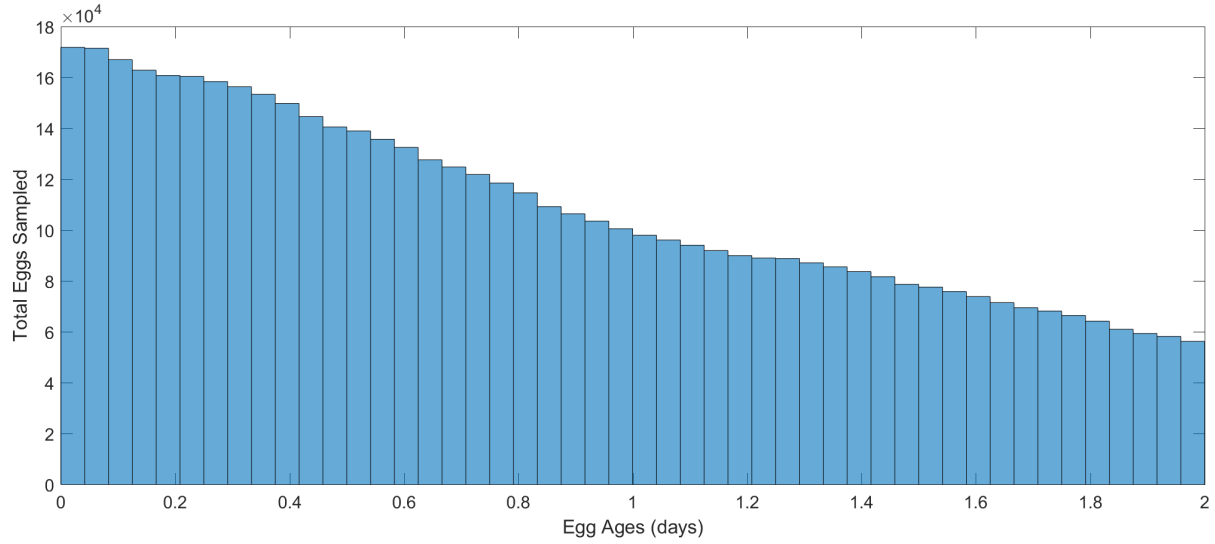

Figure 36: Histogram of total eggs counted across all MCEs (1000) as a function of egg-age of survey with H_z, H_v=∞ and Z=0.58 day^{-1}.

To investigate the effects of diffusion, egg-mortality was set at Z=0 day^{-1} and horizontal diffusion at K_h=0.5 m^2·s^{-1} and vertical diffusion at K_v=2.0x10^{-4} m^2·s^{-1}. The application of the DEPM was modified slightly to not log transform the data, since there was no exponential decay associated with egg mortality. The expectation was that as the spawn patches spread with egg-age, the probability they are sampled increases but the concentration of eggs decreases. This is shown in Figure 37 where there is a higher degree of scatter for young eggs around a mean egg density that is close to the model P_0.

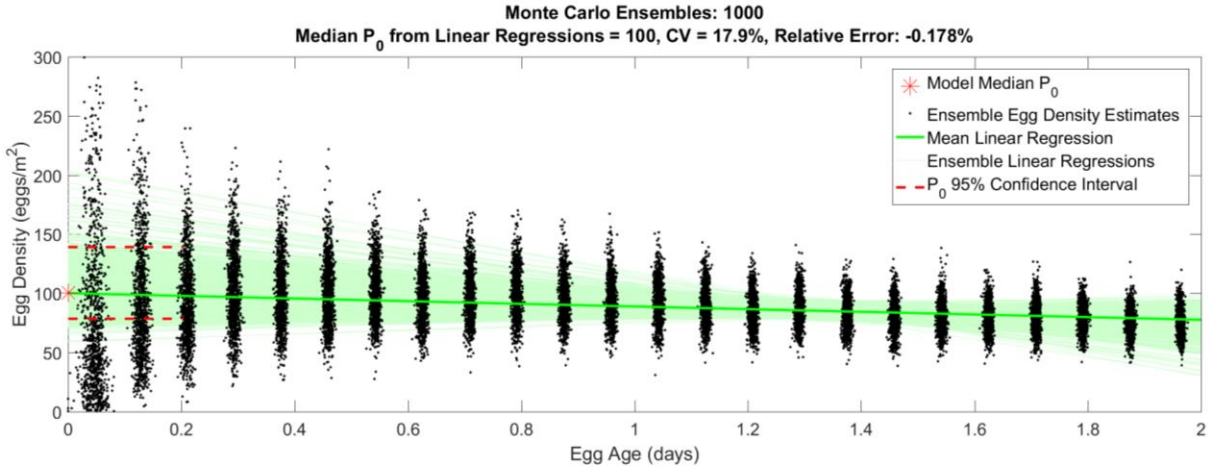

Figure 37: DEPM for nominal P_0 of 100 eggs·m^{-2} with H_z=0.5 m^2·day^{-1}, K_v=2.0x10^{-4} m^2·s^{-1} and Z=0 day^{-1}. MCE=1000.

The histogram of the egg count as function of egg-age is informative (Figure 38). Without mortality, the number of eggs sampled from older, widely-diffused spawning sites was much higher than from the younger sites. The younger sites make much smaller targets for sampling, despite having much higher concentrations of eggs.

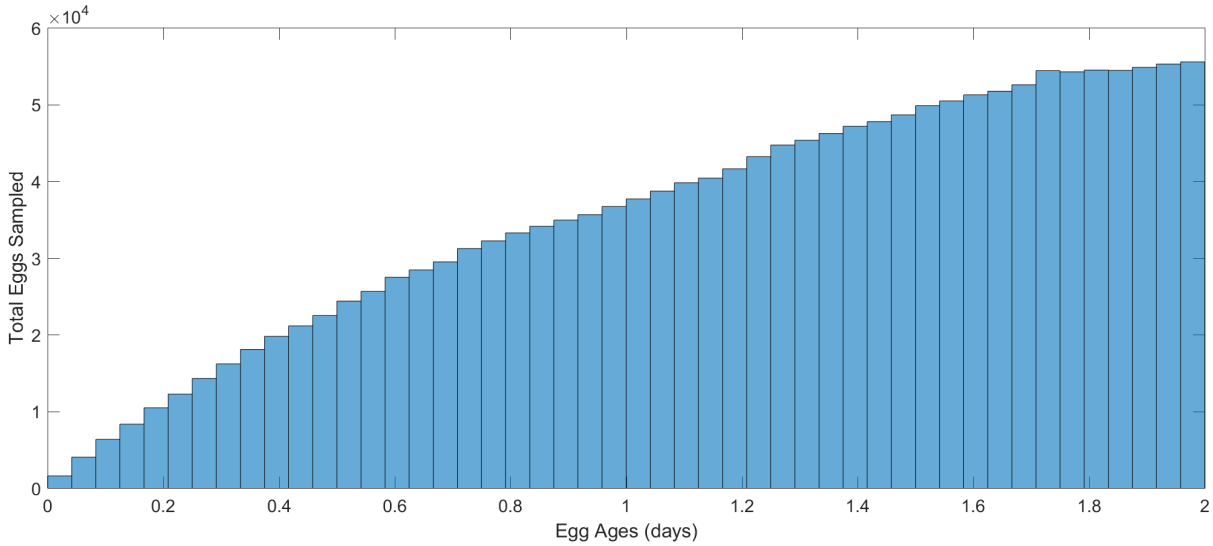

Figure 38: Histogram of total eggs counted across all MCEs (1000) as a function of egg-age of survey with H_z=0.5 m^2·s^{-1}, K_v=2.0x10^{-4} m^2·s^{-1} and Z=0 day^{-1}.

Finally, realistic diffusion and egg mortality were combined to conduct a full simulation of a field survey and apply the log transformed regression. There was much more variability in the regressions (pale green lines in Figure 39), and the CV of 23.6% reflected this uncertainty. The relative error of the average P_0 taken across all 1000 MCE was still relatively small, but the estimate from any single ensemble (representing a single survey) could be large (i.e. 100 eggs·m^{-2}).

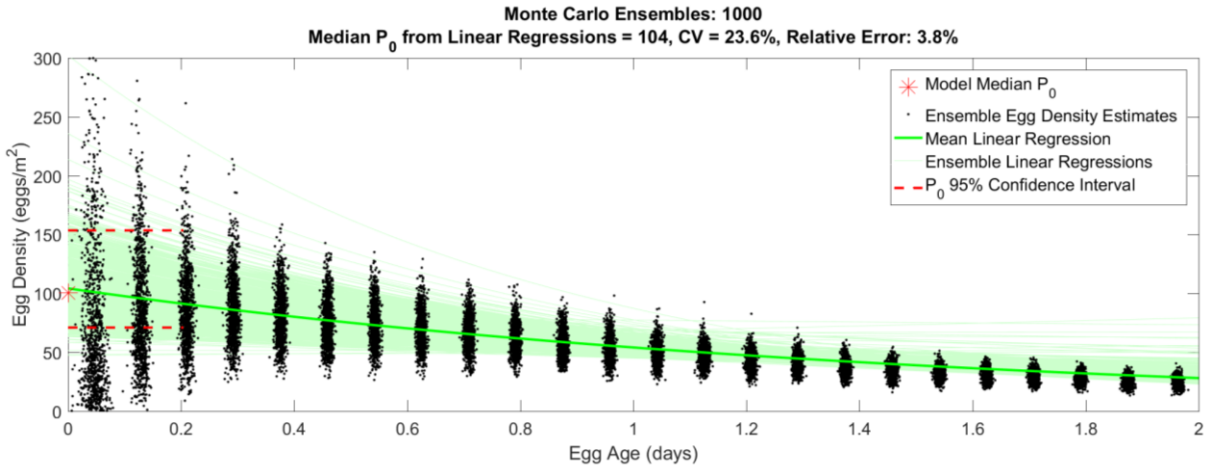

Figure 39: DEPM for nominal P_0 of 100 eggs·m^{-2} with H_z=0.5 m^2·s^{-1}, K_v=2.0x10^{-4} m^2·s^{-1} and Z=0.58 day^{-1}. MCE=1000.

The histogram of egg counts (Figure 40) showed that diffusion still led to low counts for young eggs but with a flattening off and slight decrease due to egg mortality for eggs older than 1.5 days.

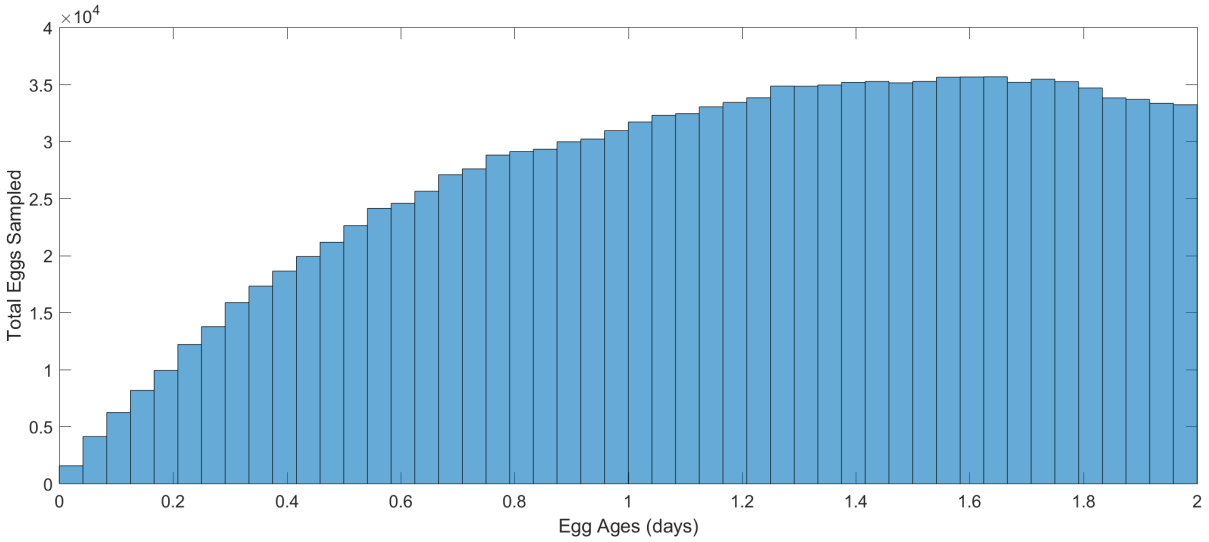

Figure 40: Histogram of total eggs counted across all MCEs (1000) as a function of egg-age of survey with H_z=0.5 m^2·s^{-1}, K_v=2.0x10^{-4} m^2·s^{-1} and Z=0.58 day^{-1}.

To summarise, the egg-space model produces a three dimensional environment which can be sampled and analysed with the DEPM and reproduces expected results for ideal and realistic scenarios. The statistical behaviour of the model occurs as expected and should be useful for testing different sampling scenarios.

Sampling Methods

The efficiency of two different ichthyoplankton sampling methods, a vertical two and oblique tow, were investigated with the model. The vertical tow method assumes a pair of CalVet nets with a mouth diameter

of 0.14 m^2 are lowered to 70 m and then raised to the surface at about 1 $m \cdot s^{-1}$ (Figure 41). The total swept volume of the vertical tow is about 9.9 m^3.

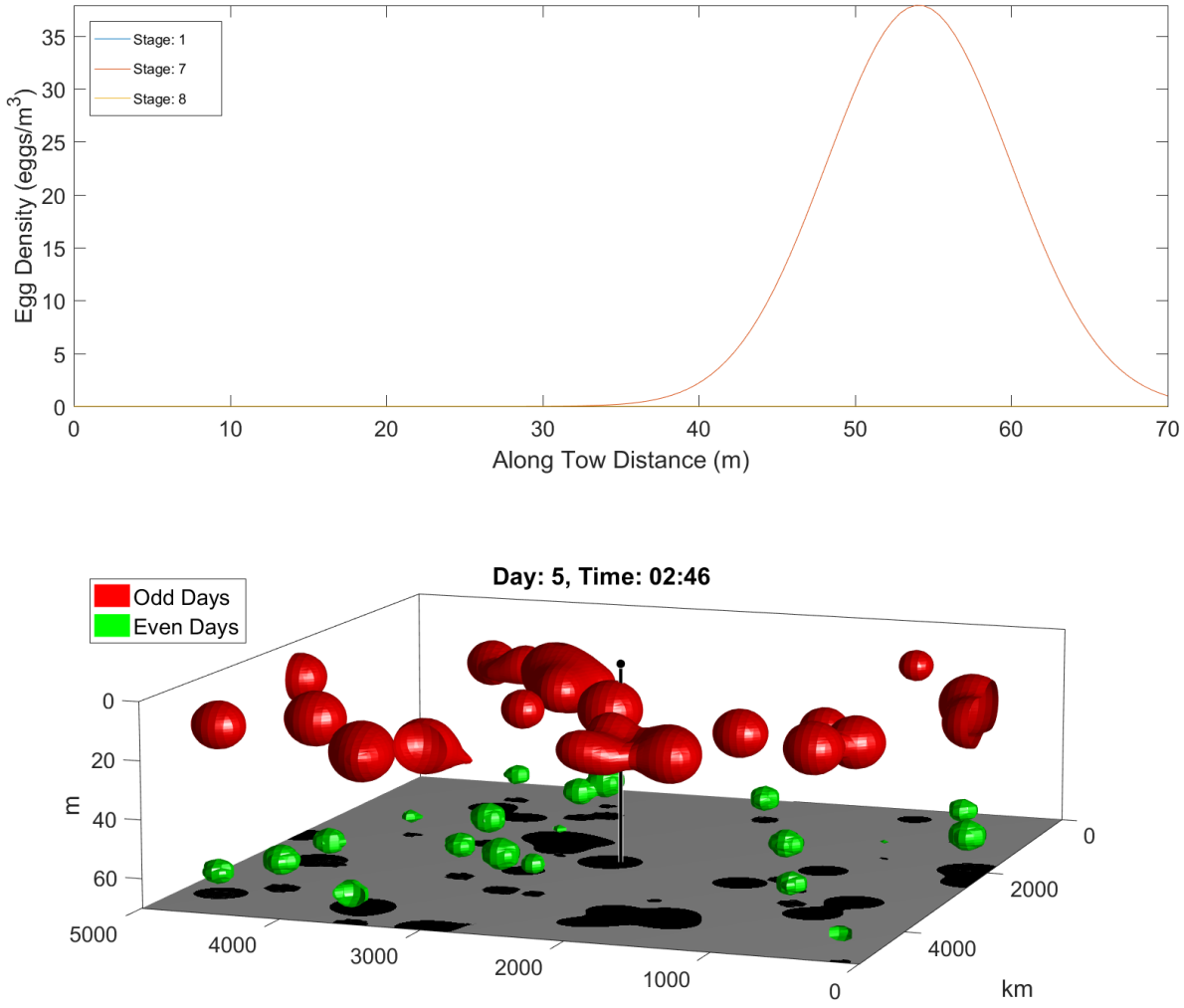

Figure 41: Vertical tow sampling strategy showing the egg-density in egg·m^{-3} along the 70 m tow in the upper panel and the local egg-space with the 10 egg·m^{-3} isosurfaces of the daily cohorts (red for odd days, green for even days) illustrated in the lower panel.

The oblique tow method uses a much smaller net (mouth diameter of 0.03 m^2) and assumes the net is lowered to 70 m and gradually brought to the surface while the sampling vessels travels 2000 m along the surface (Figure 42). Because the vessel is limited to 3 $m \cdot s^{-1}$ (~6 knots) during the tow, the tow takes a little over 11 minutes to be pulled from bottom to surface. Because of the longer towing distance, the total swept volume is nearly 63 m^3 or six times the swept volume of the vertical tow.

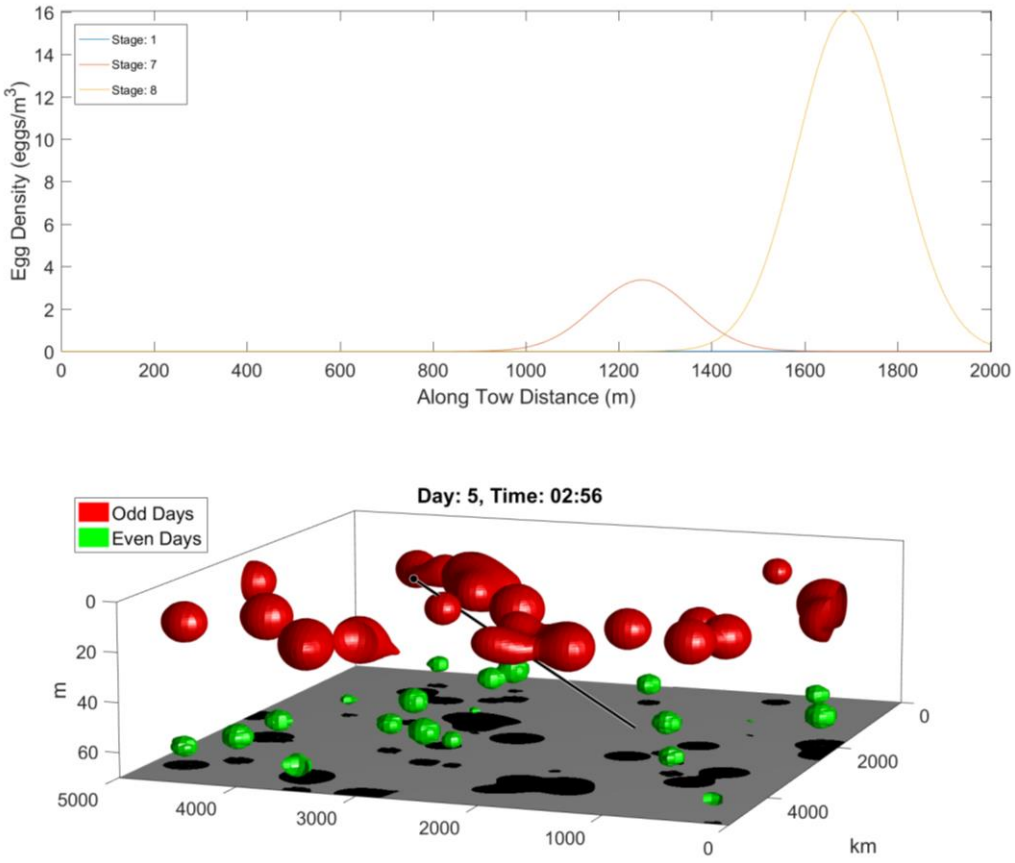

Figure 42: Oblique tow sampling strategy showing the egg-density in egg·m^{-3} along the 2000m surface tow in the upper panel and the local egg-space with the 10 egg·m^{-3} isosurfaces of the daily cohorts (red for odd days, green for even days) illustrated in the lower panel.

Model Validation

At this point, model validation is primarily subjective: a visual comparison between the distributions in historic DEPM surveys and typical distributions from the simulation. The distribution of staged Australian Sardine eggs measured during surveys along the southern shelf of Australia from 1998 to 2016 shows a high degree of variability from year to year (Figure 43). There is no marked pattern in the distribution as a function of age, but there are many empty and near empty Stage bins along with a few highly populated bins.

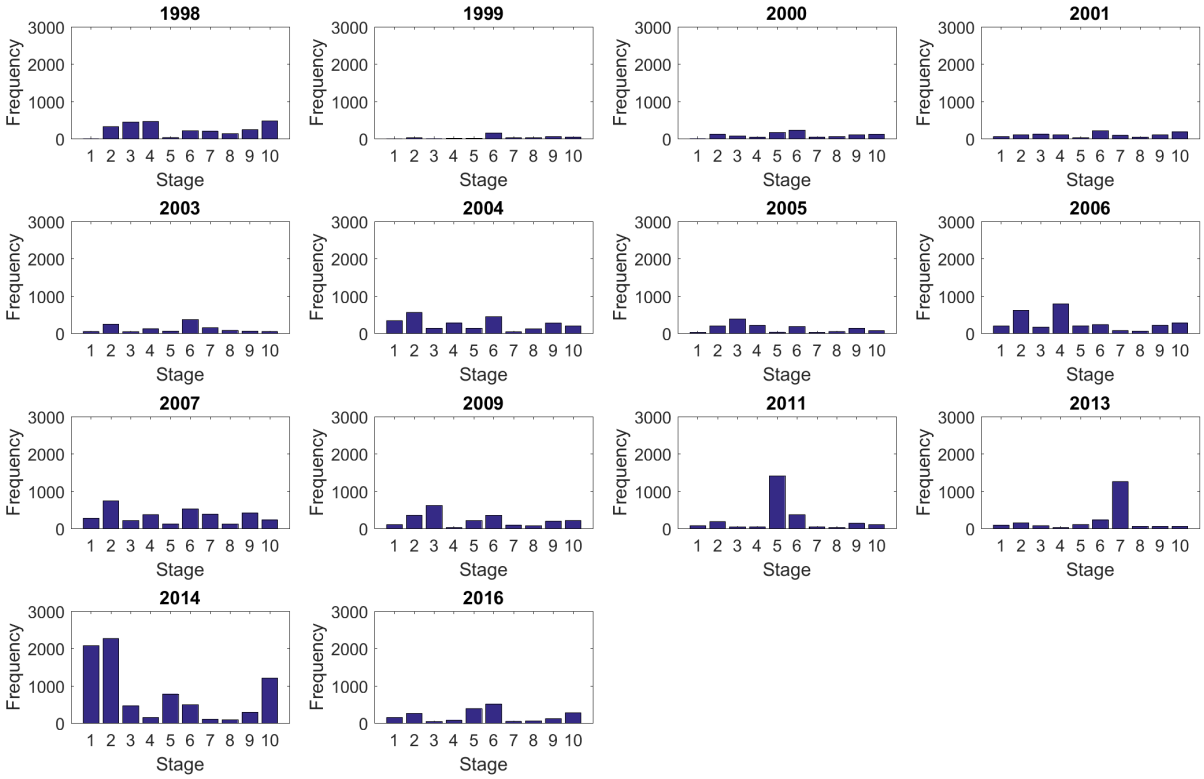

Figure 43: Distribution of staged eggs from historic sardine surveys from 1998 to 2016 showing highly variable egg frequencies.

The model is not invalidated by showing similar variability in the distributions. To test this, we ran a simulation of 1000 ensembles with settings that would create a high degree of variability in our sampling. Nominal P_0 for the model was set low at 25 eggs·m^{-2}, horizontal diffusion was also low at only 0.1 m^2·s^{-1} (only 1/5 of the default setting), strong clumping was set with a coefficient 0.1, and site to site variability was set to 100%. In this configuration of the DEPM analysis, over 68% of the vertical tows sites sampled had no eggs and the CV was nearly 60% (Figure 44). The estimate of P_0 is still a reasonable estimate (RE<2%) given the amount of variability put into the simulation. In contrast to the distributions shown in Figure 43, the effect of exponential mortality is still apparent in the binned data.

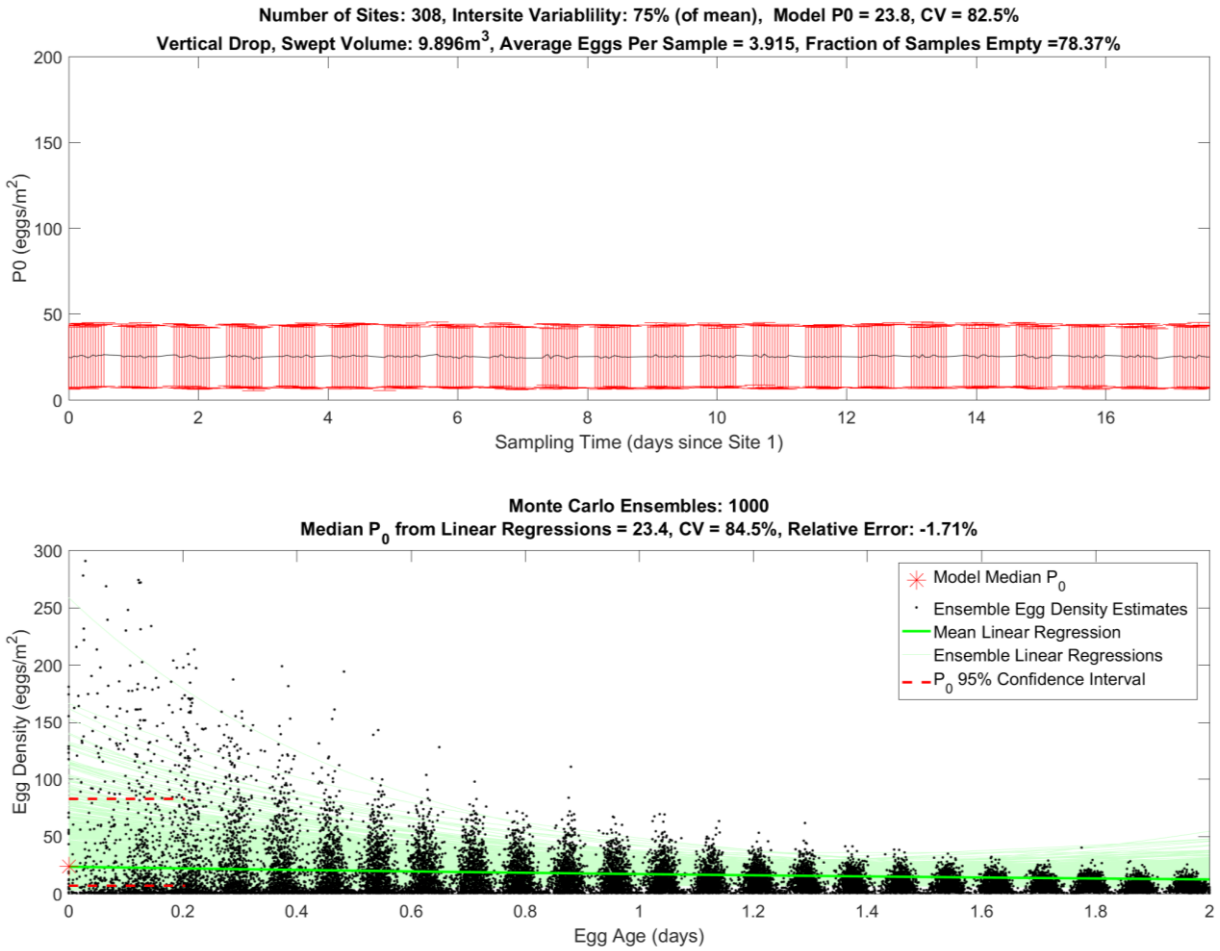

Figure 44: DEPM analysis for highly variable parameters. Top panel: Site to Site P_0 variability 100%. Bottom panel: DEPM regressions.

Since the analysis in Figure 44 is based on 1000 ensembles and 308,000 samples, a better comparison would be to look at the distribution within individual ensembles. To do this, we selected 14 ensembles at random and binned them by egg stage from Stage 1 to 10. Once removed from the large group ensembles, the individual ensembles have distributions that appear to be as skewed as the actual surveys with similar characteristics of frequent empty bins and occasional very large bins (Figure 45). The skewing of these distributions leads to the regressions shown in the lower panel of Figure 44 (the light green lines), frequently having a negative mortality coefficient, indicating an increase in egg density with age rather than a decline.

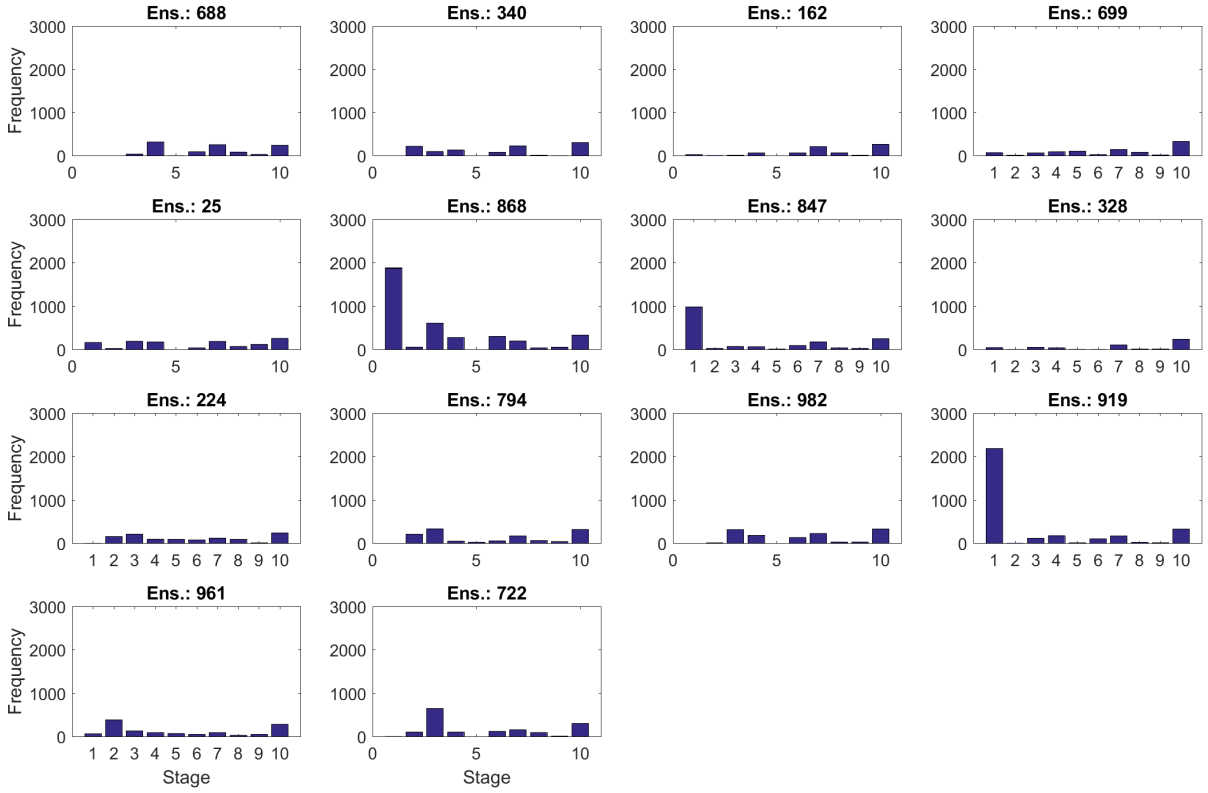

Figure 45: Distributions of Staged Eggs from randomly selected ensembles showing similar egg frequency variability to that in **Figure 43**.

Model Experiments and Results

A series of model runs with MCE set to 1000 were conducted to examine the model sensitivity to four parameters hypothesised to have the greatest influence on the estimate of DEPM: sampling strategy, nominal P_0, site-to-site variability (SSV) and clumping coefficient (CC). The model runs were conducted using default parameter values (Table 6, 7 and variations outlined in Table 10) in sequence starting with the combination of parameters that were assumed to create the most unfavourable conditions for estimating P_0 (Run 1) to the combination which should give the most favourable conditions (Run 16). The results of the 16 model runs are outlined in Table 11 with the CV for P_0 and both log-linear and least-squares fit regressions. We used the CV to characterize the amount of spread in the estimates; a better system will have a smaller spread and provide higher confidence in the final estimate of P_0 calculated from a single survey. Differences between CVs were tested for significance using modified McKay 95% confidence intervals (Vangel 1996). High SSV values (75%) tended to bias the actual P_0 to slightly less than the nominal value but overall the CV associated with actual P_0 was consistent with the SSV. The CV results are highlighted from red (highest CV) to green (lowest CV) so that for the regressions the reddest results represent the method providing the poorest estimates of P_0.

Table 10: Summary of model sensitivity experiments

Model Run	P_0 (eggs·m^{-2})	Sampling	Site to Site Variability (%)	Clumping Coefficient
1	25	Vertical Tow	75	0.1
2	25	Vertical Tow	75	1.0
3	25	Vertical Tow	25	0.1
4	25	Vertical Tow	25	1.0
5	25	Oblique Tow	75	0.1
6	25	Oblique Tow	75	1.0
7	25	Oblique Tow	25	0.1
8	25	Oblique Tow	25	1.0
9	100	Vertical Tow	75	0.1
10	100	Vertical Tow	75	1.0
11	100	Vertical Tow	25	0.1
12	100	Vertical Tow	25	1.0
13	100	Oblique Tow	75	0.1
14	100	Oblique Tow	75	1.0
15	100	Oblique Tow	25	0.1
16	100	Oblique Tow	25	1.0

Table 11: Summary of results of model simulations. Colour shaded columns reflect the scale of the CV metric; darker greens are lower while darker reds are higher.

Run	P_0 (eggs·m^{-2})		log-linear P_0 (eggs·m^{-2})			least squares fit P_0 (eggs·m^{-2})		
	median	CV	median	CV	RE	median	CV	RE
1	23.77	82%	22.82	83%	-4%	27.18	131%	14%
2	23.79	82%	21.29	79%	-10%	27.65	197%	16%
3	24.79	33%	21.52	86%	-13%	26.46	142%	7%
4	24.80	33%	21.93	77%	-12%	26.27	137%	6%
5	23.70	83%	22.06	66%	-7%	24.85	82%	5%
6	23.79	82%	20.19	70%	-15%	23.20	98%	-2%
7	24.80	33%	19.72	71%	-20%	23.03	97%	-7%
8	24.82	33%	19.96	63%	-20%	23.22	75%	-6%
9	96.87	78%	85.04	48%	-12%	93.88	57%	-3%
10	96.86	79%	86.35	46%	-11%	91.65	53%	-5%
11	100.40	27%	83.07	45%	-17%	89.93	54%	-10%
12	100.38	27%	87.95	42%	-12%	94.08	54%	-6%
13	96.88	78%	88.88	38%	-8%	89.86	41%	-7%
14	96.69	79%	89.09	38%	-8%	91.17	39%	-6%
15	100.37	27%	88.02	39%	-12%	89.37	41%	-11%
16	100.35	27%	87.76	37%	-13%	90.38	39%	-10%

Discussion

1. Improve methods used to determine the age of egg cohorts

The ‘universal’ egg staging method developed in this project has two major advantages over the staging systems used in many other DEPM studies (e.g. Lo et al. 1996). Firstly, the ‘universal’ stages are based on distinctive morphological characteristics that are found in most pelagic fish eggs and easily identified in the laboratory. As a result, the universal method is likely to result in fewer errors in the allocation of eggs to stages than other systems. Secondly, the ‘universal’ system does not include stages with short and long durations that are present in other staging systems (e.g. Lo et al. 1996). Establishing stages with similar durations meant that variations in the observed densities of different stages (ages) could not be attributed to variations in the length of time that different stages were available to be sampled. This development allowed us to demonstrate unequivocally that young Australian Sardine eggs occur infrequently in plankton samples collected using CalVet nets. This finding is important because the absence of young eggs in samples is likely to be a major factor contributing to the high levels of uncertainty associated with estimates of P_0, which creates much of the uncertainty in estimates of spawning biomass obtained using the DEPM (e.g. Stratoudakis et al. 2006; Bernal et al. 2012; Dickey-Collas 2012). The reasons that young eggs occur infrequently in samples are discussed below.

In this study, we used a combination of two methods to estimate the age and developmental rates of eggs based on data collected during the surveys. The estimates of development rates of Australian Sardine eggs obtained from field data from moderate water temperatures (18–22°C) were similar to those obtained in incubation experiments reported by Lo et al. (1996). This finding is important as it justifies the use of data from Lo et al. (1996) to estimate the age of eggs in samples of Australian Sardine eggs collected off southern Australia. These results also demonstrate that field data contain useful information on egg age, highlighting the potential for field data to be used to estimate egg development rates and age egg cohorts and/or validate incubation experiments.

It is important to note that many factors affect the age of eggs in each sample, including spawning time, ambient temperature and variations in the development rates among individual eggs. All species of pelagic fishes considered in this study spawn at depth (e.g. >50 m) and the positively buoyant eggs float to the surface (e.g. Lakser 1985). During their ascent, eggs are potentially exposed to a range of temperatures, making the assignment of a reliable ambient temperature to each sample problematic. However, this problem affects all methods currently used to estimate the age, development rate and/or period between spawning and hatching time of pelagic fish eggs collected in field samples.

Another key development made during this project was refining the method used to identify samples where a zero count should (and should not) be allocated to one or more egg cohorts. Determining which

samples should include cohorts spawned over one, two or three days (dependent on the ambient water temperature, the spawning time and sampling time) is important because the presence/absence of zeros can have considerable influence on estimates of P_0. In particular, the presence of cohorts with zero egg counts in days 24 hours and 48 hours after spawning are important for anchoring the regressions of egg density versus age. These refinements have already been incorporated into analytical procedures used to estimate P_0 for the SASF and the SPF (e.g. Ward et al. 2017).

2. Compare performance of current statistical methods on long-term datasets for several species

This discussion of the comparison of models for estimating P_0 and z focuses on data available for Australian Sardine off South Australia because of the limited sample sizes for other species and locations. This component of the study clearly shows that none of the models used to estimate P_0 and z fit the data well; all models produce implausible estimates of these parameters in some years. This finding is not new; numerous other studies have identified the difficulties associated with estimating P_0 and z during application of the DEPM (e.g. Stratoudakis et al. 2006; Bernal et al. 2012; Dickey-Collas 2012).

One of the reasons it is difficult to estimate P_0 and z is shown in the plots of densities in 4-hour age bins; young eggs (i.e. <8 hours old) are under-represented in samples. This finding does not match the assumption of an exponential decline in egg density with age caused by egg mortality. The simulation modelling discussed below helps to explain why young eggs are under-sampled. Young cohorts of eggs are sampled less often than older cohorts because they occur in smaller clumps (with higher densities) and are encountered by the net less often than the larger (lower density) clumps of older eggs. The small size and higher densities of clumps of young eggs is the result of limited time available for dispersal to occur, which also explains the high variability observed in the densities of eggs <24 hours old (i.e. variance greater than the mean). Many samples contain no eggs less than <24 hours old, and a few samples have high egg densities.

A key finding from the comparison of models is that, in almost all cases, the log-linear model provides more plausible estimates of P_0 and z than the other models. This is because log transforming the data reduces the influence of samples with very high egg densities that cause other models to produce unrealistically high estimates of P_0 and z in some years (e.g. 2014). This finding was supported by the results of the simulation modelling discussed below. The notable exception to this interpretation was 2001, when the log-linear model produced an implausible (negative) estimate of z.

The other outputs that demonstrate the log-linear model performs better than the other models tested are the 95% CIs of the estimates of P_0 and z. The 95% CI of the estimates of both P_0 and z obtained using the log-linear model are lower than or similar to the other models in all years. In some years, such as 2014, the

95% CIs for all models except the log-linear model were much greater than the mean estimate of P_0 and z. Estimates of confidence intervals obtained by jackknifing produced similar results to bootstrapping, i.e. the 95% CIs for the log-linear model were lower than or similar to the other models in all years. The RMSEs of leave one out cross validation were similar for all models; however, the log-linear model had slightly higher RMSE values than the other models. This may reflect the negative bias of estimates of P_0 and z obtained using the log-linear model (see simulation modelling below).

A major limitation of the GAM models is that a value for z needs to be assumed to allow estimation of P_0 at each site. The failure of the GAMs to merge in 50% of years for which data were available also restricts their applicability. GAMs based on estimates of z from the log-linear model produced more plausible estimates of P_0, with narrower 95% CIs, than those based on estimates of z from other models. Inclusion of environmental data in the GAMs does not appear to significantly improve estimates of egg production.

3. Simulations to evaluate performance of different approaches to sampling and data analysis

The Monte-Carlo simulations allowed us to analyse the results of thousands of independent surveys of the Australian Sardine spawning area in South Australia to determine the sensitivity of the estimate of P_0 to some physical parameters and the sampling strategy. In contrast, logistical limitations of the real world usually allow only one survey per season. The statistical results of the ensembles identified sampling techniques that would provide the best chance of making a reasonably accurate estimate of P_0. Most notably, the non-linear least-squares fit estimates of P_0 show significantly higher CVs than the log-linear regressions. This reflects the fact that the least-squares fit occasionally make extremely large over-estimates of the true P_0 (e.g. Run 2, CV: 197%). The relative error (RE) between the ensemble estimate and true P_0 suggests that the log-linear regressions tends to under-estimate the true P_0 (i.e. is negatively biased). Overall, the log-linear approach provides more precise and conservative estimates of P_0 than non-linear least-squares fitting.

Two important factors affected CV values: the nominal P_0 and sampling strategy. The nominal P_0 (egg density used to initialize the model) is the most important factor affecting the CV values. Low egg-density model runs (P_0 = 25 eggs·m^{-2}) have high CV values, especially for vertical tows. The simulations show that the egg mortality curve can be characterised better when larger numbers of eggs are sampled. Sampling strategy was the other major factor affecting CVs. The oblique tow showed a significant improvement over the vertical tow for all model comparisons (e.g. Table 10: 1 versus 5; 2 versus 6). The oblique tow method sampled about six times the volume of the vertical tow. The higher egg numbers collected at all depths provided a much better basis for estimating P_0. Two other factors, site-to-site variability (SSV) and clumping of the distributions (CC), had minimal effect on the size of the CVs.

Conclusion

This study demonstrated the well-known challenges associated with estimating P_0 and z in the application of the DEPM. It also confirmed the findings of a previous review (Ward et al. 2011): the log-linear model is the most appropriate method currently available for estimating P_0 and z for Australian Sardine, because this approach produces more precise and plausible estimates of these parameters than the other models. Importantly, the log-linear model does not produce the unrealistically large estimates of P_0 that the other models often produce when a few samples contain very high densities of eggs. The fact that estimates of P_0 obtained using the log-linear model are negatively biased means that resulting estimates of spawning biomass are likely to be conservative (i.e. precautionary).

The universal egg staging method developed during this project helped to identify one of the reasons why it is difficult to estimate P_0 and z, i.e. that young eggs are under-represented in samples. The simulation modelling helped to explain why young eggs are under sampled, i.e. because they present small targets to the egg sampler as they have not yet undergone significant dispersal. The simulation modelling study also supported the idea that estimates of egg production may be improved by sampling eggs using oblique rather than vertical tows (SARDI, unpublished). The modelling platform developed in this project is a significant legacy that will allow ongoing evaluation of options for improving methods used to estimate of P_0 and z.

Implications

Several significant developments were made during this project. The universal egg staging system that was developed and the refinements to the method used to allocate zero counts to egg cohorts have already been adopted in the application of the DEPM in the SASF and SPF (e.g. Ward et al. 2017). Historical estimates of spawning biomass for the southern stock of Australian Sardine have also been updated using the refined methods for estimating P_0 and z developed in this project (Ward et al. 2017).

As a result of this project, the use of an oblique sampler (Nackthai) for collecting samples to estimate P_0 and z will be trialled for Australian Sardine in South Australia (FRDC Project No. 2017-027) in 2017/18. This trial will be conducted in conjunction with the 2018 application of the DEPM to Australian Sardine off South Australia. A report evaluating the costs/benefits of the new oblique sampler will be completed in late 2018.

Several recent studies have highlighted the imprecision of estimates of spawning biomass obtained using the DEPM (e.g. Bernal et al. 2012, Dickey-Collas et al. 2012; Ward et al. 2011; Steer et al. 2017). The

high level of uncertainty in estimates of spawning biomass is partly driven by the difficulties associated with estimating P_0 and z identified in this study. However, for species such as those considered here (i.e. Australian Sardine, Jack Mackerel and Blue Mackerel) that have low spawning fractions (e.g. 5-20%), problems associated with estimating P_0 and z are also exacerbated by difficulties associated with the estimation of spawning fraction. At least two reviews (Alheit 1993, Stratoudakis et al. 2006) have concluded that the DEPM is better tailored to species with high spawning fractions (>50%), such as anchovies (*Engraulis* spp.) and snappers (Steer et al 2017), than to species with low spawning fractions, such as Sardine. This is because spawning biomass is inversely proportional to spawning fraction, i.e. low estimates of spawning fraction produce high estimates of spawning biomass (e.g. Alheit 1993, Stratoudakis et al. 2006). Relatively small variations (including errors) in estimates of low spawning fractions (5 to 15%) have strong effects on spawning biomass (i.e. 300%), whereas comparable variations for species with high spawning fractions (50 to 60%) have relatively less impact (i.e. 20%).

Recent studies (e.g. Ward et al. 2011; 2017) suggest that significant increases in the precision of estimates of the spawning biomass of Australian Sardine off South Australia will be difficult to achieve due to the combined challenges of estimating P_0 and spawning fraction. However, improving estimates of these two parameters remains a high priority for the SASF, because currently there is no viable alternative to the DEPM for monitoring the status of spawning stock of Australian Sardine off South Australia (e.g. Ward et al. 2017). In 2017/18, a project funded by members of the SASF and FRDC will investigate whether the precision of mean daily egg production estimates can be improved by collecting samples using oblique rather than vertical plankton tows. In addition, an industry-based program will be undertaken in 2018 to assess the potential for using samples obtained from commercial vessels to estimate spawning fraction in offshore waters of South Australia where the current adult sampling method (i.e. a gillnet) does not work effectively.

Ward et al. (2017) recommended that as well as continuing to improve the precision of estimates of spawning biomass, consideration should be given to evaluating alternative key performance indicators in the harvest strategies for the SASF. One option for tracking the future status of the Australian Sardine population off South Australia is to use spawning area as an indicator of stock status, as suggested by Mangel and Smith (1990) and Gaughan et al. (2004). It is notable that spawning area has been used as an informal proxy for spawning biomass in the SASF when difficulties estimating one or more parameters (i.e. P_0 and spawning fraction) have produced unrealistic or highly uncertain estimates of spawning biomass (e.g. Ward et al. 2014, 2016). Establishing spawning area as an indicator of stock status may address current difficulties associated with estimating both egg production and spawning fraction reliably, and may provide the most precise option for tracking year to year fluctuations in stock size. Reference points could potentially be established on the basis of the historical relationship between spawning area and spawning biomass (SARDI unpublished data).

Recommendations

- Evaluate the cost/benefits of using an oblique plankton sampler (Nakthai) to collect egg samples used to estimate P_0 and z.
- Examine the effects of dispersal on estimates of P_0 and z by collecting plankton samples from a range of depths (i.e. discreet depth sampling).
- Investigate the potential for using spawning area as a proxy for spawning biomass as outlined by Mangel and Smith (1990) and Gaughan et al. (2004).

Extension and Adoption

Objectives

- Incorporate refined methods into stock assessment of SA Sardine Fishery.
- Specialist peer review achieved through engagement with international experts and publication in international journals.

Target Audience/s

- PIRSA Fisheries and Aquaculture
- Research and Management Committee for the SASF
- South Australian Sardine Industry Association, Inc.
- AFMA, SPF Scientific Panel, quota holders in the SPF
- Australian community

Key Message/s

- Enhanced estimates of stock status to facilitate sustainable development of the SASF and SPF.

Communication/Extension Methods and Action Plan

- Workshops and presentations to PIRSA, AFMA, SASF and SPF stakeholders during project
- Methods incorporated into stock assessments
- Final report completed and scientific papers drafted.

Evaluation

- Workshops and presentations to PIRSA, AFMA, SASF and SPF industry and stakeholders have been conducted throughout the project

- Refined methods have been incorporated into stock assessments for SASF and SPF from 2017 onwards.
- International workshop and stakeholder forum on pelagic fisheries conducted in 2014.

Reports and scientific papers

- Findings incorporated in the stock assessment report for SASF (Ward et al. 2017)
- Simulation modelling paper – in prep.
- Reanalysis of sardine data and comparison of methods paper – in prep

Outcomes and Benefits

This project identified that the log-linear model is the most precise method for estimating P_0 and z in applications of the DEPM. Identifying the most precise method for estimating P_0 and z is important because resulting estimates of spawning biomass are the key performance indicator underpinning the sustainable management of Australia's main fisheries for small pelagic species (e.g. SASF, SPF).
The identification of an alternative sampling method that may further increase the precision of estimates of P_0 and z is also important and has implication for the application of the DEPM to other species, especially those where spawning fraction can be estimated reliably.

The conclusion that consideration should be given to evaluating potential alternative performance indicators for the SASF is also important. The potential for using spawning area to track inter-annual variations in Australian Sardine population off South Australia is supported by analyses by Mangel and Smith (1990) and Gaughan et al. (2004). It is notable that spawning area has been used as an informal proxy for spawning biomass in the SASF when difficulties estimating one or more parameters (i.e. P_0 and spawning fraction) have produced unrealistic or highly uncertain estimates of spawning biomass (e.g. Ward et al. 2014, 2016).

The direct beneficiaries of the outputs are: the SASF, PIRSA Fisheries and Aquaculture, the SPF, AFMA and other stakeholders (e.g. recreational fishers, conservation groups) interested in the robust assessment and sustainable management of Australia's stocks of small pelagic fishes. This study will also benefit industries, fisheries managers and stakeholders in other jurisdictions that support harvestable populations of small pelagic fishes (NSW, Victoria, NT and Tasmania). Resolving current uncertainty and lack of consensus about the best approach to estimating egg production will help address community concerns about the sustainable use of Australia’s stocks of small pelagic fishes.

Appendices

Appendix A

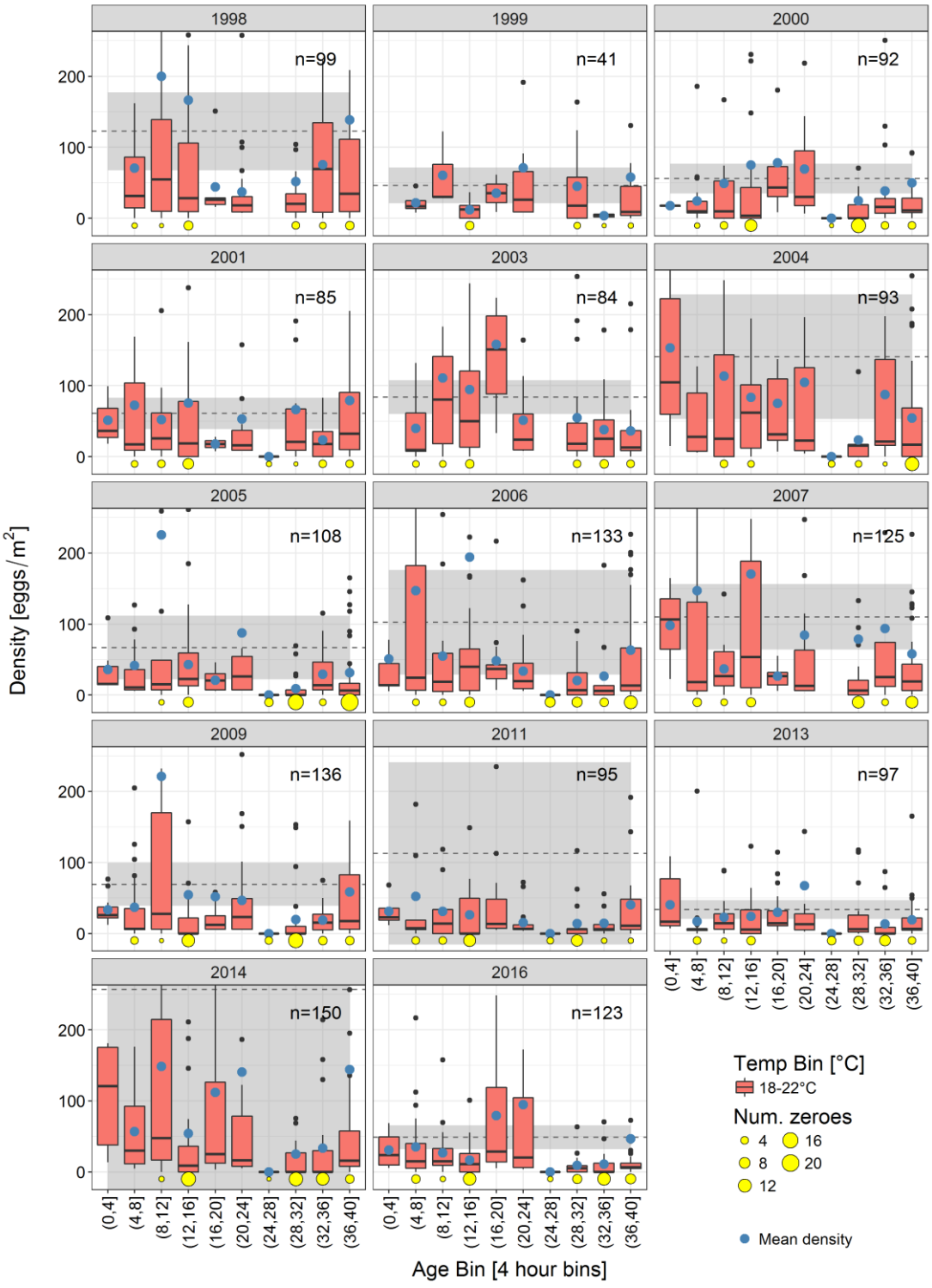

Figure A 1: Egg densities of Australian Sardines (South Australia) collected during DEPM surveys from 1998 to 2016 calculated from egg ages estimated directly from field data. Egg densities are plotted by age (4 hour bins). Yellow dots: number of zero counts in daily cohort; n: number of density points per year; dashed line and shading: mean egg density for all values with 95% CI.

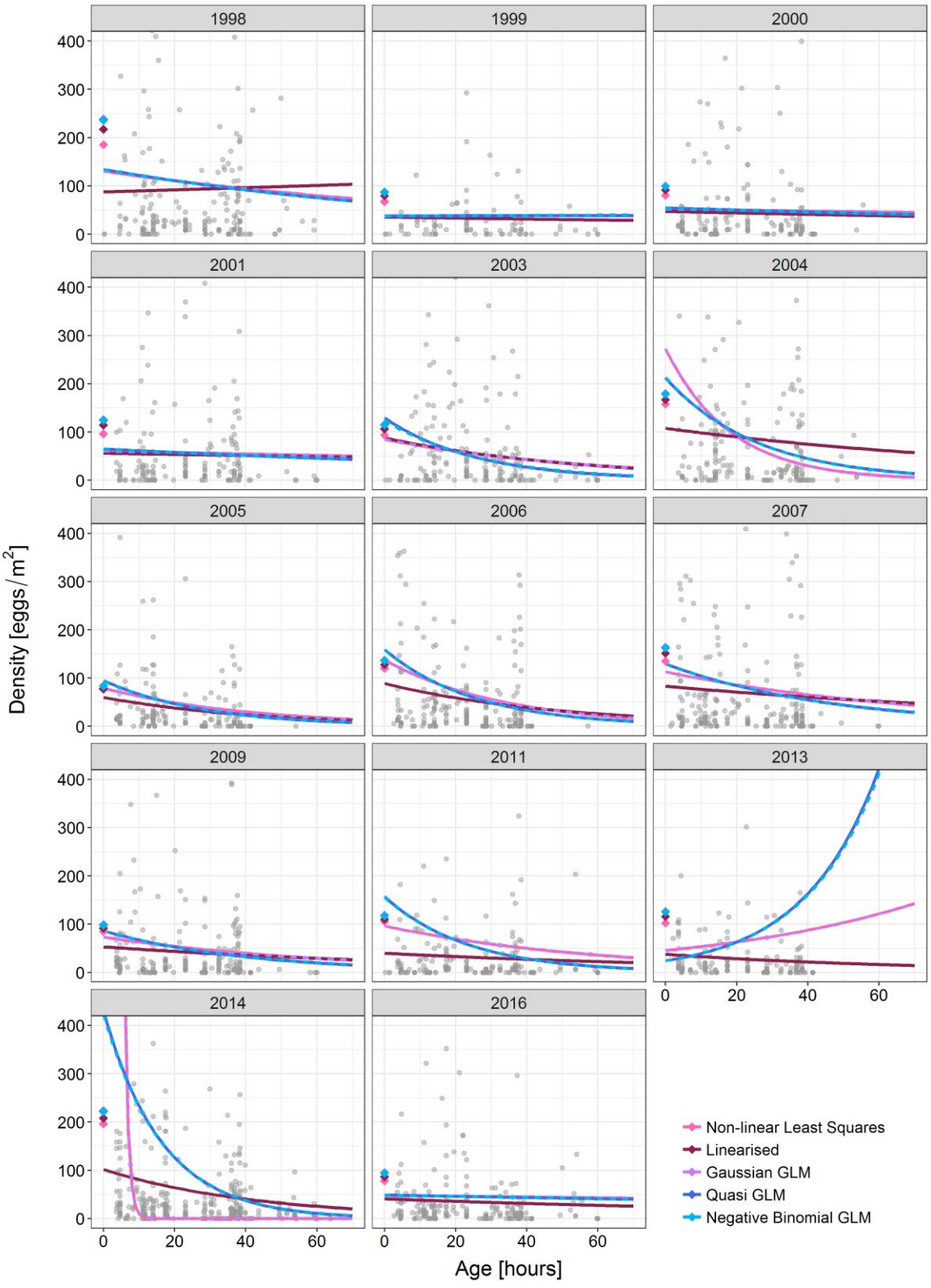

Figure A 2: Fits of non-GAM models by year to data of egg densities calculated from egg ages estimated directly from field data for Australian Sardine (South Australia). Diamonds: values of P_0 predicted using a common mortality (all years z) obtained by fitting each non-GAM model to an all-years combined data. Linearised: log-linear model.

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