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Spawning biomass of Blue Mackerel (*Scomber australasicus*) and Australian Sardine (*Sardinops sagax*) in the East sub-area of the Small Pelagic Fishery

Report to the Australian Fisheries Management Authority

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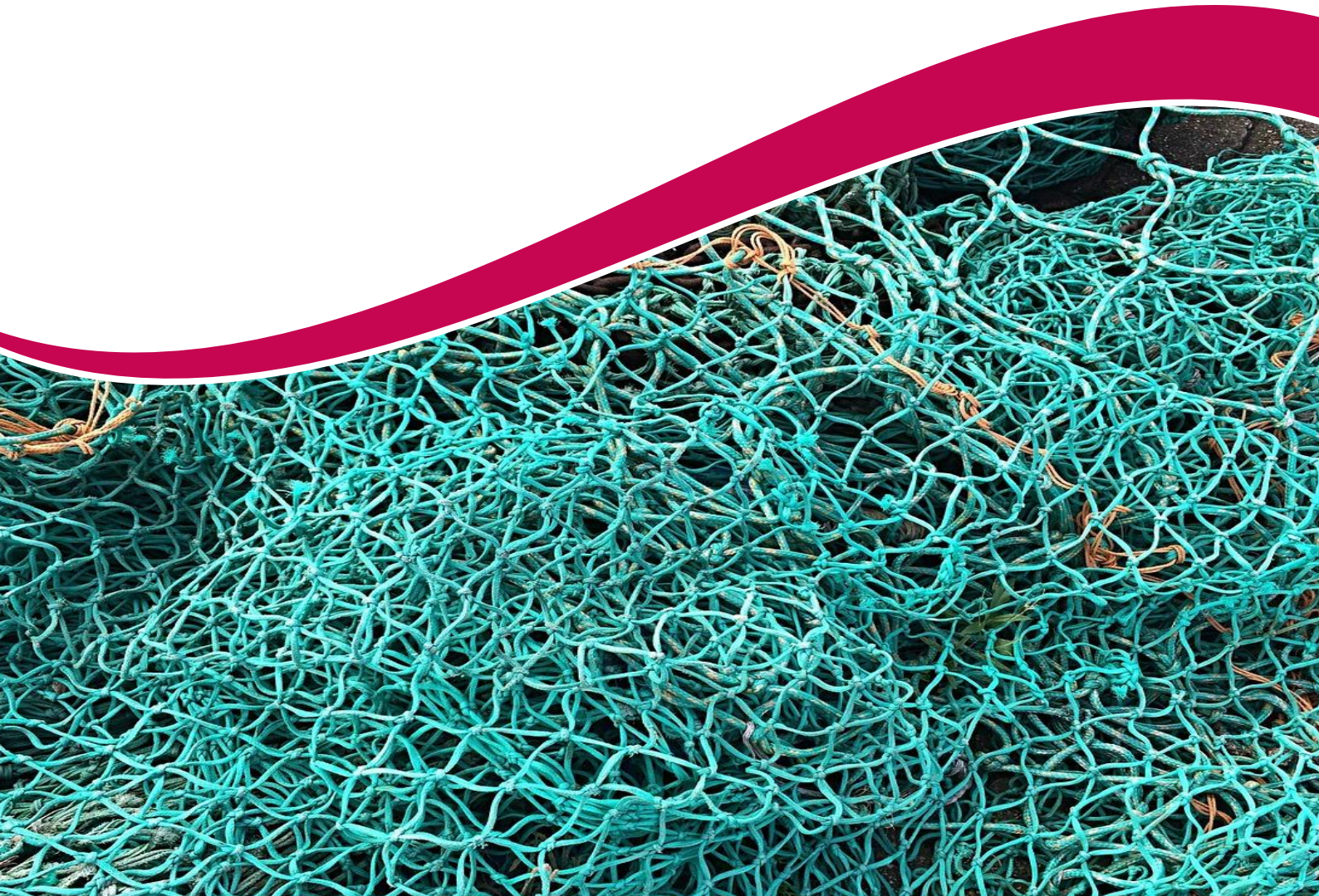

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Executive Summary

Background and Need

Estimates of spawning biomass (*SB*) obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator for target species in the Commonwealth Small Pelagic Fishery (SPF). Estimates of spawning biomass are used to set Recommended Biological Catches (RBCs) and Total Allowable Catches (TACs) under guidelines outlined in the SPF Harvest Strategy. The Harvest Strategy has three tiers with different exploitation rates.

The DEPM was previously applied to Blue Mackerel (*Scomber australasicus*) and Australian Sardine (*Sardinops sagax*, hereafter Sardine) in the East sub-area of the SPF in 2014. Blue Mackerel East would have reverted to Tier 2 in 2020/21, reducing the exploitation rate from 15% to 7.5%, unless an application of the DEPM was completed in 2019. The reduction in TAC associated with the decline to Tier 2 would have impeded the development of the fishing operation in the East sub-area in 2016/17.

The DEPM was applied to Blue Mackerel and Sardine between southern Queensland and central New South Wales in September 2019 to coincide with the peak spawning seasons of the two species.

Objectives

The objectives of this study were to:

1. Estimate egg production, spawning area and adult reproductive parameters of Blue Mackerel from egg and adult surveys conducted in the East sub-area of the SPF during September 2019.

2. Estimate the *SB* of Blue Mackerel and Sardine in the East sub-area in 2019.

Methods

The rationale for the DEPM is that *SB* can be calculated by dividing the mean number of eggs produced per day (i.e. total daily egg production) by the mean number of eggs produced per unit weight of adult fish (i.e. mean daily fecundity).

To estimate total daily egg production, ichthyoplankton samples were collected at 251 sites in shelf waters between southern Queensland and central New South Wales from 3 to 24 September 2019.

Blue Mackerel and Sardine eggs were identified using standard laboratory procedures. Morphological identifications of Blue Mackerel eggs were confirmed using the molecular

techniques described in Ward *et al.* (2015). Mean daily egg production (P_0) and spawning area (*A*) were estimated using methods described in Ward *et al.* (2020).

Adult Blue Mackerel were sampled using a modified demersal trawl net between 12 and 15 September 2019 in continental shelf and slope waters. Adult sampling for Sardine was not undertaken during this study. Adult reproductive parameters of both species, i.e. spawning fraction (*S*), sex ratio (*R*) and relative fecundity (*F'*) were estimated using methods described in Ward *et al.* (2020).

Uncertainty estimates for all parameters are 95% Confidence Intervals (CIs). Sensitivity analyses were undertaken to determine the influence of uncertainty in individual parameters on estimates of the *SB* of Sardine and Blue Mackerel.

Results, Discussion and Implications

The total area covered by the ichthyoplankton survey was 62,476 km^2.

Live Blue Mackerel eggs (n = 1,829) were collected at 81 of the 251 sites (32%). *A* was 20,387 km^2 and P_0 was 36.5 (18.6–59.3) $eggs.day^{-1}.m^{-2}$. Adult Blue Mackerel collected as part of the 2019 survey were mature, but relatively small and not actively spawning. Estimates of adult parameters (95% CI) of Blue Mackerel were obtained from samples collected off South Australia between 2002 and 2006. *S* was 0.135 (0.102–0.167); *R* was 0.448 (0.40–0.50); and *F'* was 139.9 (136.3–143.5) $eggs.g^{-1}$. *SB* of Blue Mackerel in the East sub-area of the SPF in 2019 was 88,265 (33,320–143,209) t, which is 6% higher than the estimate of 83,300 t obtained in 2014 (Ward et al. 2015).

Live Sardine eggs (n = 4,667) were found in 58 (22.9%) of samples. *A* was 14,281 km^2 and P_0 was 53.9 (29.4–95.9) $eggs.day^{-1}.m^{-2}$. Estimates of adult parameters for Sardine were obtained from samples collected off South Australia between 1998 and 2018. *S* was 0.108 (0.100–0.123), *R* was 0.55 (0.52–0.58), and *F'* was 305.0 (303.8–306.3) $eggs.g^{-1}$. *SB* of Sardine in the East sub-area of the SPF in 2019 was 42,724 (95% CI = 15,487–69,962) t, which is 14% lower than the estimate of 49,600 t obtained in 2014 (Ward et al. 2015).

The *SB* of Blue Mackerel in the East sub-area appears to have remained stable or increased between 2014 and 2019, despite recent increases in annual catches. In contrast, the *SB* of Sardine appears to have declined even though annual catches over the last decade have been relatively low. Intensive adult sampling programs to obtain robust estimates of *S* in the East sub-area are needed for both species. However, due to the recent increases in annual catches this need is most pressing for Blue Mackerel.

Keywords: Daily Egg Production Method

1 Introduction

1.1 Background

The Commonwealth Small Pelagic Fishery (SPF) was established in 2000 and is managed by the Australian Fisheries Management Authority (AFMA). It is a purse-seine and mid-water trawl fishery that operates in Commonwealth waters (3–200 nm) from southern Queensland to south-western Western Australia, including Tasmania. The fishery is divided into two sub-areas (East and West) at longitude 146°30'E (AFMA 2009). The target species are Jack Mackerel (*Trachurus declivis*), Redbait (*Emmelichthys nitidus*), Blue Mackerel (*Scomber australasicus)* and Sardine (*Sardinops sagax*, hereafter Sardine). Sardine can only be taken in the Sardine Sub-area off NSW and southern Queensland.

A detailed history of the SPF is provided by Moore and Skirtun (2012). Catch and effort in the SPF have fluctuated over time, driven by a combination of social, economic, biological and ecological factors. Catches increased in 2014/15 to 2015/16 when a factory trawler operated in both sub-areas of the SPF (Ward and Grammer 2018), and again in 2016/17 when a new fishing operation was established in the East sub-area off southern NSW.

The SPF Harvest Strategy and Management Plan were implemented in 2008/09 (AFMA 2008, 2009). The SPF Harvest Strategy was last revised in 2017. It is used to set Total Allowable Catches (TACs) for each target species and sub-area. Estimates of spawning biomass (*SB*) obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator for target species. Estimates of *SB* are used to set Recommended Biological Catches (RBCs) and Total Allowable Catches (TACs) under guidelines (i.e. exploitation rates) outlined in the Harvest Strategy. The Harvest Strategy has three tiers: Tier 1 has the highest exploitation rates and Tier 3 has the lowest.

1.2 Daily Egg Production Method (DEPM)

The rationale for the DEPM is that the adult biomass of fishes that spawn multiple batches of pelagic eggs over an extended spawning season can be calculated by dividing the mean number of eggs produced per day (i.e. total daily egg production) by the mean number of eggs produced per unit weight of adult fish (i.e. mean daily fecundity) (Parker 1980, 1985). The equation underpinning the DEPM is

$$SB = P_0 * A/(R * S * F') \qquad \text{Equation 1}$$

where *SB* is spawning biomass, P_0 is mean daily egg production, *A* is total spawning area,

R is mean sex ratio, *S* is mean spawning fraction and *F'* is mean relative fecundity (Parker 1980, Ward et al. 2021).

Parameters used to calculate total daily egg production, i.e. mean daily egg production (P_0) and spawning area (*A*), are estimated from ichthyoplankton surveys (e.g. Stratoudakis et al. 2006). Parameters used to calculate mean daily fecundity, i.e. sex ratio (*R*), spawning fraction (*S*) and mean relative fecundity (number of oocytes per gram of female weight, *F'*), are estimated from samples obtained from research or commercial vessels operating in the survey area during the study period (e.g. Stratoudakis et al. 2006).

The key assumptions of the DEPM are that: 1) surveys are conducted during the main spawning season; 2) the entire spawning area is sampled; 3) eggs are sampled without loss and identified without error; 4) levels of egg production and mortality are consistent across the spawning area; and 5) representative samples of spawning adults are collected during the survey period (Parker 1980, Alheit 1993, Hunter and Lo 1997, Stratoudakis et al. 2006). Several of these assumptions are not met in many applications of the DEPM (see Bernal et al. 2012, Dickey-Collas et al. 2012, Ward et al. 2021).

Although the DEPM has been used widely, a range of logistical and statistical challenges have been encountered and estimates of *SB* are known to be imprecise (e.g. Stratoudakis et al. 2006, Bernal et al. 2012, Dickey-Collas et al. 2012; Ward et al. 2018a, 2021). There are considerable uncertainties associated with the estimation of several parameters (Fletcher et al. 1996, McGarvey and Kinloch 2001, Gaughan et al. 2004). Recent studies have shown that inter-annual variations in estimates of several parameters can be low in comparison to statistical uncertainty (e.g. Ward et al. 2018a, 2019, 2020b). This means that precision of estimates of *SB* can be increased by estimating some parameters from historical data rather than annually (Ward et al 2021). Increases in the precision of estimates of *SB* can also be achieved by estimating *F'* as a single parameter (Ward et al. 2021), as was done in the original formulation of the DEPM by Parker (1980), rather than as two separate parameters (i.e. female weight and batch fecundity), as has been done in most recent applications of the DEPM (Stratoudakis et al. 2006).

1.3 Blue Mackerel

Blue Mackerel (*Scomber australasicus*, Cuvier 1832; *Scombridae*) is the only member of its genus that occurs in Australian waters (Gomon et al. 2008). It inhabits coastal and continental shelf waters (depths up to 200 m) throughout the Pacific Ocean and Indian Ocean. Chub Mackerel (*Scomber japonicus*), a closely related species found in neighbouring Indo-Pacific waters, is heavily fished in the northern Pacific Ocean (Collette and Nauen 1983). In Australia, Blue Mackerel occurs in subtropical and temperate waters from Queensland to Western Australia.

Commercial fishing for Blue Mackerel began off the east coast in the late 1980s (Stewart and Ferrell 2001). Total annual catches in the NSW Ocean Hauling Fishery have ranged between ~200 and 600 t over the last two decades (Ward and Grammer 2021). Catches in the SPF began to increase in 2015/16 and reached 5,652 t in 2019/20 (Ward and Grammer 2021).

Blue Mackerel may attain lengths up to 650 mm (Hutchins and Swainston 1986) and ages of 8+ years in Australia (Stevens et al. 1984), but results from Ward and Rogers (2007) suggest smaller, younger fish (200 to 300 mm TL, 1 to 2 years) are more common along the Queensland/NSW coast. In NSW, Stewart and Ferrell (2001) reported 70% of the commercial Blue Mackerel purse-seine catch was comprised of 1 year old fish.

Spawning of Blue Mackerel occurs between November and April off southern Australia and between July and October off eastern Australia (Neira and Keane 2008, Rogers et al. 2009). In southern Australia, Ward and Rogers (2007) estimated spawning frequency to be 2 to 11 days (mean: 7 days), and length at 50% maturity for males and females was 236.5 and 286.8 mm FL, respectively. Shelf waters of southern Queensland and northern NSW are the main spawning area for the eastern Blue Mackerel stock (Rogers et al. 2009). Eggs and larvae along the east coast are found in high abundances in shelf waters with mean temperatures of 18 to 21°C (Neira and Keane 2008).

Blue Mackerel is serial spawner with buoyant, pelagic eggs that make it suitable for the DEPM (e.g. Lasker 1985, Rogers et al. 2009, Ward et al. 2009). The DEPM was previously applied to Blue Mackerel in the East sub-area in 2004 (Ward et al. 2009) and 2014 (Ward et al. 2015). The preliminary application of the DEPM in 2004 suggested that the *SB* of Blue Mackerel in the East sub-area was at least 30,000 t (Ward et al. 2009). The DEPM survey undertaken off eastern Australia in August/September 2014 suggested that the *SB* of Blue Mackerel was ~83,300 (95% CI = 35,100–165,000 t).

This report builds on the results of the previous two studies (Ward et al. 2009, 2015). The survey was conducted in the area between southern Queensland and central New South Wales to ensure that it covered the entire spawning area. It was timed to coincide with the peak spawning season in this region.

1.4 Sardine

Sardine (*Sardinops sagax*, Jenyns 1842, *Clupeidae*) is found in temperate marine waters from southern Queensland to Western Australia, including northern Tasmania (Gomon et al. 2008). It is a small, stream-lined, forage species (Gomon et al. 2008) that supports some of the world's largest fisheries (Schwartzlose et al. 1999). In the Sardine sub-area of the SPF, the species is targeted by fishers operating in the SPF and NSW Ocean Hauling Fishery (Ward and Grammer 2021). Annual catches in the Sardine sub-area of the SPF

peaked at 3,761 t in 2007/08, but have been less than 800 t since 2010/11 (Ward and Grammer 2021). In 2019/20, the total catch from the Sardine sub-area was 727 t (Ward and Grammer 2021).

Off southern Queensland and northern NSW, the peak in GSI occurs in winter to early spring, i.e. August September (Ward and Staunton-Smith 2002). Off southern NSW, the peak GSI occurs between winter and summer, i.e. July to December (Stewart *et al.* 2010).

The DEPM has been applied sporadically to Sardine in the east sub-area of the SPF since the late 1990s (Staunton-Smith and Ward 2000, Ward et al. 2007). The *SB* in the Sardine sub-area during 2014 was estimated to be 49,575 (24,200–213,300) t (Ward et al. 2015).

1.5 Need

Blue Mackerel East would have reverted to Tier 2 in 2020/21 unless an application of the DEPM was completed in 2019. The reduction in the TAC associated with the decline to Tier 2 would have impeded the development of the fishing operation that was established off southern NSW in 2016/17.

1.6 Objectives

1. Estimate egg production, spawning area and adult reproductive parameters of Blue Mackerel and Sardine from egg and adult surveys conducted in the East sub-area of the SPF during September 2019.

2. Estimate the *SB* of Blue Mackerel and Sardine in the East sub-area in 2019

2 Methods

2.1 Total Daily Egg Production

2.1.1 Ichthyoplankton surveys

During September of 2019, ichthyoplankton samples were collected from the *FV Santa Rocco* at 251 sites on 45 transects in shelf waters between southern Queensland and central New South Wales (Figure 2–1, Appendix 2). The survey was undertaken during 4–24 September 2019. An additional sample was collected at every second site for genetic validation of Blue Mackerel eggs (n = 131).

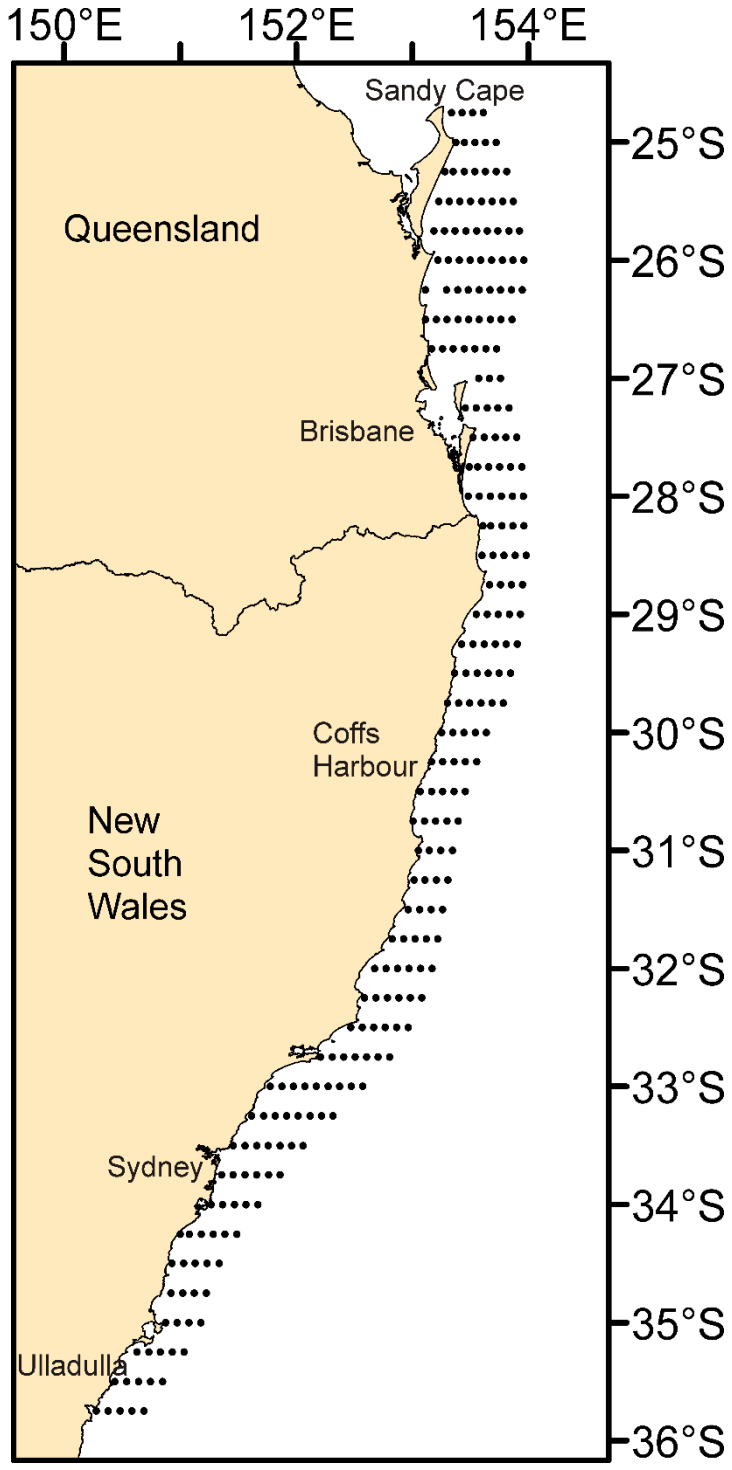
2.1.2 Plankton sampling

Paired bongo nets (0.6 m internal diameter, 500 µm mesh, plastic cod-ends) were deployed to 10 m above the sea floor or to a maximum depth of 200 m and retrieved vertically at ~1 $m \cdot s^{-1}$. Water temperature profiles were recorded with a Sea-Bird™ Conductivity-Temperature-Depth (CTD) attached below the nets. General Oceanics™ 2030 flow-meters and factory calibration coefficients were used to estimate the distance travelled by the nets during each tow. If there was >5% difference between the paired flow-meters, then the relationship between wire length released and flow-meter units was used to determine which meter was more accurate, and that value was used for both nets. At each sampling site, plankton collected in the paired net cod-ends were combined into one sample and fixed in a 5% buffered formalin and seawater solution. At every second site, a duplicate sample was collected for genetic validation; the paired cod-ends were combined and preserved in 95% ethanol. Location, sampling date/time, and depth were also recorded for each plankton sample.

2.2.3 Egg identification and validation

Eggs of Blue Mackerel were identified using the morphological features in published descriptions (Ward and Rogers 2007, Neira and Keane 2008, Ward et al. 2015). Identifications of Blue Mackerel eggs preserved in ethanol were validated using the molecular techniques developed by Perry (2011) and refined by Neira et al. (2015). These results were used to evaluate the morphological identification of the formalin preserved samples.

Figure 2–1. Area off southern Queensland to central New South Wales where the Daily Egg Production Method was applied to Blue Mackerel in September 2019.



All eggs were staged following Ward et al. (2018a, 2018b) (Figure 2–2). This method was used because the distinctive developmental characteristics of the stages reduce staging errors in the laboratory. Total counts of eggs per stage per sample were recorded. Eggs in the first and last stages were excluded from the statistical analyses because they are under- and over-represented in plankton samples, respectively (e.g. Ward et al. 2021).

2.1.4 Egg ageing and treatment of zero count egg samples

Kernel density plots were used to determine age at stage for Blue Mackerel from the combined egg density data from both the 2014 and 2019 east coast surveys (Ward et al. 2011). Data were separated into temperature bins to account for different development rates, however all egg data were in the middle bin of 17–23°C.

After eggs were assigned an age, eggs in each sample were aggregated into daily cohorts by stage. This was done because the eggs more than one night's spawning could be represented in a single sample. Total egg count and average age for each daily cohort was calculated by assigning each egg stage to a day of spawning (e.g. day 0, day 1, day 2), summing the number of eggs, and averaging their ages across the stages within the daily cohort. Average cohort ages were weighted by the number of eggs observed in each stage.

Samples were also identified where a zero count should (and should not) be allocated to one or more daily egg cohorts (Ward et al. 2018a). Samples with no eggs were excluded from the analyses and not considered to be part of the spawning area. Samples with eggs could contain several possible combinations of daily cohorts depending on water temperature, spawning time and sampling time. Since spawning occurs each night, zero counts were allocated for daily cohorts where the cohort was expected to be present but not found in the sample.

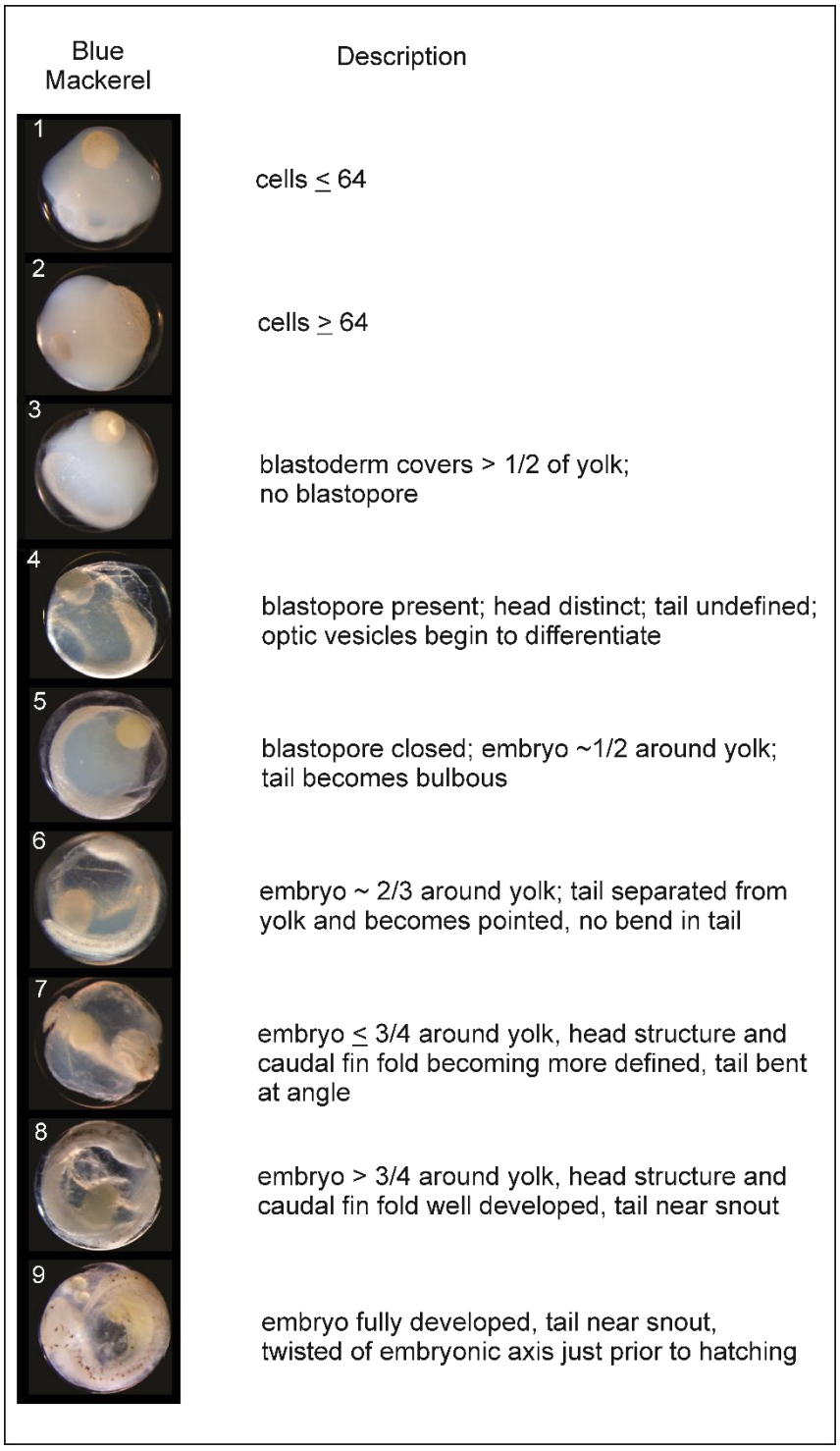
2.1.5 Egg density (P_t)

The number of eggs of each daily cohort under one square metre of water (P_t) was estimated at each site using Equation 2:

$$P_s = \frac{C\,D}{V} \qquad \text{Equation 2}$$

where C is the number of eggs at each age in a sample, V is the volume filtered (m^3), and D is the depth (m) to which the net was deployed (Smith and Richardson 1977). Plots of egg distribution and abundance were prepared using Surfer® (Ver. 8).

Figure 2–2. Egg stages of Blue Mackerel used in this study. Adapted from Ward et al. (2018a).



2.1.5 Spawning area (*A*)

The spawning area (*A*) was estimated (Lasker 1985, Somarakis et al. 2004) using the Voronoi natural neighbour method (Watson 1981). The survey area was divided into a series of contiguous polygons approximately centred on each site using the 'deldir' package 'R (R Development Core Team 2019, Turner 2016; Figure 2–3). The area represented by each site (km^2) was calculated. *A* was defined as the total area of the polygons where live Blue Mackerel eggs were present in the plankton samples.

2.1.6 Mean daily egg production (P_0) and egg mortality (z)

The model underpinning the estimation of mean daily egg production (P_0) is the exponential egg mortality model (Equation 3)

$$P_t = P_0 e^{-z\,t} \qquad \text{Equation 3}$$

where *Pt* is egg density at age *t* and *z* is the instantaneous rate of daily egg mortality.

However, previous studies have shown that this exponential model is unsuitable for estimating P_0 and *z* directly using non-linear least squares regression because egg data are not normally distributed and are often over-dispersed (Ward et al. 2011, 2021).

P_0 and *z* were therefore estimated using the linear (log-transformed) version of egg mortality model (Equation 4) with a bias correction factor (Equation 5) as described by Picquelle and Stauffer (1985).

$$\ln P_b = \ln\left(P_{i,t} + 1\right) - Zt \qquad \text{Equation 4}$$

$$P_0 = e^{\left(\ln P_b + {\sigma^2}/{2}\right)} - 1 \qquad \text{Equation 5}$$

where P_b is the negatively biased estimate of P_0, $P_{i,t}$: is the density of eggs of age *t* at site *i* and σ^2 is the variance of P_b.

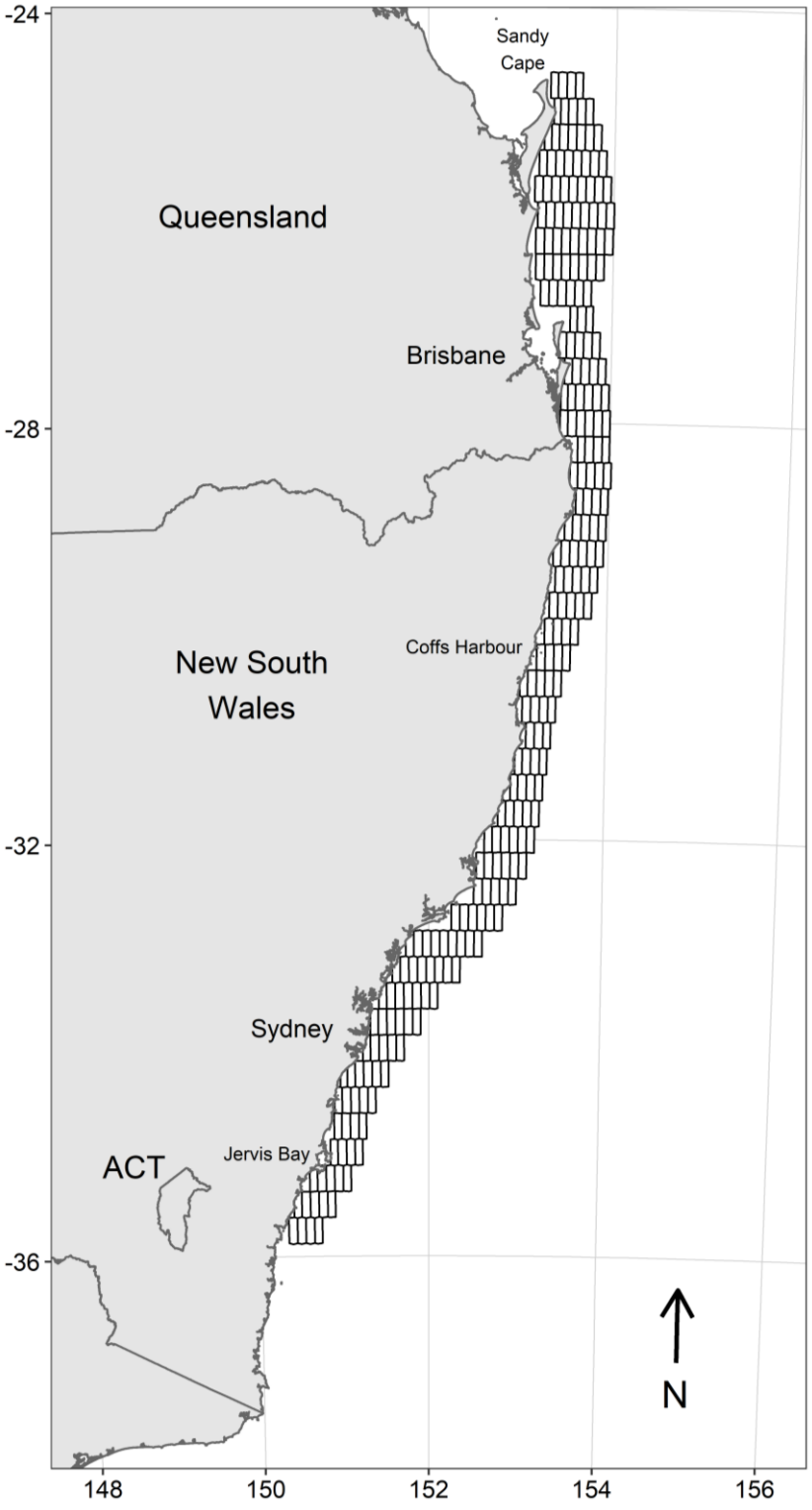


Figure 2–3. Polygons generated using the Voronoi natural neighbour method and used to estimate the spawning area of Blue Mackerel off eastern Australia in 2019.

P_0 and z were also estimated using a generalised linear model (GLM NB1) with a negative binomial error structure (Equation 6) which is considered suitable for over-dispersed count data, such as egg density by age (e.g. Ward et al. 2021)

$$E[P_0] = g^{-1}(-zt + \varepsilon) \qquad \text{Equation 6}$$

where E [P_0] is the expected value of P_0, g^{-1} is the inverse-link function, zt is the instantaneous rate of daily egg mortality at age t, and ε is the error term.

Variance of GLM NB1 increased linearly with the mean (Equation 7)

$$\sigma = \mu*(1+ \mu +\varphi)) \qquad \text{Equation 7}$$

where μ is the model estimate, σ is the model variance and φ is the overdispersion parameter.

Due to the challenges of estimating z (e.g. McGarvey et al. 2018), P_0 was also estimated using the method of McGarvey and Kinloch (2001), where z is assumed rather than estimated as a free parameter.

2.2 Mean Daily Fecundity

2.2.1 Adult Sampling

Adult Blue Mackerel were sampled using a modified demersal trawl net deployed from the *FV Saints Antonio Giuseppe* in shelf and slope waters off southern NSW between Newcastle and Jervis Bay between 12 and 15 September 2019 (Figure 3–1). Samples of Blue Mackerel collected in trawls were dissected and sexed. Mature females were labelled and fixed in a 10% buffered formaldehyde seawater solution. Males were labelled and frozen.

Samples of Blue Mackerel were not suitable for estimating adult reproductive parameters. Although fish collected were mature (yolked oocytes present) they were relatively small and showed minimal evidence of recent spawning (i.e. post-ovulatory follicles). No adult samples of Sardine were collected during the study. Samples collected off South Australia were used to estimate adult reproductive parameters of Blue Mackerel (2002-2006) and Sardine (1998–2018) (Ward et al. 2009, 2020).

2.2.2 Female weight (*W*)

Mature fish from each sample were weighed (± 0.01 g). The mean weight of mature females in the population was calculated from the average of sample means weighted by proportional sample size:

$$W = \overline{\left[\frac{\overline{W_i}\, n_i}{N}\right]} \qquad \text{Equation 8}$$

where, $\overline{W_i}$ is the mean female weight of each sample *i*; *n* is the number of fish in each sample and *N* is the total number of fish collected in all samples.

Mature males in each sample were thawed and weighed (± 0.01 g).

2.2.3 Sex ratio (*R*)

The mean sex ratio of mature individuals in each sample was calculated:

$$\overline{R_i} = \frac{F_i}{F_i + M_i} \qquad \text{Equation 9}$$

where F_i is the total weight of mature females in each sample *i,* and M_i is the total weight of mature males in each sample *i*

The sex ratio of the population was calculated from the average of sample means weighted by sample size:

$$R = \overline{\left[\frac{\overline{R_i}\, n_i}{N}\right]} \qquad \text{Equation 10}$$

where, *n* is the number of fish in each sample, *N* is the total number of fish collected in all samples and $\overline{R_i}$ is the mean sex ratio of each sample calculated from the equation:

2.2.4 Relative Fecundity (*F'*)

Batch fecundity was estimated from ovaries containing hydrated oocytes using the methods of Hunter and Macewicz (1985). Both ovaries were weighed and the number of hydrated oocytes in three weighed ovarian sub-sections counted. The total batch fecundity for each female was calculated by multiplying the mean number of oocytes per gram of ovary segment by the total weight of the ovaries. The relationship between female weight (ovaries removed) and batch fecundity was determined by linear regression analysis and used to estimate the mean batch fecundities of all mature females.

Relative fecundity, (*F'*), the number of eggs produced per gram of total female weight, was calculated for historical data from previous DEPM surveys for each female by dividing batch fecundity (i.e. *F* and $\hat{F}$) by total female weight (*W*):

$$F' = \bar{F}/\bar{W} \qquad \text{Equation 11}$$

where, *F'* is the relative fecundity, $\bar{F}$ is the mean fecundity and $\bar{W}$ is the mean weight.

The mean and variance of *F'* were calculated for each year and across all years (2002–2006 for Blue Mackerel and 1998–2018 for Sardine) using a ratio estimator (Rice 1995, Equation 12):

$$Var(F') = \frac{1}{n_{fish}} \cdot \frac{1}{\bar{W}^2} \cdot \{(F')^2 \cdot \sigma_W^2 + \sigma_F^2 - 2 \cdot F' \cdot cov(F,W)\} \qquad \text{Equation 12}$$

where σ_F^2 and σ_W^2 are the variances of $\bar{F}$ and $\bar{W}$, $cov(F,W)$ is the covariance of *F* and *W* and n_{fish} is the number of female fish.

2.2.5 Spawning Fraction (*S*)

Histological slides prepared from the ovaries of mature females were examined to estimate spawning fraction. Ovaries were sectioned and stained with haematoxylin and eosin using standard histological techniques. Several sections from each ovary were examined to determine the presence/absence of post-ovulatory follicles (POFs). POFs were assigned ages according to the criteria developed by Hunter and Goldberg (1980) and Hunter and Macewicz (1985). The spawning fraction of each sample was estimated as the mean proportion of females with day-0 POFs (*d*0) (assumed to be spawning or have spawned on the night of capture), day-1 POFs (*d*1) (assumed to have spawned the previous night) and day-2 POFs (*d*2) (assumed to have spawned two nights prior). The mean spawning fraction of the population was then calculated from the average of sample means weighted by proportional sample size (Equation 13).

$$S = \left[\overline{\overline{S_i} * \frac{n_i}{N}} \right] \qquad \text{Equation 13}$$

where, *n* is the number of fish in each sample, *N* is the total number of fish collected in all samples and $\overline{S_i}$ is the mean spawning fraction of each sample calculated from the equation:

$$\overline{S_i} = \frac{[(d0 + d1 + d2POFs)/3]}{n_i} \qquad \text{Equation 14}$$

where, *d*0, *d*1 and *d*2 POFs are the number of mature females with POFs in each sample and n_i is the total number of females within a sample.

2.3 Spawning biomass (*SB*)

Spawning biomass (*SB*) was calculated according to Equation 1 using the estimate of P_0 obtained from the method of McGarvey and Kinloch (2002) for Blue Mackerel and the log-linear model for Sardine. The estimates of *A* were obtained during this study. The estimates of *R, S* and *F'* were obtained from samples collected off South Australia.

The reliability of model fits, 95% confidence intervals (CIs) and coefficients of variation (CVs) for P_0 were estimated using bootstrap resampling methods with 10,000 iterations. Coefficients of variation and CIs for *R, S and F'* were calculated from all adult data. A ratio estimator was used calculate the coefficients of variation (CVs) for *S*, *R*, and *F*' (see Rice 1995, Equation 13). The variance around the *SB* estimate was calculated by the summing the squared CVs for each parameter and multiplying by the square of the estimate of *SB*. Uncertainty estimates for all parameters are 95% CIs. Data analyses were done in the R programming environment (R Core Team, 2019).

2.4 Sensitivity Analysis

Sensitivity analyses were conducted to assess the effects of varying parameter values used to calculate *SB* on the estimate of *SB*. Each parameter in Equation 1 was varied in turn, while keeping all other variables constant. Estimates of P_0 and *A* for the sensitivity analyses were the values estimated during the current survey. Values of adult parameters (*R, S* and *F'*) were those estimated from historical DEPM surveys and literature reviews.

3 Results

3.1 Environmental Variables

Sea surface temperatures (SSTs) ranged from 15.6°C in the south of the survey to 23.1°C in the north (Figure 3–1). The offshore sites were consistently several degrees warmer than the inside sites.

3.2 Blue Mackerel

3.2.1 Egg distribution and abundance

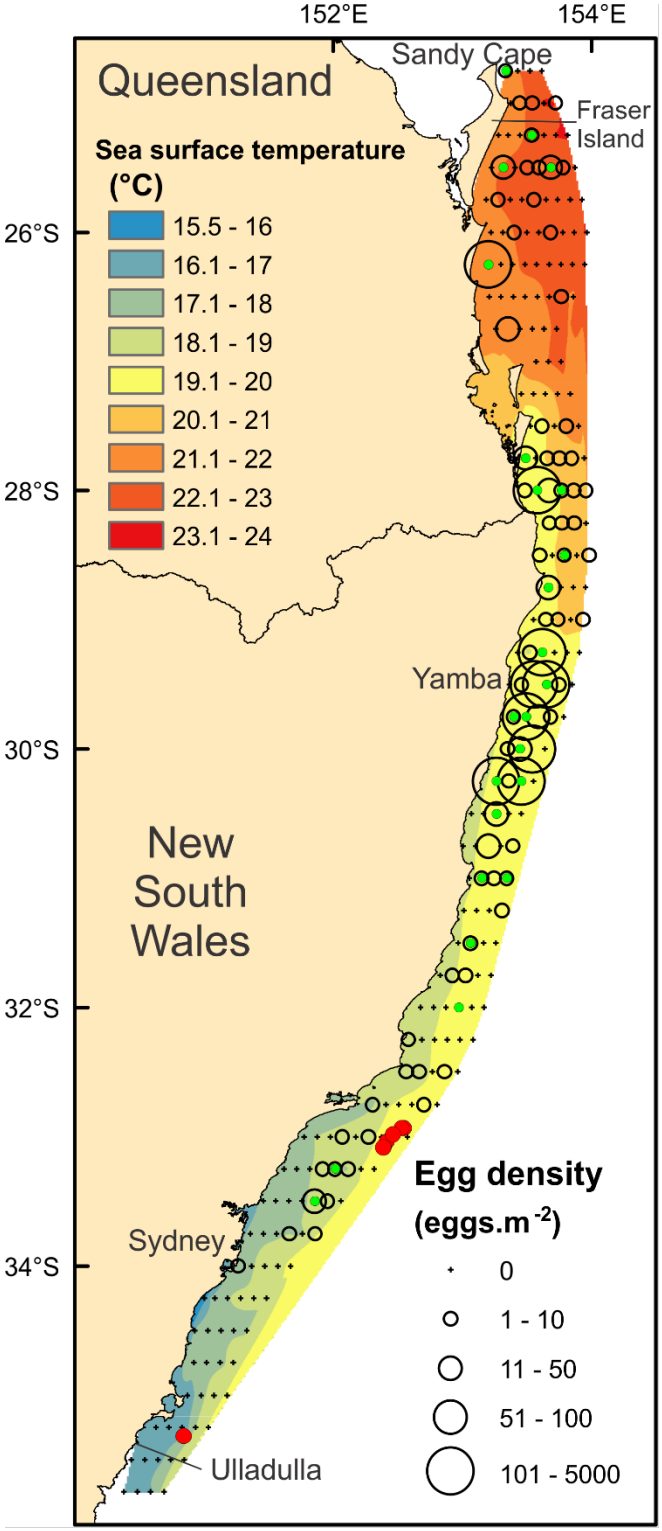
A total of 1,829 live Blue Mackerel eggs were collected at 81 of 251 (32.3%) sites on 45 transects between Sandy Cape, Queensland and Ulladulla, New South Wales (Figure 3–1). Eggs were found in samples collected from Sandy Cape south to 34°S. Blue Mackerel eggs were found in waters ranging from 24–1,756 m deep and SSTs from 17.8 to 23.0°C. High egg densities (>10 eggs.m^{-2}) were mostly found off the mid-north of NSW, in depths of 24–170 m (mean 75 m) and SSTs of 19–20°C.

The morphological identifications of Blue Mackerel eggs were confirmed by molecular identifications (Appendix 1) off eggs preserved in ethanol during the survey (Figure 3–1).

3.2.1.1 Egg density (P_t)

Egg densities were high in mid to outer shelf waters between southern Queensland and the northern NSW (Figure 3–1). High densities of eggs (10 eggs·m^{-2}) were recorded where depths were 24–170 m (mean: 75 m). The sea surface temperatures at locations where eggs were collected ranged from 17.8–23.0°C (mean 20.0°C).

Figure 3–1. Distribution and densities (egg·m-2) of live Blue Mackerel eggs between Sandy Cape, Queensland and southern New South Wales during September 2019. Densities are overlaid on sea surface temperatures (SST; °C) measured during the survey. Green circles indicate locations where Blue Mackerel were confirmed using genetics, red circles are locations of trawls for adults were undertaken.



3.2.1.2 Spawning area (*A*)

The estimated spawning area for Blue Mackerel was 20,387 km^2, which comprised 32.6% of the total area sampled of 62,476 km^2.

3.2.2 Mean daily egg production (P_0)

The estimate of P_0 obtained using the log-linear model (Equation 3) was 16.5 (10.1–27.6) eggs·day^{-1}·m^{-2} and z was 0.305 day^{-1} (Table 3–1; Figures 3–2, 3–3). The estimate of P_0 obtained using the negative binomial GLM NB1 (Equation 5) was 43.6 (21.5–75.5) eggs·day^{-1}·m^{-2} and z was 0.476 day^{-1}. The method of McGarvey and Kinloch (2002), with z set at 0.3 produced an estimate of P_0 of 36.5 (18.6–59.3) eggs·day^{-1}·m^{-2}.

Egg Production Model	P_0 eggs·day^{-1}·m^{-2} (95% CI)	z
Linear version of exponential model, corrected	16.5 (10.1–27.6)	0.305
GLM, Negative Binomial (NB1), log link	43.6 (21.5–75.5)	0.476
McGarvey and Kinloch (2002)	36.5 (18.6–59.3)	0.300

Table 3–1. Point estimates of mean daily egg production (P_0, eggs·day^{-1}·m^{-2}) and instantaneous daily mortality (z, day^{-1}) for Blue Mackerel in September 2019 generated by the two egg production models fits and the set mortality method of McGarvey and Kinloch (2002).

Figure 3–2. (A) Models fitted to egg densities (eggs.m^{-2}) and egg age (hours) of Blue Mackerel cohorts in August / September 2014 and September 2019. GLM with negative binomial error structures; GLM NB1. Dashed horizontal line: mean egg density for survey. (B) Set mortality (z = 0.2, 0.3 and 0.4) using the method of McGarvey and Kinloch (2002).

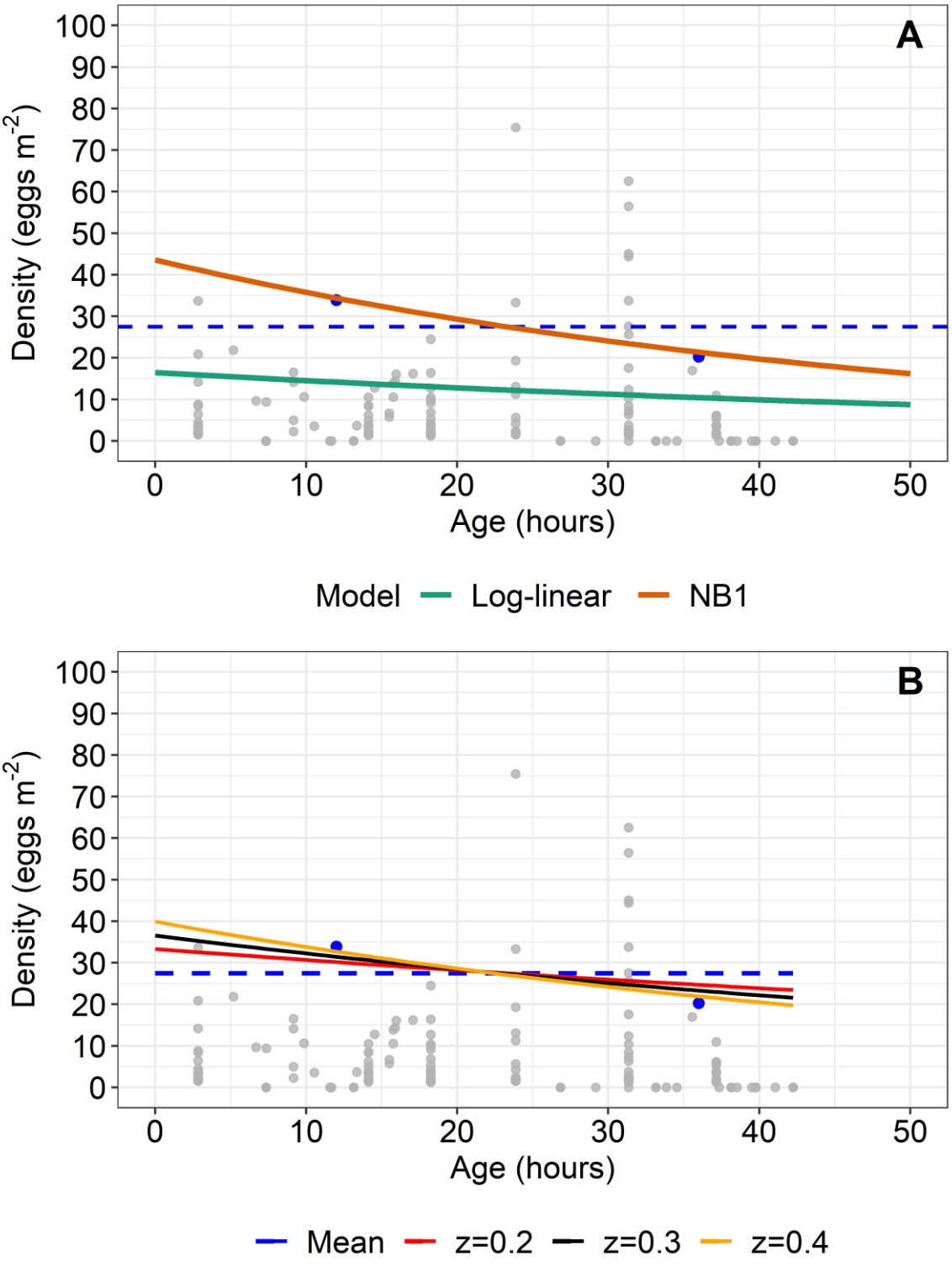
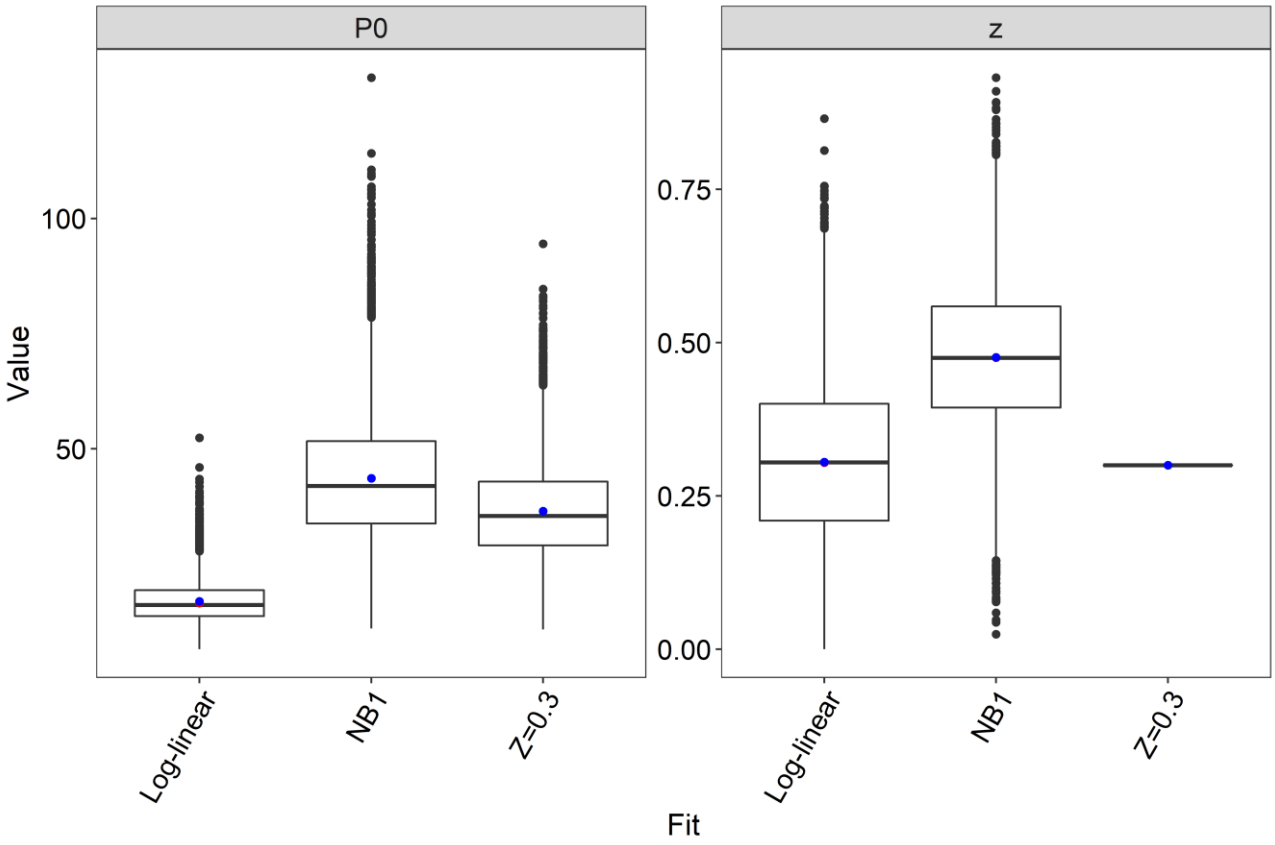


Figure 3–3. Mean daily egg production (P_0, eggs.day^{-1}.m^{-2}) and instantaneous daily mortality (z, day^{-1}) for Blue Mackerel from the two egg production models for data collected in August / September 2014 and September 2019. Horizontal black line is the median and box is the quartiles. Blue dot: bootstrapped mean; solid line: 99% Confidence Interval, black dots: outliers.



3.2.2 Mean Daily Fecundity

A total of 355 mature Blue Mackerel were caught in the six trawls undertaken from the *FV Saints Antonio & Guiseppe*. All trawls contained mature females (Table 3–2). Estimates of adult reproductive parameters used in calculations of *SB* are provided in Tables 3–2, 3–3 and 3–4.

3.2.2.1 Mean female weight (*W*)

The *W* of Blue Mackerel in samples collected in 2019 ranged from 141 to 253 g (Table 3–2). The *W* of mature females in 2019 was 202.8 g (Tables 3–2). The *W* of Blue Mackerel collected off South Australia between 2002 and 2006 was 460.3 g (n = 1,858).

	Male			Female			
Sample	n	Average FL (mm)	Average weight (g)	n	Average FL (mm)	Average weight (g)	R
1	12	300	246	23	289	252	0.66
2	27	291	224	54	297	253	0.69
3	21	293	229	21	260	170	0.43
4	47	256	187	47	265	193	0.51
5	35	248	156	28	248	150	0.43
6	20	241	139	20	242	141	0.50
Total	162*	266#	190#	193*	271#	**202.8**#	0.56#

Table 3–2. Number of males and females of Blue Mackerel in samples and estimates of female weight (*W*). Values in last row are sums (*) and weighted means (#).

Species Comparison: *Scomber japonicus*	S (range)	F' (range)
Dickerson et al. 1992	0.087 (0.010–0.206)	168 (53–315)
Penna et al. 1992 (in Dickerson et al. 1992)		278
Shiraishi et al. 2009	0.169 (0.072–0.250)	
Yamada et al. 1998	0.174 (0.076–0.333)	158 (32–250)
Watanabe & Nishida 2002	0.386 (0.090–0.620)	
MacGreggor (1975)		264 (141–457)

Table 3–3. Spawning fraction (*S*) and relative fecundity (*F'*, eggs.g^{-1}) reported for *Scomber japonicus*.

Parameter	Symbol	Units	Value	95% CI
Egg Production	$P_{0\ (z=0.3)}$	eggs·day^{-1}·m^{-2}	36.5	18.6–59.3
Spawning Area	***A***	km^2	20,387	-
Sex Ratio	***R***	-	0.448	0.400–0.497
Spawning Fraction	***S***	-	0.135	0.102–0.167
Fecundity	***F***	eggs·female^{-1}	64,420	62,291–66,548
Female Weight	***W***	g	460.3	450.2–470.5
Relative Fecundity	***F'***	eggs·g^{-1}	139.9	136.3–143.5
Spawning Biomass		t	88,265	33,320–143,209

Table 3–4. Estimates of egg production and spawning area derived from the September 2019 survey, adult parameters and bootstrapped 95% confidence intervals for Blue Mackerel sampled in South Australia between 2002–2006.

3.2.2.2 Sex ratio (*R*)

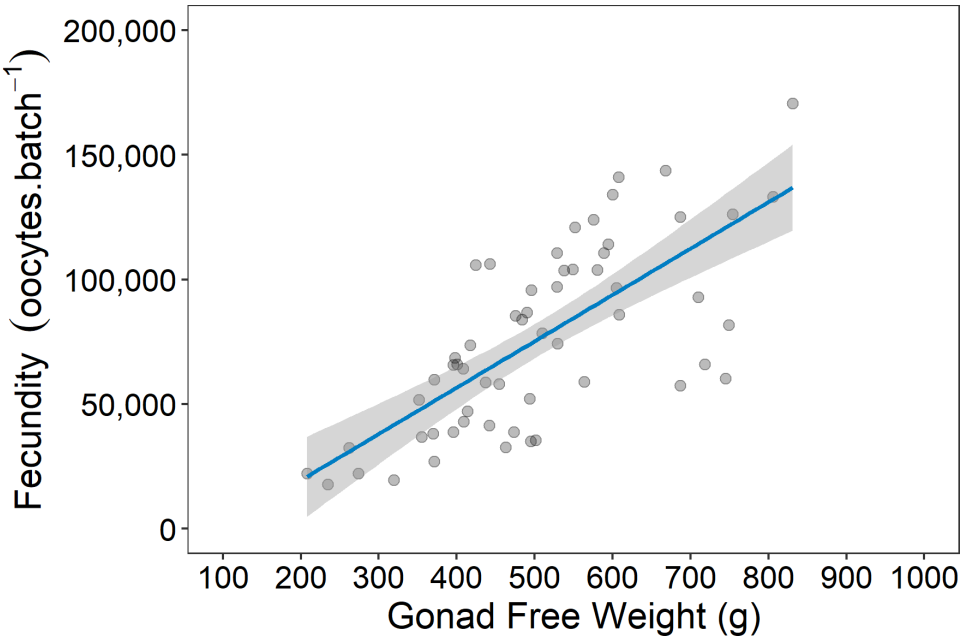
The *R* by weight calculated from all fish collected in 2019 was 0.56 (0.49–0.60) (Table 3–2). The total numbers of females and males collected were 193 (54.4% of fish) and 162 (45.6%), respectively. Estimates of *R* for individual samples ranged from 0.43 to 0.69 (Table 3–2). The estimate of *R* for mature Blue Mackerel collected off South Australia between 2002 and 2006 was 0.45 (Table 3–4).

3.2.2.3 Batch fecundity (*F*)

In 2019, no females with hydrated oocytes were collected. Between 2002–2006 in South Australia, 57 hydrated Blue Mackerel were collected and the batch fecundity relationship (Figure 3–4) was: Batch Fecundity = 186 * Gonad Free Weight – 17,795 (R^2 = 0.51).

The estimate of relative fecundity (*F'*; eggs per gram of female weight) for female Blue Mackerel collected in South Australia between 2002–2006 was 139.9 (136.3–143.5) eggs.g^{-1} (Table 3–4; Figure 3–4, 3–5A). *F'* was almost constant across the range of *W* of mature females (Figure 3–5B).

Figure 3–4. Relationship between gonad-free weight and batch fecundity (*F*) for all hydrated Blue Mackerel collected between 2002–2006 (shading = 95% CI). F = 186 * Gonad Free Weight – 17,795, (R^2 = 0.51).



3.2.2.4 Spawning fraction (*S*)

No POFs were detected in the 193 mature females collected off NSW in 2019. Mean *S* for samples of Blue Mackerel collected off South Australia in 2002–2006 was 0.135 from 803 females examined (Table 3–4). Estimates of *S* reported for *S. japonicus* ranged from 0.087 to 0.386 (Table 3–3).

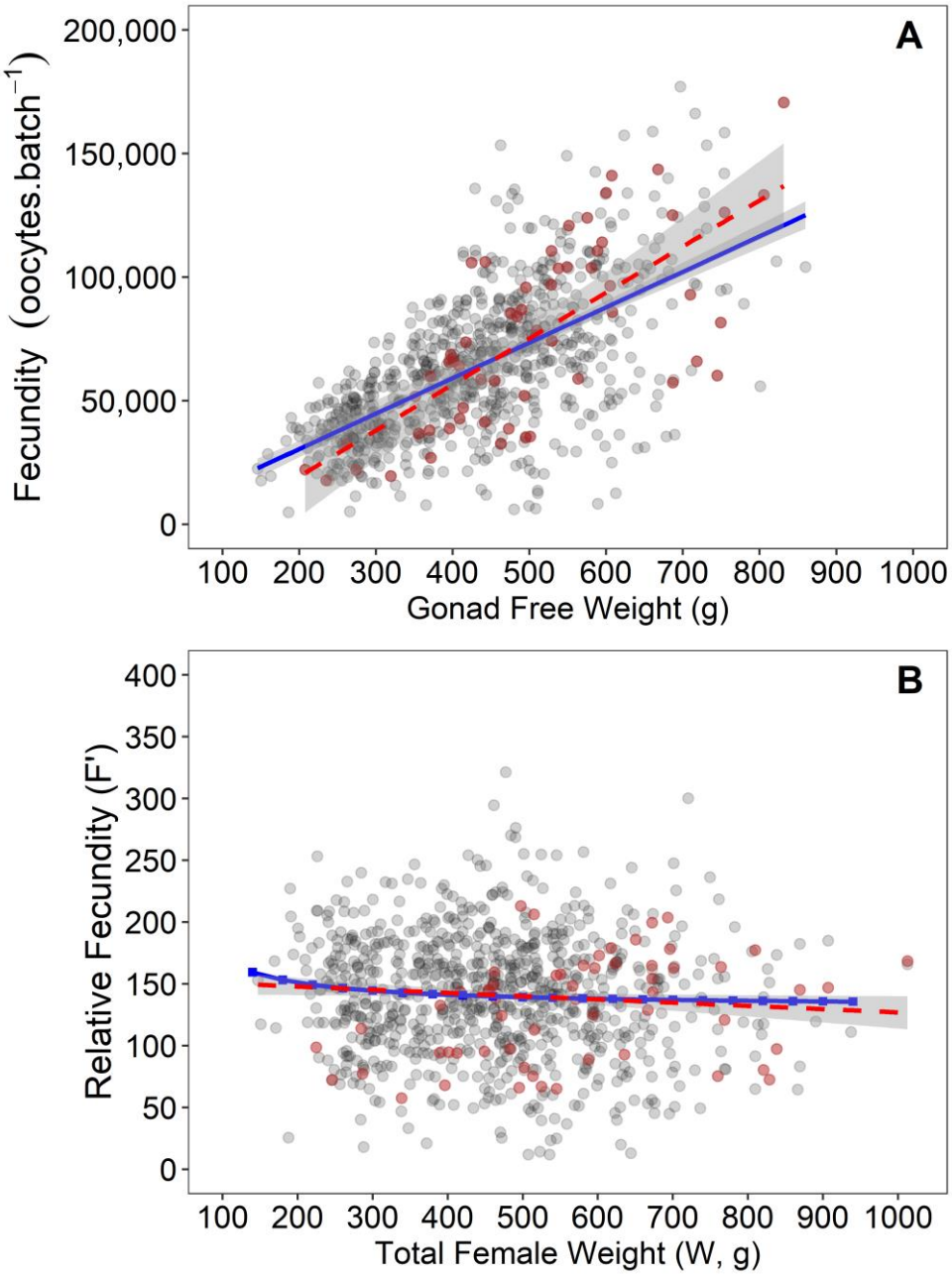
3.2.2.5 Spawning biomass (*SB*)

The estimate of *SB* for Blue Mackerel was 88,265 t (33,320–143,209). This estimate was calculated using the method of McGarvey and Kinloch 2002 (z = 0.3) to estimate P_0 and values of adult parameters obtained from samples collected off South Australia from 2002–2006 (Table 3–4).

3.2.3 Sensitivity analysis

The sensitivity analysis showed the effects of variability in *A*, P_0, *R, S,* and *F'* on the estimate of *SB* for Blue Mackerel in 2019 (Table 3–4; Figures 3–6).

Figure 3–5. A) Batch fecundity and gonad-free weight for Blue Mackerel, red circles batch counts, grey circles estimated values relationship from females with hydrated oocytes (dashed red). The regression slope for females without hydrated oocytes is shown for comparison (blue). B) Relationship (blue) between relative fecundity (*F'*, egg.g^{-1}) and female weight (*W, g*). This relationship is obtained from dividing fecundity estimates plotted in A by female weight (*W*) and then regressing against *W* to show the relationship (blue) between *F'* and *W*. Red: measured *F'* values; grey: *F'* values resulting from the estimates of $\hat{F}$ produced in plot A divided by *W*. Shading around regression slopes are 95% CIs.



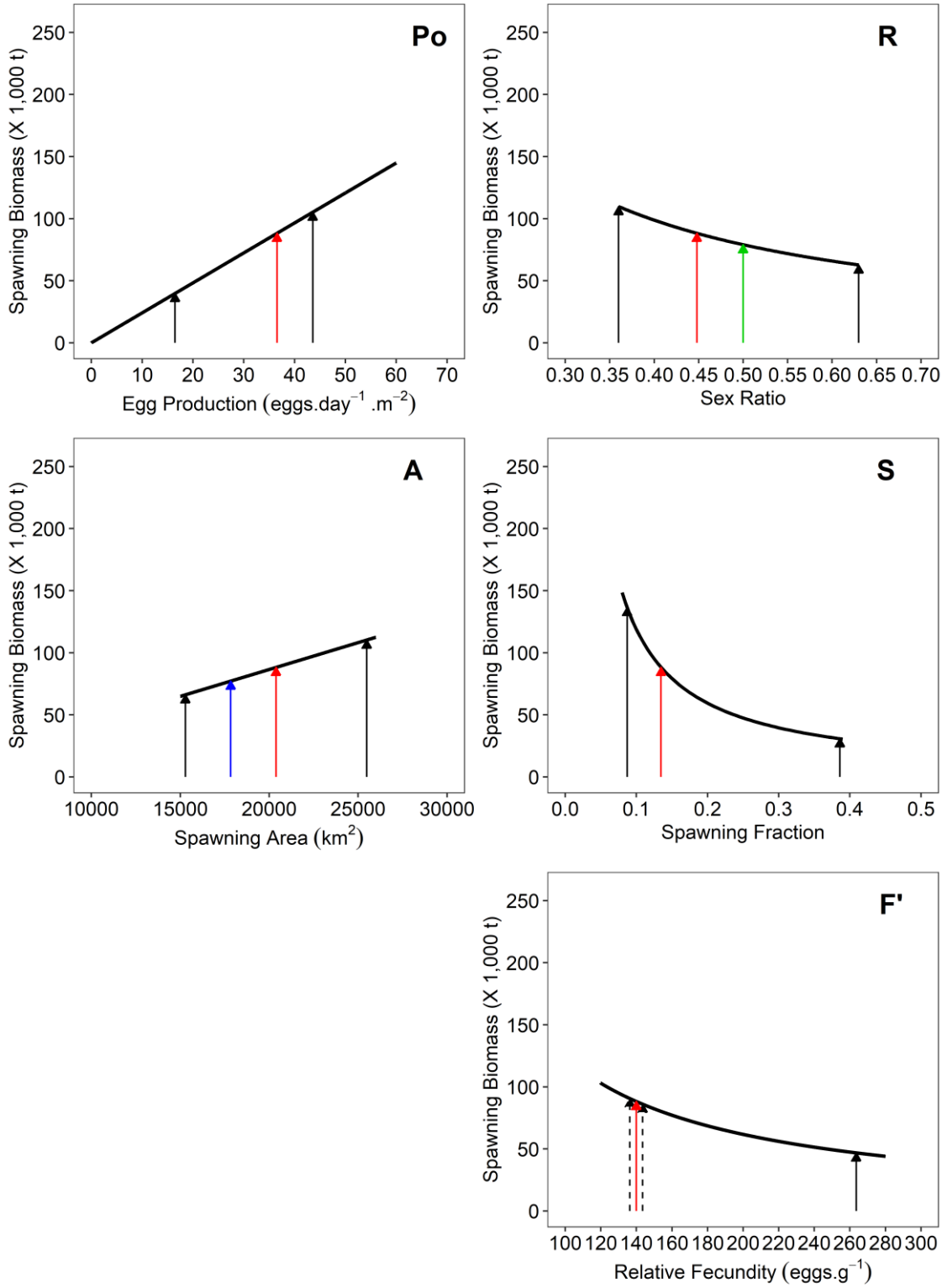
It demonstrates the strong influence that the model used to estimate P_0 had on estimates of *SB* of Blue Mackerel (Figure 3–7). *SB* increased linearly with *A* (Figure 3–6). The 2019 survey had a larger *A* than the 2014 survey, despite the similar design and coverage of the surveys, so the estimate of *SB* in 2019 was higher than in 2014.

Estimates of *SB* increased as estimates *R* decreased (Figure 3–7). The fluctuations in *R* between surveys may reflect the limitations of the adult sampling programs (e.g. Ward et al. 2021).

Estimates of *SB* increased as estimates *S* decreased The estimate of *S* for Blue Mackerel collected in South Australia between 2002–2006 (0.135) was based on a relatively large (N = 54) number of samples. However, there was a wide range of estimates of *S* (0.087–0.386) reported for *Scomber japonicus* in the literature which produced a wide range of *SB*. It is unclear whether the differences between studies reflect real differences between populations or sampling error.

F' was similar across the size range of females sampled in the 2002–2006 period. Variations in this parameter had a relatively small effect on *SB*. The estimate of *F'* for *Scomber japonicus* (264.0 eggs.g^{-1}, MacGreggor 1975) is higher than the estimate reported here (139.9 eggs.g^{-1}, Figure 3–6).

Figure 3–6. Sensitivity plots showing effects of variability in adult parameters and egg production on estimates of spawning biomass. Solid red arrows: parameter used for 2019 estimate; solid black arrows: minimum and maximum parameter estimates from reviews of literature (P_0, log-linear model and NB1, *A* ± 25%); Blue arrow 2014 estimate; Dashed arrows: 95% confidence intervals; green arrow: *R* = 0.5.



3.3 Sardine

3.3.1 Egg distribution and abundance

A total of 4,667 live Sardine eggs were collected at 58 of 251 (23.1%) sites between Sandy Cape, Queensland and Ulladulla, New South Wales. Eggs were found at sites from Sandy Cape to the southern end of the survey (Figure 3–7). Spawning occurred in at least three distinct regions; in relatively warm water (SST ~22°C) off the southern end of Fraser Island, in water with SSTs around 19°C between southern Queensland and northern NSW and cooler water (SST ~16°C) south of 33°S.

3.3.1.1 Egg density (P_t)

Egg densities were high at sites on the mid to outer shelf in the northern part of the survey, at inshore to the mid shelf sites in the central part of the survey and at mainly inshore sites in the southern part of the survey (Figure 3–7). The SSTs at sites where eggs were collected ranged from 15.6–22.3°C (mean 18.7°C).

3.3.1.2 Spawning area (A)

The estimated spawning area for Sardine was 14,281 km^2, comprising 22.9% of the total area sampled (62,476 km^2).

3.3.1.3 Mean daily egg production (P_0)

The estimate of P_0 obtained using the log-linear model (Equation 3) using egg density data from both 2014 and 2019 was 53.9 (29.4–95.9) $eggs.day^{-1}.m^{-2}$and z was 0.403 day^{-1} (Table 3–5; Figures 3–8, 3–9). The estimate of P_0 obtained using the negative binomial GLM NB1 (Equation 5) was 67.9 (37.8–111.1) $eggs.day^{-1}.m^{-2}$ and z was 0.235 day^{-1}.

Figure 3–7. Distribution and densities (egg·m^{-2}) of live Sardine eggs between Sandy Cape, Queensland and southern New South Wales during September 2019. Densities are overlaid on sea surface temperatures (SST; °C) measured during the survey.

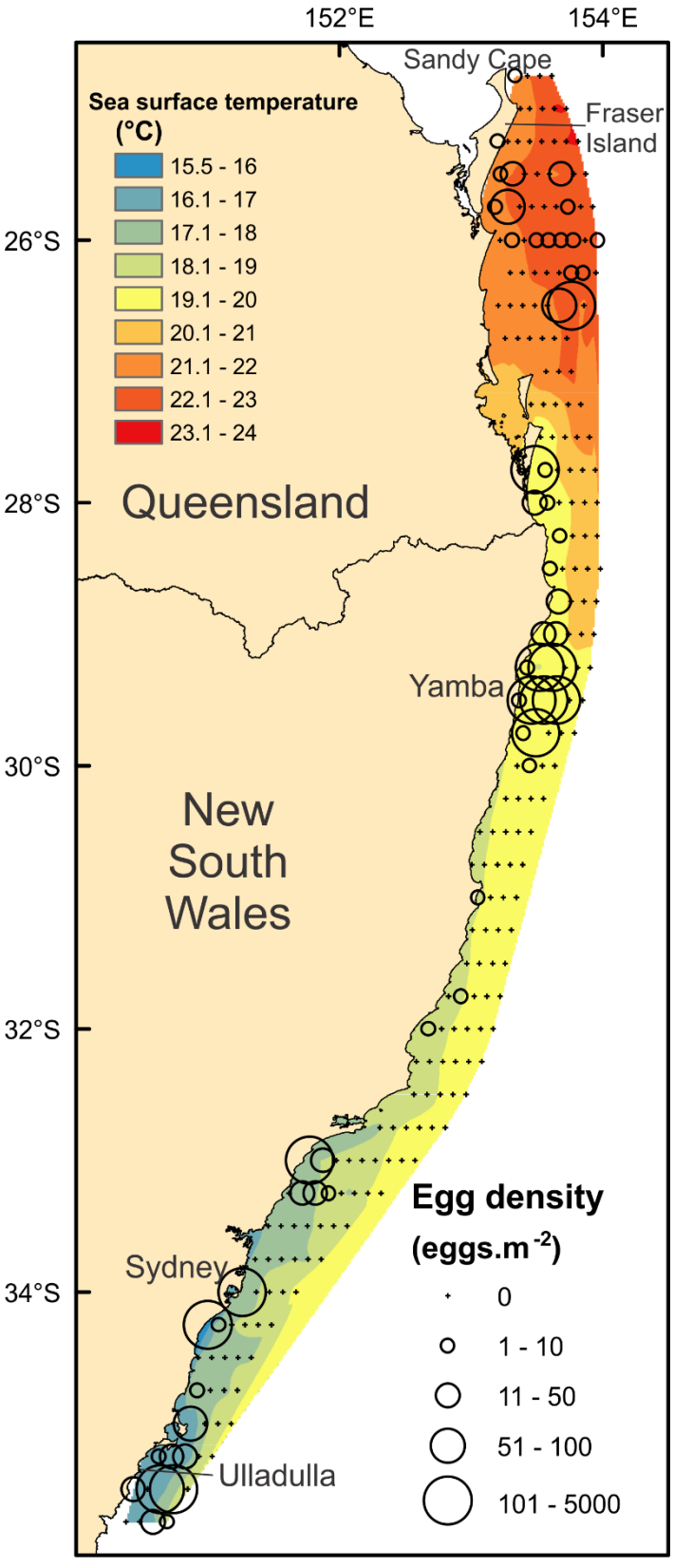


Figure 3–8. Models fitted to egg densities (eggs.m^{-2}) and egg age (hours) of Sardine cohorts in eastern Australia during August-September 2014 and September 2019. NB1: GLM with negative binomial error structures. Grey horizontal line: mean egg density for survey.

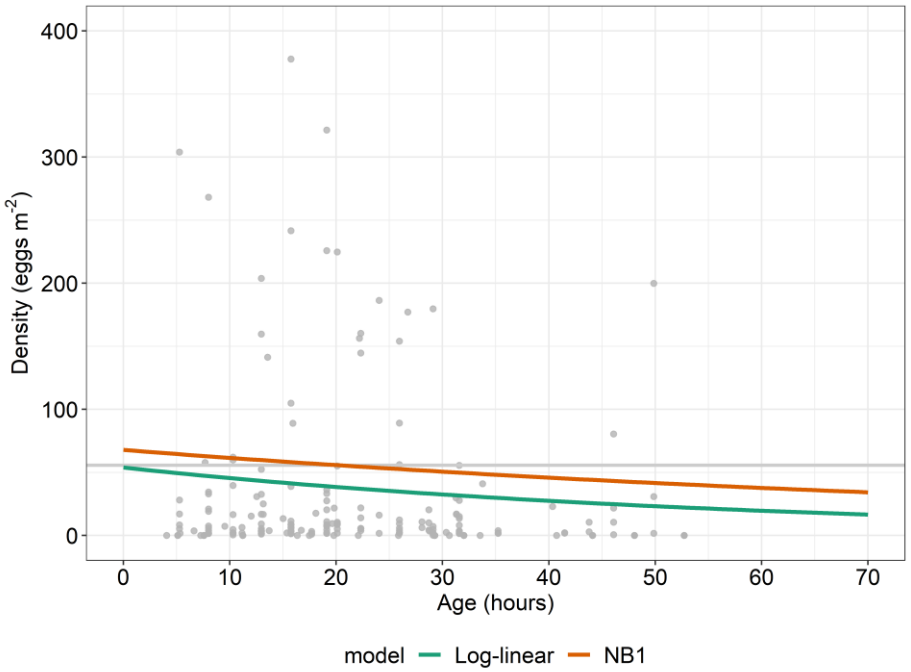
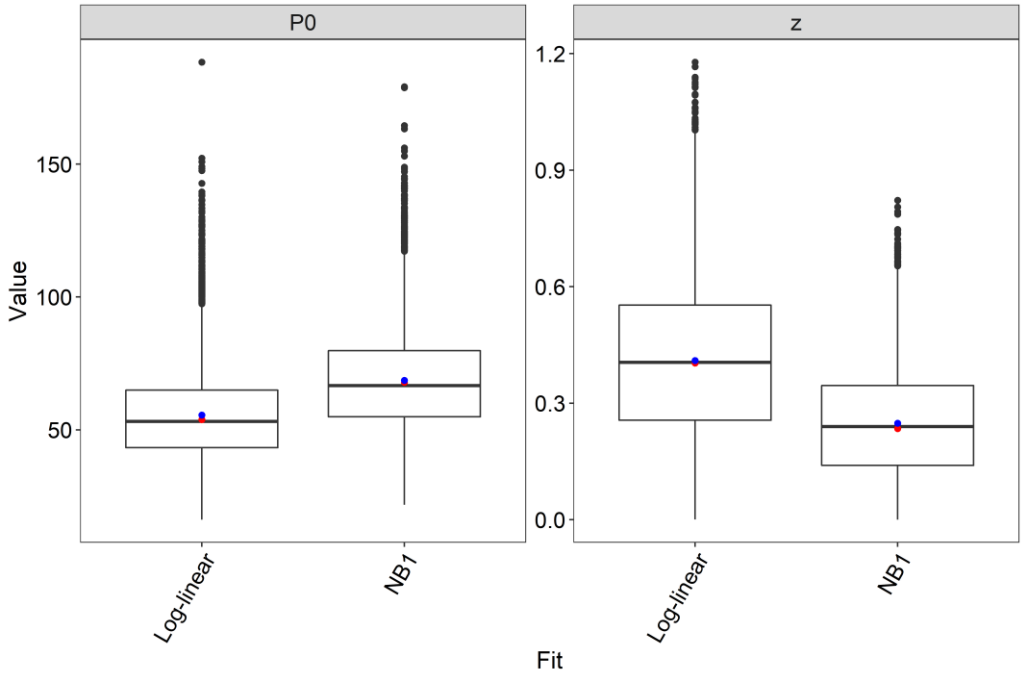


Figure 3–9. Mean daily egg production (P_0, egg·day^{-1}·m^{-2}) and instantaneous daily mortality (z, day^{-1}) for Blue Mackerel from the two egg production models for data collected in eastern Australia during August-September 2014 and September 2019. Horizontal black line is the median and box is the quartiles. Blue dot: bootstrapped mean; solid line: 99% Confidence Interval, black dots: outliers.



Egg Production Model	P_0 eggs·day^{-1}·m^{-2} (95% CI)	z
Linear version of exponential model, corrected	53.9 (29.4–95.9)	0.403
GLM, Negative Binomial (NB1), log link	67.9 (37.8–111.1)	0.235

Table 3–5. Point estimates of mean daily egg production (P_0, eggs·day^{-1}·m^{-2}) and instantaneous daily mortality (z, day^{-1}) for Sardine in September 2019 generated by the two egg production models fits.

3.3.2 Mean Daily Fecundity

No Sardine were caught during this survey. Estimates of the adult reproductive parameters used in calculations of spawning biomass were obtained from samples collected off South Australia (Table 3–6).

Parameter	All Years	95% CI	CV	Range (among years)
Sex Ratio (R)	0.55	0.52–0.58	0.03	0.36–0.70
Spawning Fraction (S)	0.108	0.100–0.123	0.05	0.041–0.179
Female Weight (W, g)	58.4	23.1–93.7	0.31	46.5–78.7
Fecundity (F, eggs.female^{-1})	17,816	3,819–31,813	0.40	14,107–23,601
Relative Fecundity (F' eggs.g^{-1})	305.0	303.8–306.3	<0.01	292.5–312.9

Table 3–6. Estimates of adult parameters, bootstrapped 95% confidence intervals and the range of annual estimates for Sardine sampled off South Australia between 1998–2018.

3.3.2.1 Mean female weight (*W*)

The *W* of Sardine collected off South Australia between 1998–2018 was 58.4 g (23.1–93.7, Table 3–6). Annual estimates of *W* ranged from 46.5 to 78.7 g.

3.3.2.2 Sex ratio (*R*)

The *R* of Sardine collected off South Australia between 1998–2018 was 0.55 (0.52–0.58, Table 3–6). Annual estimates of *R* ranged from 0.36–0.70.

3.3.2.3 Batch fecundity (*F*)

The relationship calculated from all females with hydrated oocytes collected between 1998 and 2018 was: *F* = 335 x Gonad Free Female Weight – 797 (R^2 = 0.53, Figure 3–10A). *F'* was 305.1 (303.8–306.3) eggs.g^{-1} (Table 3–6; Figure 3–10B) and was almost constant across the range of *W* (Figure 3–10).

3.3.2.4 Spawning fraction (*S*)

The estimate of *S* of Sardine collected off South Australia between 1998–2018 was 0.108 (95% CI: 0.100–0.123, Table 3–6). Annual estimates of *S* ranged from 0.041 to 0.179.

3.3.2.5 Spawning biomass

The estimate of *SB* for Sardine in September 2019 was 42,724 t (95% CI = 15,487–69,962). This value was calculated using the log-linear model to estimate P_0 from data collected in 2014 and 2019 combined and values of adult parameters estimated from samples collected off South Australia from 1998–2018 (Table 3–6).

3.3.3 Sensitivity analysis

The sensitivity analysis shows the effects of variability in parameters (i.e. P_0, *A*, *R, S* and *F'*) on the estimate of *SB* for 2019 (Table 3–6; Figures 3–11).

The relationship between P_0 and *SB* was linear, and the sensitivity analysis showed the strong influence that the model used to estimate P_0 has on estimates of *SB* (Figure 3–11).

SB increased linearly with *A* (Figure 3–11). The 2019 survey had a lower *A* than the 2014 survey which was of a very similar design, so the estimate of *SB* was lower.

Estimates of *SB* increased as *R* decreased (Figure 3–11). The fluctuations in *R* between years may reflect limitations of the adult sampling program (e.g. Ward et al. 2020).

Estimates of *SB* also increased as *S* decreased (Figure 3–11). The estimate of S used for the 2019 estimate (South Australian samples between 1998–2018) was lower but based on a far larger data set than that used in the 2014 east coast estimate (five samples).

Relative fecundity (i.e. eggs per gram of female weight, F') was almost constant across the range of *W* obtained in samples (Figure 3–11).

Figure 3–10. A) Measured fecundities (oocytes.batch^{-1}; red) from female Sardine with hydrated oocytes collected between 1998 and 2018 (n = 1099) were used to estimate the fecundity of females without hydrated oocytes (black; n = 15,662). Batch fecundity relationship from females with hydrated oocytes (dashed red): *F* = 335*Gonad Free Weight - 797, (R^2 = 0.53). The regression slope for females without hydrated oocytes is shown for comparison (blue). B) Relationship (blue) between relative fecundity (*F'*, egg.g ^{1}) and female weight (*W*, g) of sardine collected between 1998 and 2018. This relationship results from dividing fecundity estimates plotted in A by female weight (*W*) and then regressing against *W* to show the relationship (blue) between *F'* and *W*. Red: measured *F'* values; Black: *F'* values resulting from the estimates of $\hat{F}$ produced in plot A divided by *W*. Shading around regression slopes are 95% CIs (narrow and difficult to see).

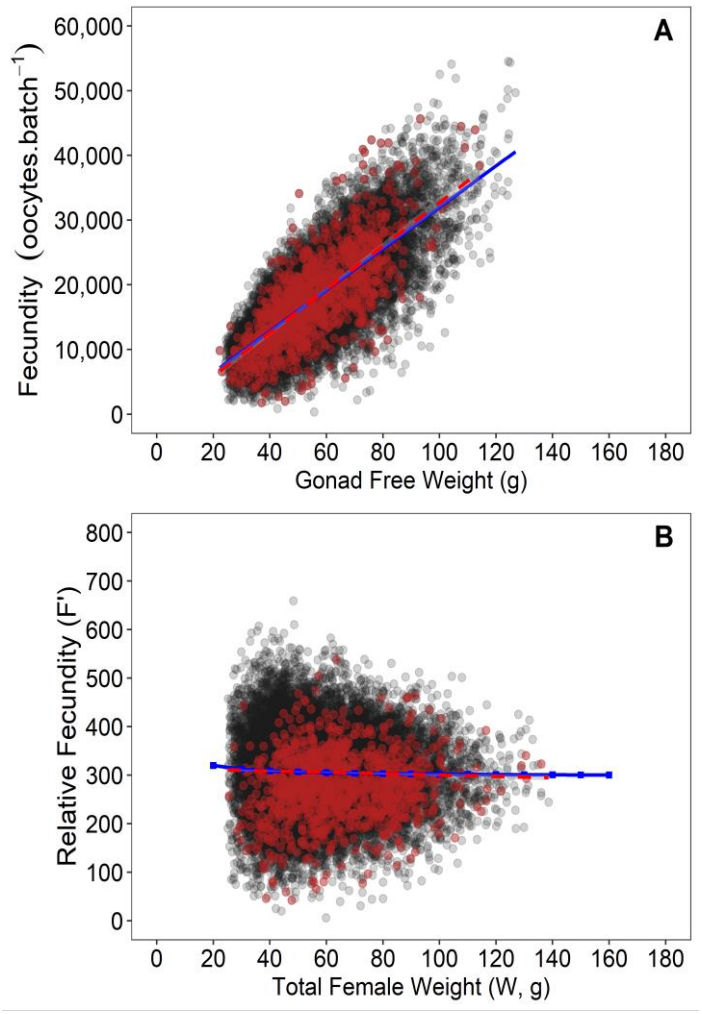
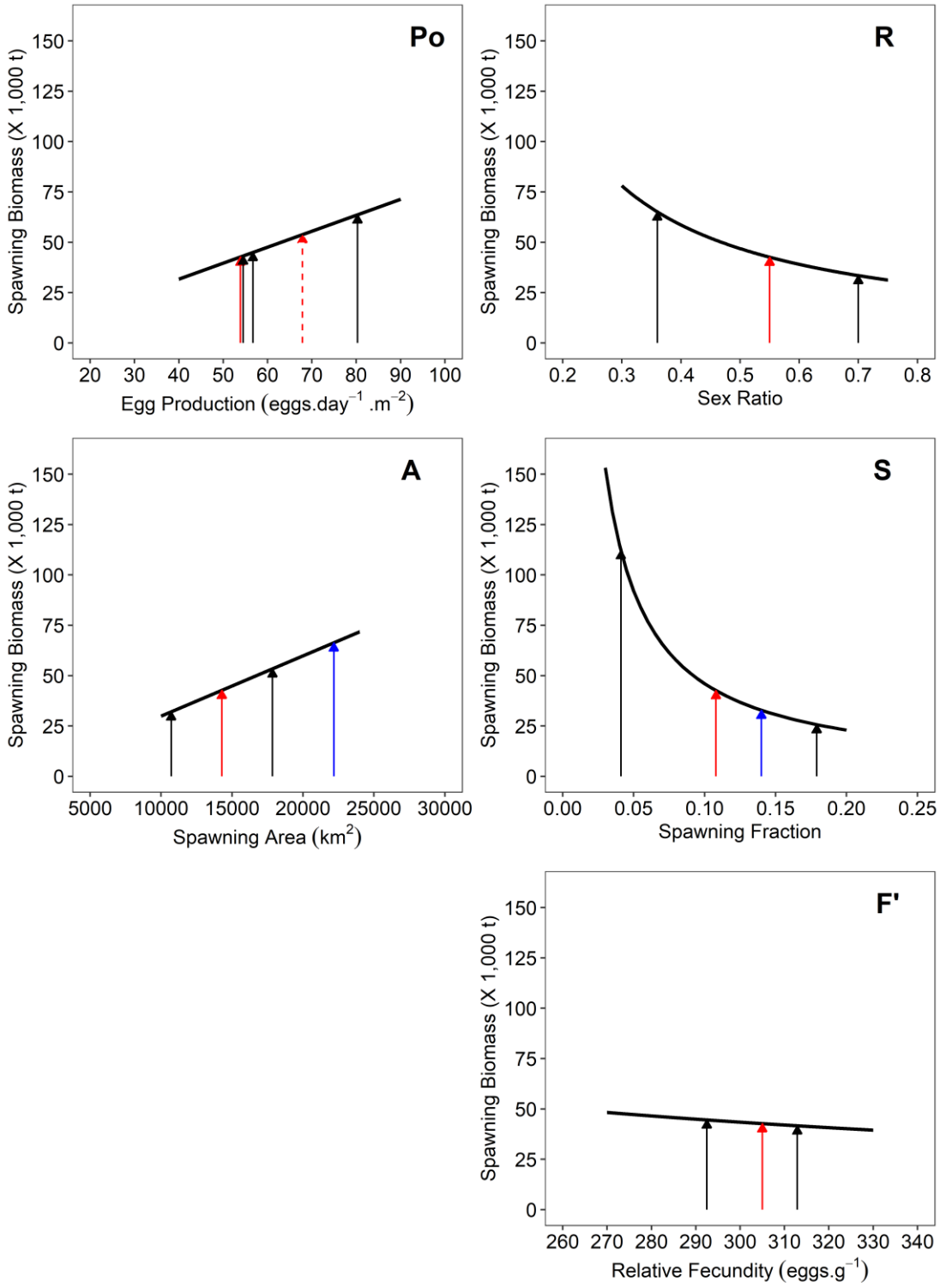


Figure 3–11. Sensitivity plots showing effects of variability in adult parameters and egg production on estimates of spawning biomass of Sardine. Solid red arrows: parameter used for 2019 estimate; solid black arrows: minimum and maximum annual parameter estimates for Sardine from South Australian DEPM surveys between 1998 and 2018 (*A*: ± 25%, P_0: 2014 and 2019 estimates). Dashed arrows: GLM P_0 estimate; blue arrow: 2014 estimate.



4 Discussion

4.1 Blue Mackerel

4.1.1 Egg distribution and spawning area

The distribution of Blue Mackerel eggs observed in this study was broadly similar to the results of previous studies, with most eggs collected north and central parts of the survey (Ward and Rogers 2009, Ward et al. 2015). The estimate of A in 2019 of 20,387 km^2 was 14% larger than the estimate of 17,911 km^2 reported in 2014. Both estimates were obtained using the same sampling methods and survey design. In both cases, the absence of eggs at sites on the seaward end of most transects, limited evidence of spawning near the southern end of the surveys and relatively low egg densities in the far north, suggested that the surveys covered most of the spawning habitat. For these reasons, it is likely that the estimates of A for 2014 and 2019 are robust. As A is strongly correlated with adult abundance in many species of small pelagic fishes (e.g. Barange et al. 2009; Ward et al. 2021), the increase in A between 2014 and 2019 suggests that the increase in fishing effort that has occurred East sub-area since the last survey (e.g. Ward and Grammer 2021) has not caused a significant decline in stock abundance.

4.1.2 Egg abundance and mean daily egg production

Estimating P_0 and z of Blue Mackerel in the East sub-area remains challenging (see Ward et al. 2015) for several reasons: 1) young eggs are underrepresented in samples; 2) relatively small numbers of egg samples have been collected and 3) different models produce diverging estimates of the two parameters.

The first issue was largely addressed in this study by removing Stage 1 eggs from the analyses, as has been done in many other DEPM studies worldwide (e.g. Stratoudakis et al. 2006; Ward et al. 2021). The second issue was addressed by combining the datasets collected in 2014 and 2019. This was done because Ward et al. (2021) showed that inter-annual variability in P_0 can be lower than the uncertainty of annual estimates for some species (e.g. Sardine) and that, if multiple years of data are available, precision can be sometimes be increased by combining data across years. In the case of Blue Mackerel off eastern Australia, the fits of the two models were similar in 2014 and 2019, with point estimates marginally higher in 2019. The similarity of the data for 2014 and 2019 justified combining the two data sets.

Choosing which model to use to estimate P_0 and z was more challenging because none of the models fitted the data well. The log-linear model fitted the data poorly and produced

estimates of P_0 that did not approximate the mean daily egg densities. The GLM NB1 fitted the data better than the log-linear model and produced estimates of P_0 that were more similar to the mean daily egg densities. In the end, we decided to use the method of McGarvey and Kinloch (2002) with a fixed z of 0.3, because it limited the potential for over- or under-estimating P_0 and z.

4.1.3 Adult parameters

Adult Blue Mackerel collected as part of this study were relatively small (around the size of maturity). Although these fish macroscopically mature (ovaries with yolked oocytes), they showed no histological signs of recent spawning (POFs). The estimates of adult parameters obtained from samples collected off South Australia are robust. However, the potential for temporal and spatial variation in adult parameters cannot be discounted. As R is likely to be stable and close to 0.5 and F' is relatively constant across a range of W, the main concern about the lack of concurrent adult samples relates to the estimation of S. The sensitivity analyses show that this parameter has a strong influence on estimates of SB**.** The importance of obtaining representative samples of adult Blue Mackerel from the east coast of Australia has been identified as a high priority in previous studies (e.g. Ward et al. 2015). Although the DEPM is relatively robust to inadequate adult sampling (e.g. Ward et al. 2021) obtaining reliable estimates of S in the East sub-area of the SPF remains a high research priority for the fishery.

4.1.4 Spawning biomass

The estimate of SB of Blue Mackerel for 2019 of 88,265 (33,320–143,209) t is similar to the estimate obtained of 83,300 in 2014. The estimate of SB for 2019 is suitable for setting RBCs for Blue Mackerel in the east sub-area. The sustainability risks associated with uncertainties in estimates of SB resulting from lack of concurrent samples to estimate S are offset by the conservative exploitation rate established for Blue Mackerel in the SPF Harvest Strategy (i.e. 15%), which is well below the 23% that Smith et al (2015) suggested was sustainable for this species.

4.2. Sardine

4.2.1 Egg distribution and spawning area

The presence of high egg densities across the entire survey area from Sandy Cape, Queensland to Ulladulla, NSW supports the hypothesis that the peak spawning season of Sardine in this region is late winter and early spring. The distribution of Sardine eggs in 2019 was similar to 2014 with the notable exception of an area without significant

spawning between 30–33°S and increased spawning in deeper water off southern Queensland. The 36% reduction in spawning area from 22,400 km^2 in 2014 to 14,281 km^2 in 2019 reflects the reduced number of sites at which Sardine eggs were collected in 2019. As *A* is strongly correlated with adult abundance in many species of small pelagic fishes including Sardine (e.g. Barange et al. 2009; Ward et al. 2021), this decrease in *A* between 2014 and 2019 suggests that the population may have declined over this period. As total annual catches of Sardine from this region have been less than 800 t (i.e. <2% of the *SB* in 2014), it seems unlikely that this apparent decline in abundance has been driven primarily by fishing pressure (e.g. Ward and Grammer 2021a). This apparent change in abundance may be due to natural fluctuations in recruitment and mortality which are known to occur in this species (e.g. Barange et al. 2009; Ward et al. 2021).

4.2.2 Egg abundance and mean daily egg production

Both the egg production models fitted the Sardine data better than the Blue Mackerel data. Ward et al. (2021) showed that the log-linear model produces more precise estimates of P_0 for Sardine than the GLM NB1, but that estimates from the log-linear model may be negatively biased whereas GLM NB1 may be unbiased. The decision about which model should be used to estimate P_0 is a trade-off between precision and accuracy. The log-linear model has been adopted as the model of choice in the South Australian Sardine Fishery and has been used previously in the SPF (e.g. by Ward et al. 2015, before the GLM NB1 was fully developed). The estimate of P_0 from the log-linear model for both 2014 and 2019, and for both datasets combined, were very similar (~54 eggs.m^{-2}).

4.2.3 Adult parameters

The limited number of adult samples of Sardine that have been collected from the East sub-area (e.g. Ward et al. 2015) do not suggest that adult reproductive parameters (especially *R* and *F'*) are likely to be different to those reported off South Australia. However, given the strong influence that S has on estimates of spawning biomass more rigorous estimation of this parameter in the East sub-area is warranted.

4.2.4 Spawning biomass

The 14% decline in the estimate of *SB* of Sardine from 49,575 (24,200–213,300) in 2014 to 42,724 t (95% CI = 15,487–69,962) in 2019, combined with the 36% decline in *A* over the same period, suggest that stock abundance has declined over this period. As noted above, because of the low level of recent catches in this sub-area, it seems likely that this decline reflects a natural fluctuation in abundance rather than a fishing induced impact.

4.3. Conclusions, implications and recommendations

The *SB* of Blue Mackerel in the East sub-area appears to have remained stable or increased between 2014 and 2019, despite recent increases in annual catches. In contrast, the *SB* of Sardine appears to have declined even though annual catches over the last decade have been relatively low. Intensive adult sampling programs to obtain robust estimates of *S* off eastern Australia are needed for both species. However, due to the recent increases in annual catches this need is most pressing for Blue Mackerel.

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Appendices

Appendix 1: Genetic identification of Blue Mackerel eggs

Molecular Identification

A molecular approach of Mitochondrial DNA (mtDNA) extraction, amplification, and sequencing for *Scomber* spp. developed by Perry (2011) and refined by Neira et al. (2014) was employed to identify eggs of Blue Mackerel. DNA extractions from eggs identified based on morphological characters were carried out using the QIAamp DNA Micro Kit (QIAGEN, USA) following the manufacturer's protocol for tissue extraction. Amplification by polymerase chain reactions (PCRs) were performed using MyTaq HSTM DNA Polymerase (Bioline) with PCR product purification and bi-directional sequencing performed by Macrogen Inc. (Seoul, Republic of Korea) (see Neira et al. 2014 for full methods). An additional run using general fish primers, FishF2 (5′TCGACTAATCATAAAGATATCGGCAC3′) and FishR2 (5′ACTTCAGGGTGACCGAAGAATCAGAA3′), were used in the PCRs to amplify a fragment (~ 655 bp) from the 5′ region of the *cox1* gene to identify species of fish with similar characteristics to Jack Mackerel. Sequences were aligned to reference data in the Fish Barcode of Life Database (BOLD) using BioEdit biological sequence alignment editor.

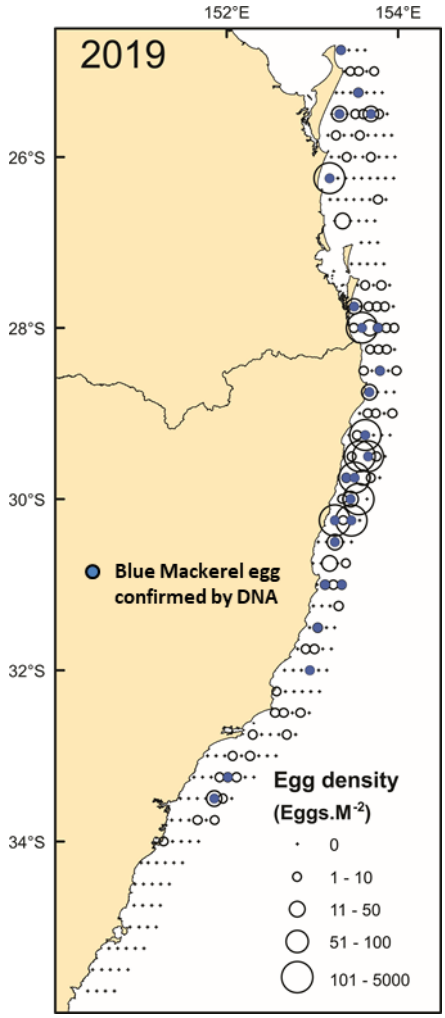
A total of 79 eggs were selected for mtDNA analysis; 25 identified morphologically as Blue Mackerel, 33 indeterminable, and 21 similar but morphologically different to Blue Mackerel (Tables A1-1). Indeterminable eggs consisted of early stage eggs whose morphological characteristics were masked by ethanol preservation, making morphological identification problematic.

Molecular analyses successfully validated eggs identified as Blue Mackerel using species-specific morphological characters. The analysis further confirmed the presence of Blue Mackerel eggs where morphological identification was problematic in ethanol preserved samples, especially with early stage eggs. The molecular analyses confirmed the presence of Blue Mackerel eggs across the survey area from Sandy Cape, Queensland to central New South Wales (Figure A1-1). Results of molecular identifications were used to aid identifications of formalin preserved eggs.

Morphological identification	n tested	Genetic Identification			Notes
		Blue Mackerel	Other	No DNA	
Blue Mackerel	25	23	1	1	Misidentified egg aligned with: *Lepidotrigla argus* (n = 1)
Possible Blue Mackerel: early stage eggs with limited characteristics for ID	33	16	16	1	Other eggs aligned with: *Lepidotrigla mulhalli* (n = 2) *Lepidotrigla argus* (n = 8) *Lepidotrigla alata (n = 1)* *Etrumeus jacksoniensis* (n = 2) *Seriola lalandi* (n = 1) *Rexea solandri* (n = 2)
Not Jack Mackerel, but possessing some similar characteristics	21	4	17		Other eggs aligned with: *Lepidotrigla mulhalli* (n = 3) *Lepidotrigla argus* (n = 7) *Lepidotrigla sp. (n = 1)* *Trachurus japonicus* (n = 2) *Chelidonichthys spinosus* (n = 1) *Rexea solandri* (n = 2) *Centrolophus niger* (n = 1)

Table A1-1. Molecular identifications of morphologically identified Blue Mackerel and similar eggs collected between Sandy Cape, Queensland and southern New South Wales during September 2019.

Figure A1–1. Distribution and densities (egg·m^{-2}) of live Blue Mackerel eggs between Sandy Cape, Queensland and southern New South Wales during September 2019. Blue circles: Blue Mackerel eggs confirmed by genetic analysis.



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