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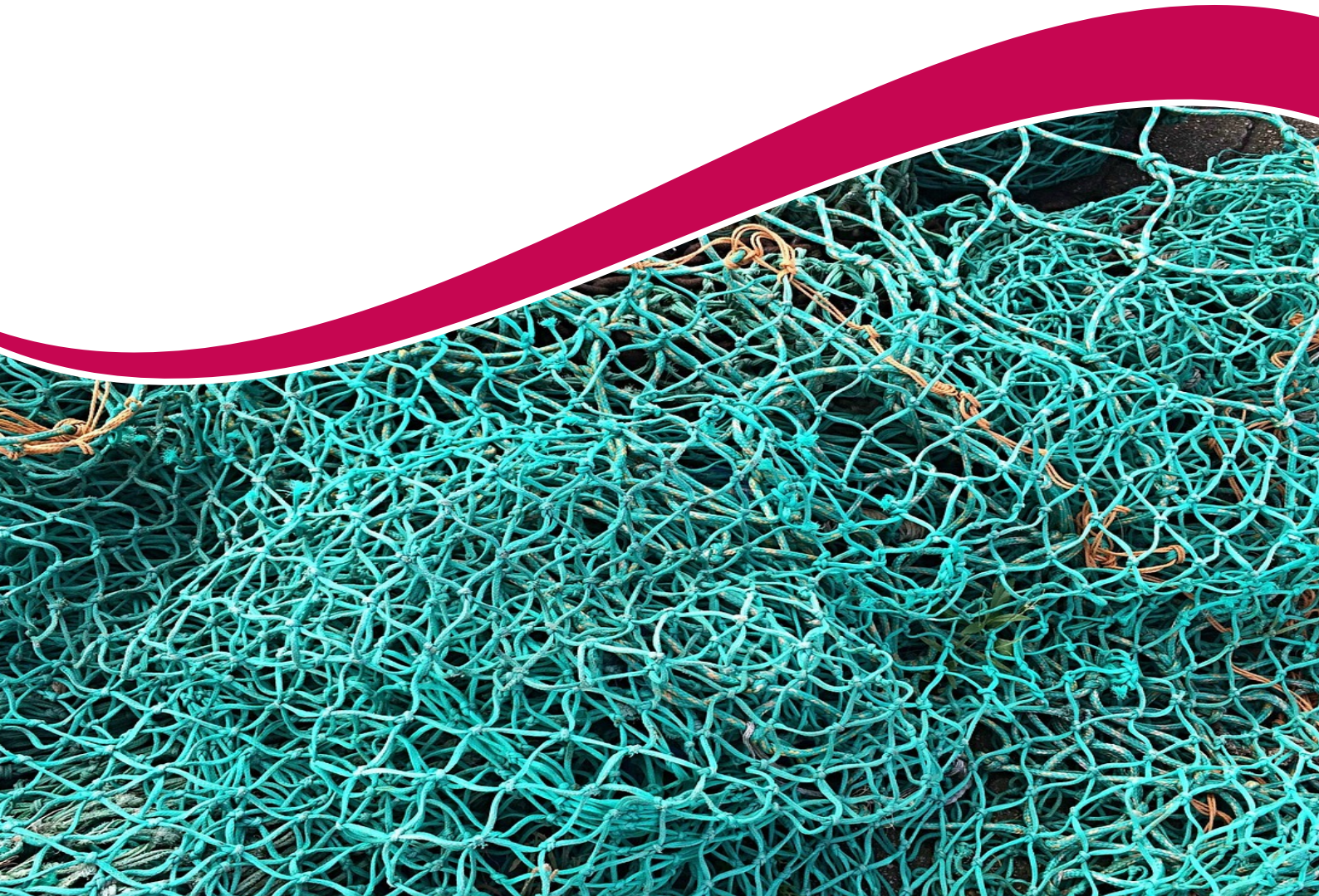


Spawning biomass of Jack Mackerel (*Trachurus declivis*) in the East sub-area of the Small Pelagic Fishery during summer 2019

Report to the Australian Fisheries Management Authority

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

RR2018/0810 June 2020



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SPAWNING BIOMASS OF JACK MACKEREL EAST IN 2019

Version	Updates	Approver

About this document

This publication may be cited as:

Ward, T. M., Grammer, G. L. Ivey, A. R. and Keane, J. (2020). Spawning biomass of Jack Mackerel (*Trachurus declivis*) in the East sub-area of the Small Pelagic Fishery during summer 2019. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2020/000206-01. 42pp.

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Acknowledgements

This fishery assessment report was funded by the Australian Fisheries Management Authority (AFMA). We thank the master, crew, owner and staff of the *FV Santo Rocco* for their professional and cheerful assistance. The efforts of Mr Tony Muollo to ensure the success of the survey are recognised with gratitude. We thank Mr Nathavong Navong for working diligently in the field to collect the samples. We thank Mr Kriston Bott, Ms Sonja Hoare and Mr Sam Foster for processing samples in the laboratory. This report was formally reviewed by Dr Jonathan Smart and Dr Katherine Heldt and approved for release by Dr Stephen Mayfield (SARDI Aquatic Sciences).

Executive Summary

Background and Need

Estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator for quota species in the 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 DEPM was previously applied to Jack Mackerel (*Trachurus declivis*) in the East sub-area of the SPF in 2014. Jack 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 new fishing operation that has recently been established in the East sub-area.

Egg and adult surveys of Jack Mackerel East (funded by industry through FRDC) were conducted in January-February 2019 between south-eastern Tasmania and central New South Wales, including Bass Strait as far west as 146°30'E. This area was surveyed because previous surveys have shown that this is the likely spawning area of Jack Mackerel in the East sub-area during the peak spawning season (Ward et al. 2015, 2018).

Objectives

The objectives of this study were to:

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

Methods

The rationale for the DEPM is that spawning biomass 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 from the *FV Santo Rocco* from 206 in shelf waters between south-eastern Tasmania and central New South Wales from 15 January to 7 February 2019.

Jack Mackerel eggs were identified using standard laboratory procedures. Morphological identifications of Jack Mackerel eggs were confirmed using standard molecular techniques. Spawning area was estimated using the Voronoi nearest neighbour method.

Five models were tested to estimate egg production (P_0). The value of P_0 used to estimate spawning biomass was the Generalised Linear Model with a negative binomial error structure where the variance increases linearly with the mean (GLM NB1).

Modified demersal trawls for adult Jack Mackerel were undertaken from the *FV Santo Rocco* at 19 sites in shelf and slope waters between south-eastern Tasmania and southern New South Wales during 15 January to 7 February 2019. Jack Mackerel were caught in 13 of the 19 trawl, and 11 of the trawls contained mature females. Estimates of the adult reproductive parameters calculated from these samples were used in calculations of spawning biomass.

Sensitivity analyses were undertaken to determine the influence of uncertainty in individual parameters on estimates of spawning biomass.

Results, Discussion and Implications

The total survey area was 68,295 km². Live Jack Mackerel eggs (n = 921) were collected at 107 of the sites (51.9%). The spawning area (A) was 36,100 km². Mean daily egg production (P_0 , 95% CI) was 15.1 (8.0–22.2) eggs.day⁻¹.m⁻².

Trawl samples included 1,080 adult fish (505 males, 575 females). Only four fish with hydrated oocytes were collected in 2019. Data from these four fish were combined with data from hydrated females collected in 2014. A total of 17 hydrated females were used to estimate the fecundity-weight relationship. The relationship obtained was similar to that obtained for *Trachurus trachurus*.

Estimates of adult parameters (95% CI) were: spawning fraction (S): 0.032 (0.016–0.048); sex ratio (R): 0.546 (0.49–0.60); mean female weight (W): 126.3 (68.3–184.3) g; and batch fecundity (F): 25,212 (13,570–36,854) oocytes. The ratio of \hat{F}/W , i.e. 199.6 (179.1–220.1) eggs.g⁻¹, was used instead of the individual parameters of F and W to calculate spawning biomass because this approach increased precision.

This is the second dedicated application of the DEPM to Jack Mackerel in the East sub-area of the SPF. The estimate of spawning biomass for 2019 of 156,292 t (95% CI = 49,120–263,496) is similar to that obtained in 2014 (157,805 t; Ward et al. 2015). The estimate of spawning biomass for 2019 is suitable for setting RBCs, because it is based on robust and/or conservative estimates of key parameters.

Keywords: Jack Mackerel, *Trachurus declivis*, East sub-area, Spawning Biomass, Small Pelagic Fishery, Daily Egg Production Method

1 Introduction

1.1 Background

A large purse-seine fishery for small pelagic fishes developed off Tasmania in the mid-1980s. The majority of the catch was Jack Mackerel (*Trachurus declivis*), with relatively small quantities of Redbait (*Emmelichthys nitidus*) and Blue Mackerel (*Scomber australasicus*) taken as by-product. Catches of Jack Mackerel peaked at ~40,000 t in 1986/87, making it Australia's largest fishery by weight at that time (Kailola et al. 1993, Pullen 1994, Ward and Grammer 2018).

The Commonwealth Small Pelagic Fishery (SPF) was established in 2000. The SPF is a purse-seine and mid-water trawl fishery. It is managed by the Australian Fisheries Management Authority (AFMA) and 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, Redbait, Blue Mackerel and Australian Sardine (*Sardinops sagax*).

A detailed history of the SPF is described in Moore and Skirtun (2012). Catch and effort in the SPF have fluctuated over time, driven by a combination of social, economic and biological factors. Catch and effort increased in 2014/15 to 2015/16 when a factory trawler operated in both sub-areas (Ward and Grammer 2018).

The SPF Harvest Strategy and Management Plan were implemented in 2008/09 (AFMA 2008, 2009). The SPF Harvest Strategy was last revised in 2017. The SPF Harvest Strategy is used to set Total Allowable Catches (TACs) for each species and sub-area. Estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator for target species. Estimates of spawning biomass are used to set Recommended Biological Catches (RBCs) and Total Allowable Catches (TACs) under guidelines outlined in the Harvest Strategy.

The DEPM has previously been applied to Jack Mackerel in the East sub-area in 2014 (Ward et al. 2015, Ward et al. 2016). The present study is the second dedicated application of the DEPM in the East sub-area of the SPF. It was conducted in the area between south-eastern Tasmania and central New South Wales and extended into Bass Strait to 146°30'E to ensure the spawning area was covered. The extension of the survey into Bass Strait was based on evidence from both the 2014 DEPM survey and a 2017 DEPM survey for Jack Mackerel in the West sub-area of the SPF (Ward et al. 2018b)

1.2 Daily Egg Production Method (DEPM)

The rationale for the DEPM is that the adult biomass of a species present in the spawning area during the 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). The equation underpinning the DEPM and definitions of the key parameters are shown in Table 1 (Equation 1).

The DEPM is applied to determinate or indeterminate spawning fishes that spawn multiple batches of pelagic eggs over an extended spawning season (Parker 1980, Ganius 2013). Parameters used to calculate total daily egg production, i.e. mean daily egg production (P_0) and spawning area (A), are estimated from structured ichthyoplankton surveys, typically undertaken from research vessels (e.g. Stratoudakis et al. 2006). Adult samples 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/W), can be collected from the vessel undertaking the ichthyoplankton survey, or chartered or commercial vessels operating in the survey area during the study period (e.g. Stratoudakis et al. 2006).

Model Name	Equation	Eq. No.	Parameters	Reference
Daily Egg Production Method	$SB = P_0 * A / (R * S * F/W)$	(1)	SB: spawning biomass P_0 : mean daily egg production A: total spawning area R: mean sex ratio S: mean spawning fraction F/W: mean relative fecundity	Parker 1985, Ward et al. 2020

Table 1-1. The equation for the Daily Egg Production Method (DEPM) used to calculate the spawning biomass (SB) of Jack Mackerel in waters off eastern Tasmania to southern New South Wales during January-February 2019.

The key assumptions of the DEPM are that: 1) surveys are conducted during the main (preferably peak) 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).

Although the DEPM is used widely, a range of logistical and statistical challenges have been encountered and estimates of spawning biomass are known to be imprecise (e.g. Stratoudakis et al. 2006; Bernal et al. 2012, Dickey-Collas et al. 2012; Ward et al. 2018a). There are considerable uncertainties associated with the estimation of several parameters, especially P_0 (Fletcher et al. 1996, McGarvey and Kinloch 2001, Gaughan et al. 2004).

Recent studies have shown that inter-annual variations in estimates of P_0 for Sardine off South Australia are low in comparison to statistical uncertainty (e.g. Ward et al. 2018a, 2019, 2020). These findings support previous studies (e.g. Mangel and Smith 1990; Gaughan et al. 2004) that have shown that spawning biomass of Sardine is not correlated with P_0 , but is strongly correlated with A . Studies by both Mangel and Smith (1990) and Gaughan et al. (2004) showed that inter-annual variations in total daily egg production are driven primarily by variations in A . This finding may apply to other small pelagic fishes, such as Jack Mackerel, with similar reproductive strategies and warrants further investigation.

Uncertainties in the estimation of S mainly relate to difficulties obtaining representative samples of the adult population. However, uncertainty also arises from the challenge of estimating the age of post-ovulatory follicles (Ganias 2012). Uncertainties associated with estimation of S are most problematic for species with low spawning fraction, where small changes in S (e.g. from 5% to 15%) can have a major impact on estimates of biomass (Stratoudakis et al. 2006). S has also been shown to change with latitude for Jack Mackerel off south-eastern Australia (Sexton et al. 2017), and this adds to the difficulty of obtaining representative adult samples.

1.3 Jack Mackerel

Egg production methods have been applied to several trachurid species. For example, since 1995, annual egg production surveys have been applied to Horse Mackerel (*Trachurus trachurus*) off the Iberian Peninsula (Goncalves et al. 2009). The DEPM was first applied to this stock in 2007. The DEPM has been successfully applied to Chilean Jack Mackerel (*Trachurus murphyi*) off the central coast of Chile (1999-2006; Ruiz et al. 2008) and also to Yellowtail Scad (*Trachurus novaezelandiae*) off eastern Australia in 2009 (Neira 2009).

Jack Mackerel (*Trachurus declivis*) is widely distributed throughout coastal waters of southern Australia and New Zealand (Gomon et al. 2008). It occurs in depths up to 500 m but is most common in shelf waters <200 m (Pullen 1994), where it feeds primarily on krill and other aquatic crustaceans (Stevens et al. 1984, Bulman et al. 2008, McLeod et al. 2012). A review by Bulman et al. (2008) concluded that it was likely that there are two separate sub-populations of Jack Mackerel in Australian waters; one off eastern Australia, including eastern Tasmania, and one west of Tasmania, including the Great Australian Bight and Western Australia.

Jack Mackerel are serial spawners (Marshall et al. 1993, Neira 2011). The fish spawn in spring along the New South Wales (NSW) coast (Maxwell 1979, Keane 2009) and during summer further south off Tasmania and in the Great Australian Bight (Stevens et al. 1984, Jordan et al. 1995, Ward et al. 2016, Sexton et al. 2017). The main spawning area is

thought to be located off south-eastern Australia from western Victoria through Bass Strait to eastern Tasmania and southern NSW (Bulman et al. 2015, Ward et al. 2018b). Off eastern Tasmania, spawning occurs continuously from December to February (Williams and Pullen 1986, Jordan 1994, Neira 2011).

Jack Mackerel eggs are positively buoyant and 0.97–1.03 mm in diameter (Neira 2011). They are morphologically similar to Yellowtail Scad eggs, but slightly larger (Yellowtail Scad egg diameter: 0.78–0.88 mm; Neira 2009). Previous studies have demonstrated a high level of success in identifying Jack Mackerel eggs from morphological characteristics (Neira 2011, Ward et al. 2015, Ward et al. 2018b).

The first dedicated application of the DEPM to Jack Mackerel off the south-east coast of Australia was done in 2014 (Ward et al. 2015). The estimate of spawning biomass of 157,805 t (95% CI = 59,570–358,731) was based on reliable estimates of key adult parameters and considered robust. A preliminary study based on samples collected off south-eastern Australia in 2002–2004 provided estimates of spawning biomass in the range of 114,000–169,000 t (Neira 2011). The first dedicated application of the DEPM to Jack Mackerel in the West sub-area of the SPF (Kangaroo Island to western Tasmania) was done in 2017 (Ward et al. 2018b). This estimate of spawning biomass of 31,069 t was an underestimate as key spawning habitat in western Bass Strait was not included in the survey. Ecosystem modelling estimated the spawning biomass of Jack Mackerel of south-east Australia to be 130,000–170,000 t (Fulton 2013). Similar ecosystem modelling suggested the biomass of Jack Mackerel west of Tasmania was approximately 60,000–110,000 t (Smith et al. 2015).

1.4 Need

Estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator for quota species in the 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.

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because previous surveys have shown that this is the likely spawning area of Jack Mackerel in the East sub-area during the peak spawning season (Ward et al. 2015, 2018).

1.5 Objectives

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

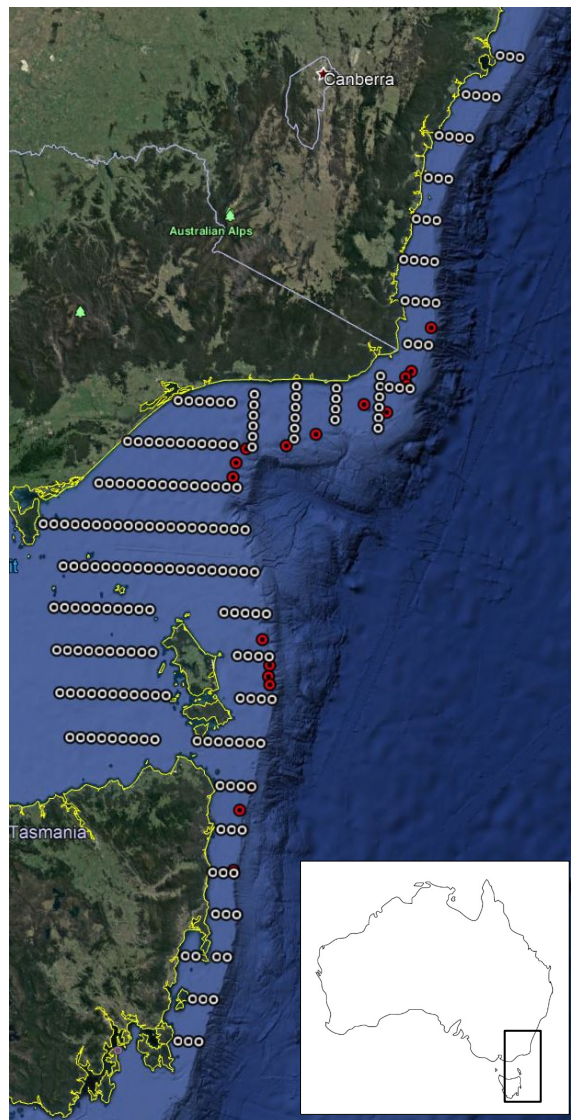
2 Methods

2.1 Total Daily Egg Production

2.1.1 Ichthyoplankton surveys

During the summer of 2019, ichthyoplankton samples were collected from the *FV Santa Rocco* at 206 sites on 29 transects in shelf waters between south-eastern Tasmania and central New South Wales (Figure 2–1, Appendix 2). The survey was undertaken from 15 January to 7 February 2019. An additional sample was taken at every second site for genetic validation of Jack Mackerel eggs (n = 102).

Figure 2–1. Area off south-eastern Tasmania to central New South Wales where the Daily Egg Production Method was applied to Jack Mackerel in summer 2019. Locations shown are the main egg sampling sites (yellow) and adult trawl sites (red).



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·s⁻¹. Water temperature profiles were recorded with a Sea-Bird™ Conductivity-Temperature-Depth (CTD) attached to 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.

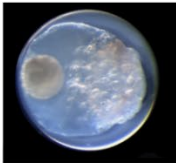
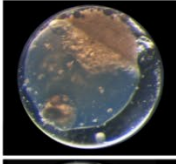
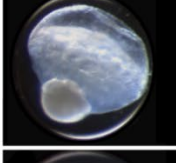
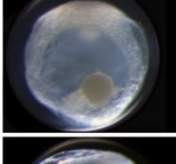
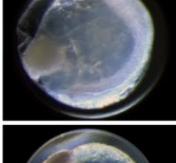
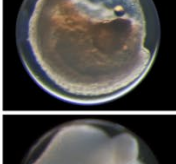
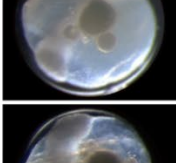
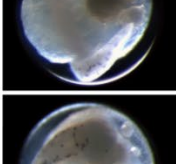
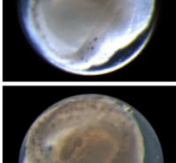
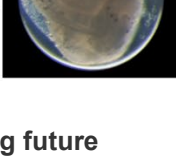
2.1.3 Egg identification and validation

Eggs of Jack Mackerel were identified using the morphological features in published descriptions for the same or closely related species (Ahlstrom and Ball 1954, Crossland 1981, Cunha et al. 2008, Ward et al. 2015, Ward et al. 2018b). Identifications of Jack 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. This validation was done because Jack Mackerel eggs have similar characteristics to other common species, especially Yellowtail Scad (*Trachurus novaezelandiae*) (See Appendix 1).

All eggs were staged following Ward et al. (2018a, 2018b) (Figure 2-2). This method was used because the distinctive developmental characteristics of the 'universal' 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 have been shown to be under- and over-represented in plankton samples, respectively (Ward et al. 2020, Stratoudakis et al. 2006, Bernal et al. 2012, Dicky-Collas et al. 2012).

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Figure 2–2. Egg stages of Jack Mackerel delineated by Ward et al. (2015, 2018b) using the ‘universal’ egg stages of Ward et al. (2018a).

	Jack Mackerel	<u>‘Universal’ Egg Stage Description</u>
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

2.1.4 Egg ageing and treatment of zero count egg samples

Based on CTD data, egg samples were allocated to three temperature bins that covered the range of temperatures typically sampled during DEPM surveys off eastern and southern Australia (14–18°C, 18–22°C, and 22–26°C). Temperature egg development rates of Cunha et al. (2008) for Horse Mackerel, a closely related species, were used to assign the mean age to each egg (Ward et al. 2018b). Generally, pelagic eggs of marine fish that are ~1 mm diameter 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).

After the eggs were assigned an age, eggs in each sample were aggregated into daily cohorts by stage. This was done because more than one night’s spawning could be represented in a 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 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:

$$P_t = \frac{C \cdot D}{V} \quad \text{Equation 2}$$

Where C is the number of eggs at each age in a sample, V is the volume filtered (m³), 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).

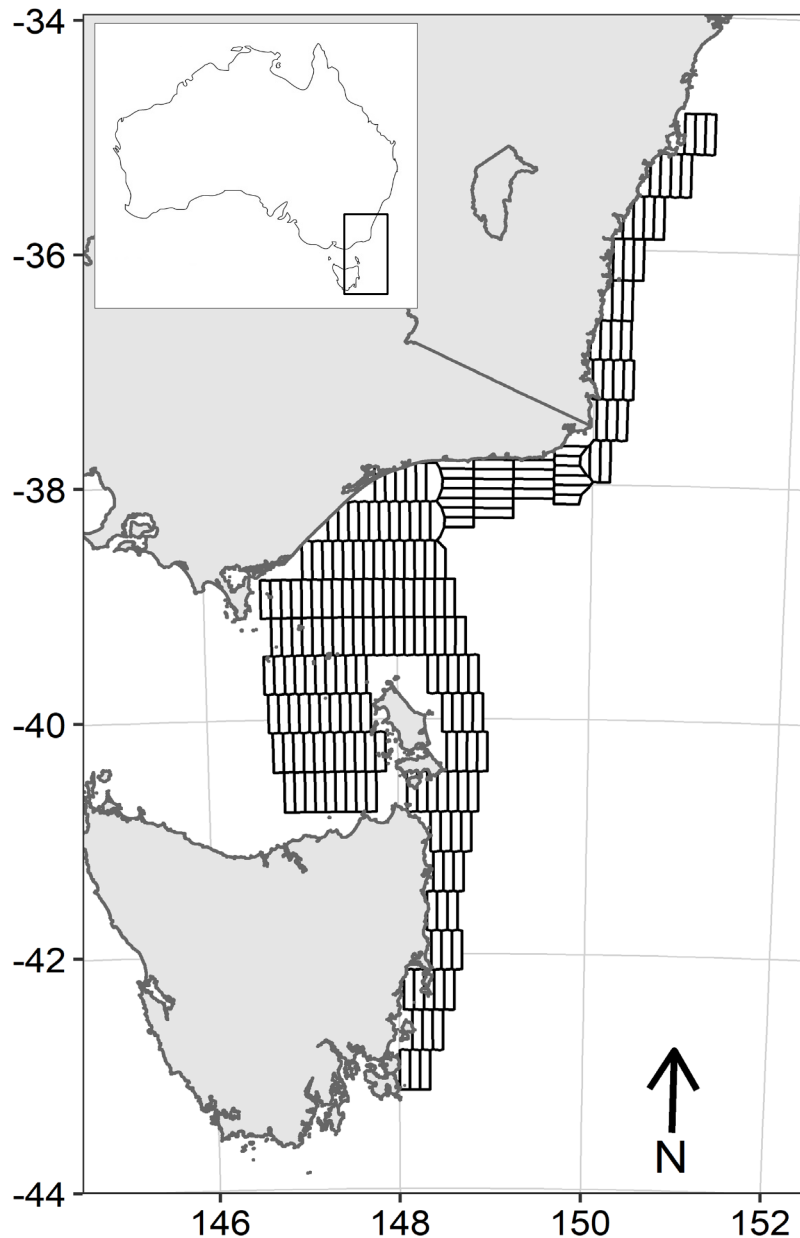
2.1.6 Spawning area (A)

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’

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package in the statistical program 'R' (R Development Core Team 2019, Turner 2016; Figure 2–2). The area represented by each site (km^2) was calculated. A was defined as the total area of the polygons where live Jack Mackerel eggs were present in the plankton samples.

Figure 2–1. Polygons generated using the Voronoi natural neighbour method and used to estimate the spawning area of Jack Mackerel along eastern Tasmania to southern New South Wales in 2019.



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

The underlying model used to calculate P_0 was the exponential egg mortality model (Equation 3) with a bias correction factor (Equation 4, the ‘log-linear model’) which was adapted from Picquelle and Stauffer (1985). The linear version of the exponential egg mortality model is:

$$\log(y_t) = \ln \left(P_0^{bias} + \frac{1}{n} \sum_{t=1}^n \frac{P_t}{C_t} \right) + Z + x_t \quad \text{Equation 3}$$

where y_t is the density of eggs of each daily cohort t , x_t is the age of each cohort in days P_0^{bias} is the negatively biased estimate of egg production (y axis intercept) and Z is the instantaneous rate of egg mortality (slope of the linear model). y_t has a normally distributed variance σ .

Estimates of P_0^{bias} obtained using the linear version of the exponential mortality model have a strong negative bias, therefore a bias correction factor was applied following the equation of Picquelle and Stauffer (1985):

$$P_0 = e^{\left(P_0^{bias} + \sigma^2/2 \right)} - \frac{1}{n} \sum_{t=1}^n \frac{P_t}{C_t} \quad \text{Equation 4}$$

where, σ^2 is the variance of the estimate of biased mean daily egg production (P_0^{bias}).

P_0 was also estimated using three general linear models (GLMs) that included a quasi-error structure, (NB1) a negative binomial error structure where the variance increases linearly with the mean ($\sigma = \mu^*(1 + \mu + \varphi)$) and (NB2) a negative binomial error structure where variance increases quadratically with the mean ($\sigma = \mu^*(1 + \mu / \varphi)$). Where μ is the model estimate, σ is the model variance and φ is the overdispersion parameter. Each GLM was used to estimate P_0 and Z using a log-link function (Equation 5):

$$E[P_0] = g^{-1}(-zt + \varepsilon) \quad \text{Equation 5}$$

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. Negative binomial and quasi error structures are considered suitable for over-dispersed count data, such as egg density by age (e.g. Ward et al. 2011, 2018a). Instantaneous egg mortality rate (Z) was estimated as a free parameter in each of the models. The value of P_0 from the GLM NB1 was used to estimate spawning biomass for Jack Mackerel following recommendations by Ward et al. (2018a, 2020).

2.2 Mean Daily Fecundity

2.2.1 Adult Sampling

Adult Jack Mackerel were sampled using a modified demersal trawl net deployed from the *FV Santo Rocco* in shelf and slope waters between eastern Tasmania and southern New South Wales from 15 January to 7 February (Figure 2–1). Where Jack Mackerel were present in trawls, fish were dissected and sexed. The ovaries of mature were removed, labelled and fixed in a 10% buffered formaldehyde seawater solution. Females, with ovaries removed, and mature males were labelled and frozen. Female weight (W) and male weight

Mature females from each sample were thawed and 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:

$$W = \left[\overline{W}_i * \frac{n_i}{N} \right] \quad \text{Equation 5}$$

where, \overline{W}_i is the mean female weight of each sample i ; n_i 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).

2.2.2 Sex ratio (R)

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

$$R = \left[\overline{R}_i * \frac{n_i}{N} \right] \quad \text{Equation 6}$$

where, n_i 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:

$$\overline{R}_i = \frac{F}{(F + M)} \quad \text{Equation 7}$$

where, F and M are the respective total weights of mature females and males in each sample i .

2.2.3 Batch 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. Hydrated females collected in the current survey and during the 2014 DEPM survey (Ward et al. 2015) were used to produce the batch fecundity relationship (see Ward et al. 2019).

Eggs per gram of female weight (\hat{F}/W) was calculated by predicting batch fecundity from the weight of each fish from both surveys (2014 and 2019). Then mean \hat{F} was then divided by the mean weight of all mature females collected (W).

2.2.4 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 aged 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.

$$S = \left[\overline{S_i} * \frac{n_i}{N} \right] \tag{Equation 8}$$

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:

$$\overline{S_i} = \frac{[(d_0 + d_1 + d_2 POFs) / 3]}{n_i} \tag{Equation 9}$$

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

Spawning biomass was calculated according to Equation 1 using the estimate of P_0 obtained from the GLM NB1, spawning area (A) estimated in 2019 and estimates of S , R and F/W obtained from adult samples collected during 2019.

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 , F , W and \hat{F}/W were calculated from all adult data. A ratio estimator was used calculate the coefficients of variation (CVs) for S , R , and \hat{F}/W (see Rice 1995). The variance around the spawning biomass estimate was calculated by the summing the squared CVs for each parameter and multiplying by the square of the estimate of spawning biomass. Uncertainty estimates presented 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 the parameter values used to calculate spawning biomass on the estimate of spawning biomass. Each parameter in Equation 1 was varied in turn, while keeping all other variables constant. Estimates of adult parameters and A for the sensitivity analyses were the values estimated during the current survey and those from the 2014 Jack Mackerel survey off south-eastern Australia. Values of egg production (P_0) resulted from egg production models in the current extended survey. The P_0 value from the 2014 DEPM off south-east Australian was added as an additional comparison for Jack Mackerel.

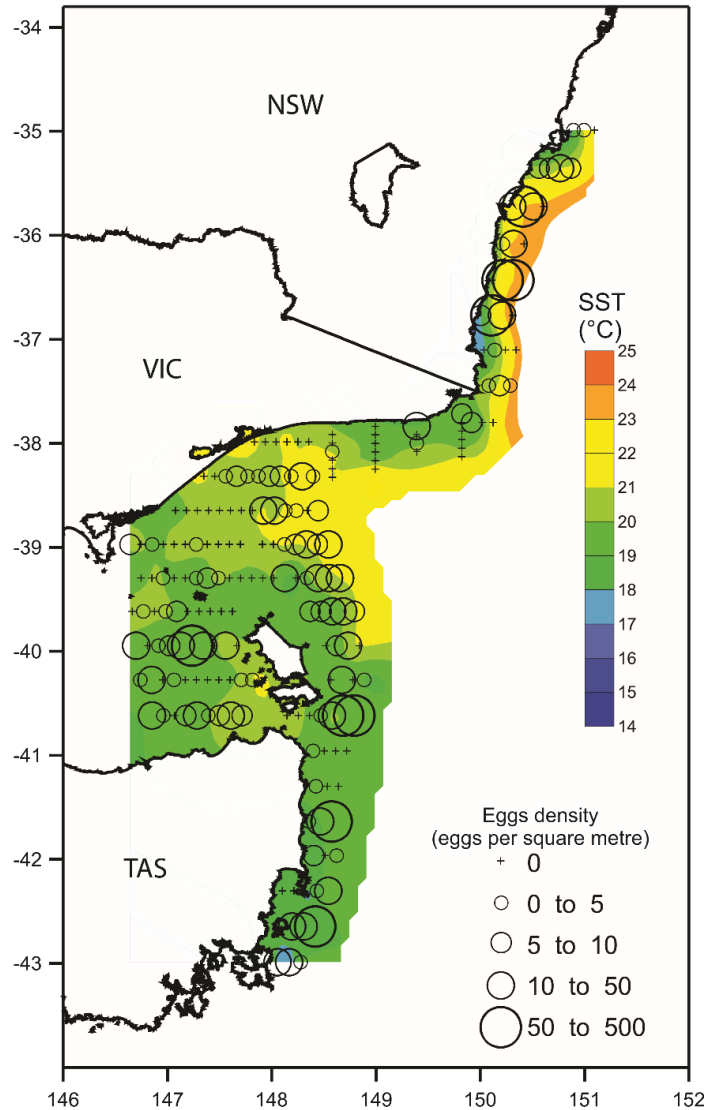
3 Results

3.1 Total Daily Egg Production

3.1.1 Egg distribution and abundance

A total of 921 live Jack Mackerel eggs were collected at 107 of 206 (51.9%) sites on 29 transects between south-eastern Tasmania and Jervis Bay, New South Wales. Eggs were found in Bass Strait on transects extending west from the shelf edge to 146°30'E. These findings were confirmed by molecular identification (see Appendix 1) of Jack Mackerel eggs in ethanol preserved samples taken during the survey (Figure 3–1).

Figure 3–1. Distribution and densities (egg·m⁻²) of live Jack Mackerel eggs between south-eastern Tasmania and central New South Wales during January to February 2019. Densities are overlaid on sea surface temperatures (SST; °C) measured during the survey.



3.1.2 Egg density (P_t)

Egg densities were higher in mid to outer shelf waters and in Bass Strait (Figure 3–1). Higher densities of eggs ($P_t > 10$ eggs·m⁻²) were collected where the depth was 41–350 m (mean: 99.5 m). The highest egg densities were in Bass Strait (281 eggs·m⁻²; 19.6°C SST) and off Batemans Bay, New South Wales (132 eggs·m⁻²; 23.0°C SST). Bottom depths where live eggs were collected ranged from 30–350 m (mean: 91.0 m), and SSTs were 17.7–23.3°C (mean 20.2°C).

3.1.3 Spawning area (A)

The estimated spawning area for Jack Mackerel was 36,100 km², comprising 52.9% of the total area sampled (68,295 km², Table 4).

Survey Area (km ²)	Spawning Area (A)	Area with Eggs (%)
68,295	36,100	52.9

Table 3–1. Spawning Area (A) and total area surveyed for Jack Mackerel between south-eastern Tasmania and central New South Wales in 2019.

3.1.1 Mean daily egg production (P_0)

The estimate of mean daily egg production (P_0) obtained using the negative binomial GLM NB1 (Equation 5) was 15.1 eggs·day⁻¹·m⁻² (95% CI: 9.4–23.5) and instantaneous daily egg mortality (z , day⁻¹) was 0.014 (Table 3–2; Figures 3–2, 3–3). The other models produced estimates of P_0 between 14.5 and 24.4 eggs·day⁻¹·m⁻² (Table 3–2; Figures 3–2, 3–3).

Egg Production Model	P_0 eggs·day ⁻¹ ·m ⁻² (95% CI)	z
Linear version of exponential model, corrected	14.5 (8.8-23.8)	0.017
Exponential Model, NLS	21.2 (-)*	0.032
GLM, Quasi, log link	24.4 (8.3-54.3)	0.041
GLM, Negative Binomial (NB1), log link	15.1 (9.4-23.5)	0.014
GLM, Negative Binomial (NB2), log link	23.8 (8.4-53.2)	0.040

Table 3–2. Point estimates of mean daily egg production (P_0 , eggs·day⁻¹·m⁻²) and instantaneous daily mortality (z , day⁻¹) for Jack Mackerel in summer 2019 generated by the five egg production models fits. *NLS model fit produced negative estimates when bootstrapped and CI was not calculated.

Figure 3–2. Models fitted to egg densities (eggs.m⁻²) and egg age (hours) of Jack Mackerel cohorts in summer 2019. NLS: Non-linear Least Squares; Quasi and NB: GLMs with quasi and negative binomial error structures; GLM NB1 and GLM NB2. Grey horizontal line: mean egg density for survey.

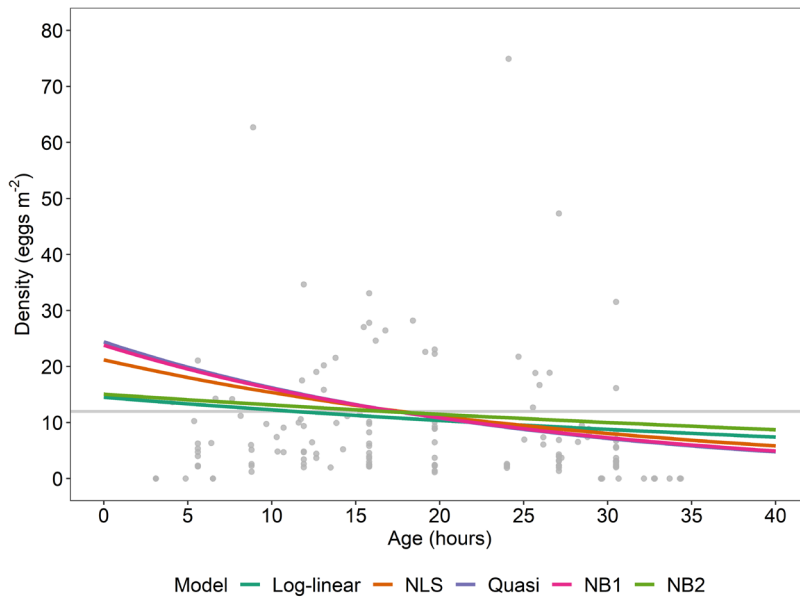
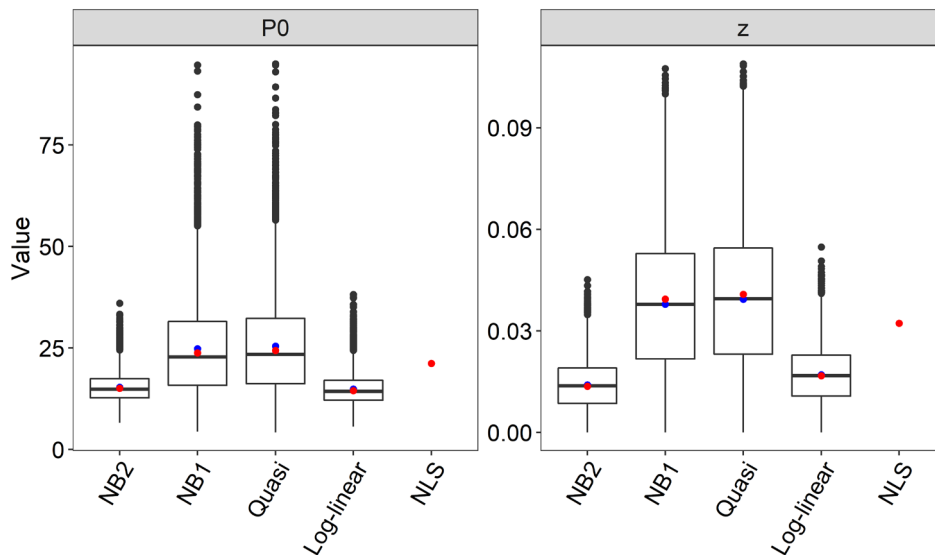


Figure 3–3. Mean daily egg production (P_0 , egg·day⁻¹·m⁻²) and instantaneous daily mortality (z , day⁻¹) for Jack Mackerel from the five egg production models for data collected in summer 2019. Horizontal black line is the median and box is the quartiles. Red dot: model point estimate; blue dot: bootstrapped mean; solid line: 99% Confidence Interval, black dots: outliers. *NLS model fit produced negative estimates when bootstrapped and CI was not calculated.



3.2 Mean Daily Fecundity

Jack Mackerel were caught in 13 of the 19 trawl undertaken from the *FV Santo Rocco*, and 11 of the trawls contained mature females. A total of 1,080 mature Jack Mackerel were sampled across the 11 sites (Table 3-3). Estimates of the adult reproductive parameters used in calculations of spawning biomass are provided in Tables 3-3, 3-4 and 3-5. The means and bootstrapped 95% confidence intervals are shown in Table 3-5.

3.2.1 Mean female weight (*W*)

The mean weight of mature female Jack Mackerel in samples collected in 2019 ranged from 88.7 to 181.9 g (Table 3-3). The weighted mean weight of mature females in 2019 was 126.3 g (95% CI 68.3–184.3, Tables 3-3, 3-5).

Trawl	Male		Female		R
	n	Average weight (g)	n	Average weight (g)	
2	39	80	18	97	0.36
3	61	90	67	89	0.52
4	13	112	17	109	0.56
5	2	117	3	102	0.57
6	132	124	137	121	0.50
7	74	130	98	142	0.59
8	30	133	43	144	0.61
9	53	148	98	148	0.65
10	3	184	4	181	0.57
11	11	173	7	143	0.35
12	87	112	83	119	0.50
Total	505*	119#	575*	126.3#	0.546#

Table 3-3. Number of males and females of Jack Mackerel in samples and estimates of female weight (*W*) and sex ratio (*R*, proportion of females by weight). Values in last row are sums (*) and weighted means (#).

3.2.2 Sex ratio (*R*)

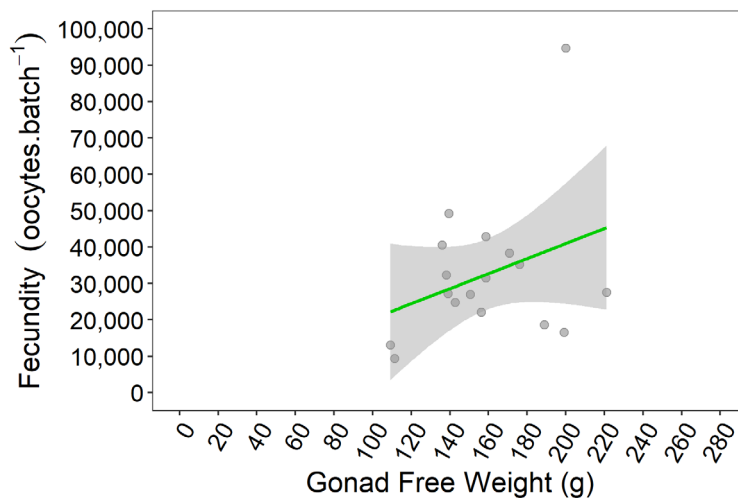
The mean sex ratio by weight (*R*, 95% CI) calculated from all fish collected in 2019 was 0.55 (0.49–0.60) (Table 3-3). The total numbers of females and males collected were 575 (53.7% of fish) and 505 (46.3%), respectively. Estimates of *R* for individual samples ranged from 0.35 and 0.65 (Table 3-3).

3.2.3 Batch fecundity (F)

In 2019, four females with hydrated oocytes were collected. Data from these fish were combined with data from hydrated females collected during the survey in 2014 (Ward et al. 2015) to calculate the batch fecundity relationship (see Ward et al. 2019) (Figure 3-4). A total of 17 hydrated females were included in the analysis. The fecundity-weight relationship estimated from these samples was: Batch Fecundity = $207 \times \text{Gonad Free Female Weight} - 332$ ($R^2 = 0.12$). Using this relationship to calculate mean batch fecundity (F , 95% CI) for 2019 gave an estimate of 25,212 (13,570–36,854) oocytes (Table 3-5). Mean gonad free female weight for 2019 was 124.0 g and ranged between 61.0 and 250.7 g.

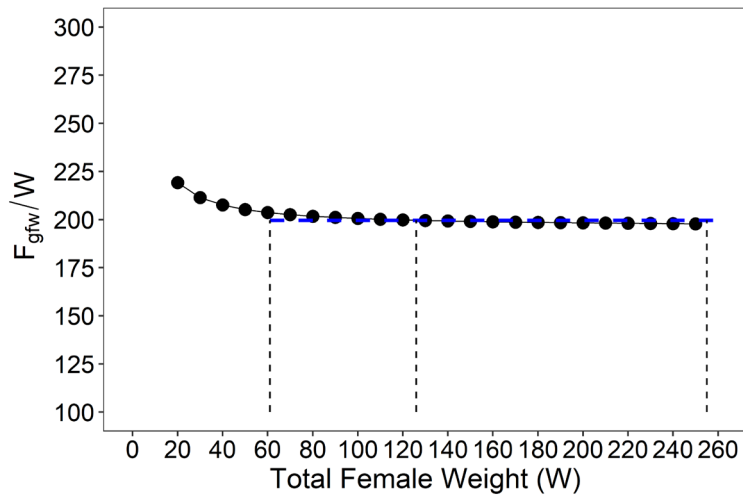
The estimate of relative fecundity (\hat{F}/W ; eggs per gram of female weight) for 2019 was 199.6 (179.1–220.1) eggs.g⁻¹ (Table 3-5; Figure 3-5). Relative fecundity (\hat{F}/W) is almost constant across the range of weight (W) of mature females obtained in samples in 2019 (Figure 3-5). In other words, fecundity increases 200 eggs for every gram of increase in total female weight.

Figure 3-4. Relationship between gonad-free weight and batch fecundity (F) for all hydrated Jack Mackerel collected in 2014 and 2019 (shading = 95% CI). $F = 207 \times \text{Gonad Free Weight} - 332$, ($R^2 = 0.12$).



SPAWNING BIOMASS OF JACK MACKEREL EAST IN 2019

Figure 3-5. Correlation between eggs per gram of female weight (F/W) and female weight (W) of Jack Mackerel collected in 2019. Dashed lines are minimum, mean and maximum female weights in 2019; Blue line: F/W value for 2019. Fecundity = $196 * \text{Weight} + 465$.



3.2.4 Spawning fraction (S)

The spawning fraction (S, 95% CI) calculated from mature females collected in 2019 was 0.032 (0.016–0.048) (Tables 3-4, 3-5). A total of 575 ovaries were examined; 15 had day-0 POFs, 25 had day-1 POFs and 16 day-2 POFs. The spawning fraction of females in each sample ranged from 0.00 to 0.111.

Trawl	n	Total POFs	S
2	18	0	0.000
3	67	0	0.000
4	17	0	0.000
5	3	1	0.111
6	137	7	0.017
7	98	16	0.054
8	43	3	0.023
9	98	12	0.041
10	4	1	0.083
11	7	2	0.095
12	83	14	0.056
Total	575*	56*	0.032#

Table 3-4. Number of female Jack Mackerel per sample and estimates of spawning fraction (S) for fish collected between south-eastern Tasmania and central New South Wales in summer 2019. Values in bottom row are sums (*) and weighted mean (#). POF: Post ovulatory follicles.

3.3 Spawning biomass

The estimate of spawning biomass for Jack Mackerel was 156,292 t (95% CI = 49,120–263,496). This value was calculated using the GLM NB1 to estimate P_0 and values of adult parameters estimated from the current survey (Table 3-5).

Parameter	Symbol	Units	Value	95% CI
Egg Production	P_0 (<i>Nbinom1 GLM</i>)	eggs·day ⁻¹ ·m ⁻²	15.1	8.0–22.2
Spawning Area	A	km ²	36100	-
Sex Ratio	R	-	0.546	0.49–0.60
Spawning Fraction	S	-	0.032	0.016–0.048
Fecundity	F	eggs·female ⁻¹	25,212	13,570–36,854
Female Weight	W	g	126.3	68.3–184.3
	F/W	eggs·g ⁻¹	199.6	179.1–220.1

Table 3-5. Estimates of adult parameters and bootstrapped 95% confidence intervals for Jack Mackerel sampled between south-eastern Tasmania and central New South Wales during summer 2019.

3.4 Sensitivity analysis

The sensitivity analysis shows the effects of variability in parameters (i.e. A , P_0 , R , S , F , W and F/W) on the estimate of spawning biomass for 2019 (Table 3-5; Figures 3-6 and 3-7). The parameter estimates used to calculate spawning biomass were those that were considered to be robust and/or produced conservative estimates of the size of the adult population.

Spawning biomass increased linearly with A (Figure 3-6). The 2019 survey had a larger A and covered a greater portion of Bass Strait than the 2014 survey but did not extend as far to the north. This suggests Bass Strait is an important spawning area for Jack Mackerel and that A was not under-estimated in 2019. If A was under-estimated, it would have reduced the estimate of spawning biomass.

The parameters W and F and their effects on estimates of spawning biomass are inter-related. Estimates of spawning biomass increased as W increased and decreased as F increased. Fish collected during the current survey were smaller than those collected during the 2014 survey. Using the batch fecundity relationship that included fish collected in 2014 and 2019 to estimate F produced a higher estimate of spawning biomass than the 2014 value of F . However, the ratio of F/W was similar between the two years and inter-

SPAWNING BIOMASS OF JACK MACKEREL EAST IN 2019

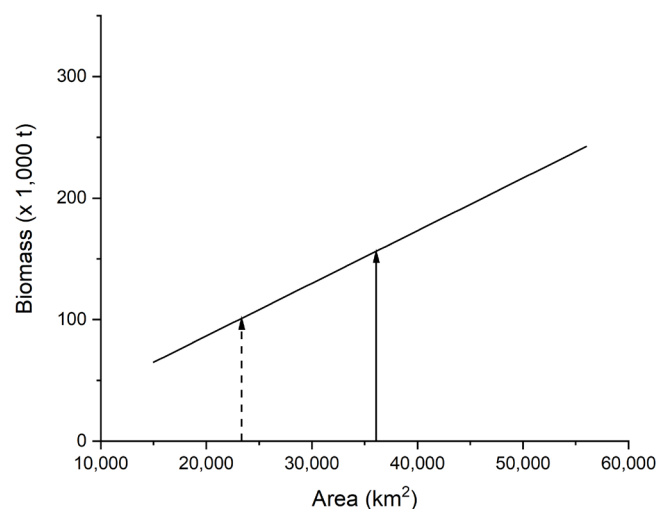
annual variation in this combined parameter had a much smaller effect on spawning biomass (i.e. 156,292 (2019) to 191,218 t (2014) (Figure 3-7).

Estimates of spawning biomass increased as R decreased (Figure 3-7). The fluctuations in R between the 2014 and 2019 surveys are more reflective of the limitations of the adult sampling program than the relative abundance of sexes in the population (e.g. Ward et al. 2019).

Estimates of spawning biomass also increased as S decreased (Figure 3-7). The estimate of S obtained in the present study was based on a smaller number of females than the 2014 survey. High rates of atresia were present in the ovaries, which suggests that the peak spawning season have been missed during the 2019 survey. Inter-annual variations in estimates of S often reflect limitations of the adult sampling program rather than differences in the spawning rates occurring in the population (e.g. Ward et al. 2019).

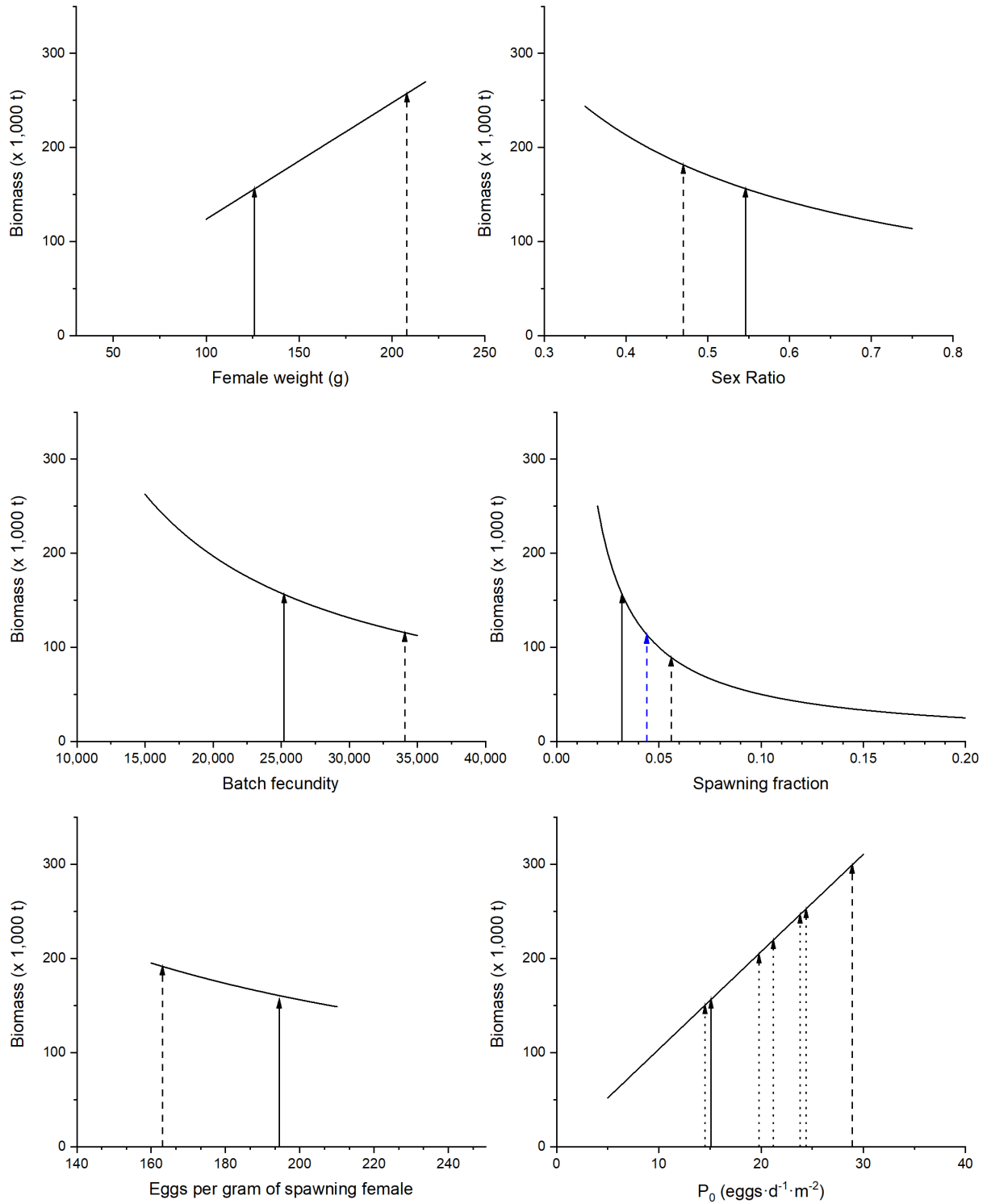
The relationship between P_0 and spawning biomass was linear, and the sensitivity analysis showed the strong influence that the model used to estimate P_0 has on estimates of spawning biomass (Figure 3-7). Other studies have shown that the log-linear model provides estimates of P_0 that are more precise and lower (likely negatively biased) than other models (Ward et al. 2018a). Recent studies suggest that the only other model that is not unduly influenced by a few samples with large numbers of eggs is the GLM NB2 which has a negative binomial error structure (Ward et al. 2019, 2020). Previous studies have shown that all the other models, including non-linear least squares, sometimes produce implausibly high estimates of P_0 (Ward et al. 2018a). In the present study, the negative binomial and quasi GLMs produced high estimates of P_0 .

Figure 3-6. Sensitivity plot showing effects of variability in spawning area (A) on estimates of spawning biomass. Solid arrow: A for 2019; Dashed arrow: A for 2014.



SPAWNING BIOMASS OF JACK MACKEREL EAST IN 2019

Figure 3-6. Sensitivity plots showing effects of variability in adult parameters and egg production on estimates of spawning biomass. Solid black arrows: parameter estimates for 2019; Dashed arrows: parameter estimates for 2014; Dotted arrows: alternate model estimates of P_0 in 2019; Blue arrow: mean S (2014 and 2019).



4 Discussion

4.1 Egg distribution

In response to the large numbers of Jack Mackerel eggs found in Bass Strait during previous surveys (Sexton et al. 2017; Ward et al. 2015, 2018b), the 2019 egg survey was extended west into Bass Strait as far as the line dividing the West and East sub-zones of the Small Pelagic Fishery (AFMA 2009). Jack Mackerel eggs were found throughout much of the additional survey area, confirming that Bass Strait is an important spawning area for Jack Mackerel during summer off south-eastern Australia.

Due to the relatively small numbers of Jack Mackerel eggs collected off central NSW during 2014 (Ward et al 2015), the northern extent of the 2019 survey was reduced from Port Stephens to Jervis Bay, in part to accommodate the extension of the survey into central Bass Strait. However, larger numbers of Jack Mackerel eggs were found in waters off southern NSW in 2019 than in 2014, even though water temperatures in this area were higher in 2019 than 2014. As some eggs were present on the northern-most transect off Jervis Bay in 2019, it is likely that some spawning occurred north of the survey area and that the total spawning area was under-estimated.

SSTs recorded in the 2019 were higher than those recorded in 2014 across all parts of the survey. For example, SSTs of 17–18°C were recorded off south-eastern Tasmania in 2019, whereas SSTs of 14–15°C were recorded in the same location in 2014. Jack Mackerel eggs were also found in higher temperature ranges in 2019 than in 2014. In 2019, egg densities $>10 \text{ egg}\cdot\text{m}^{-2}$ were recorded at sites with SSTs from 17.7 to 23.3°C (mean 20.3°C), whereas in 2014 egg densities $>10 \text{ egg}\cdot\text{m}^{-2}$ occurred at sites with SSTs of 14.5–22.0°C (mean 17.4 °C) (Ward et al. 2015).

4.2 Egg abundance, spawning area and mean daily egg production

Fewer live Jack Mackerel eggs were collected in 2019 (921 eggs) than in 2014 (3,530 eggs), but from over a larger area (i.e. 36,100 km² compared to 23,553 km²). The large area from which eggs were collected in 2019 partly reflects the increase in the survey area from 63,355 km² in 2014 (Ward et al. 2015) to 68,295 km² in 2019, especially the expansion into central Bass Strait where eggs were found at most sites. However, the refinement of the survey area in 2019 compared to 2014 was also an important factor driving the increase in the spawning area (see Sexton et al 2017). The effect of this refinement was reflected in the increase in the percentage of sites where eggs were found (i.e. positive stations) from 40.1% in 2014 to 51.9% in 2019.

It is notable that over the area covered in both surveys, the estimate of spawning area was similar in both years (23,553 km² in 2014 and 23,639 km² in 2019). This is because the additional spawning area recorded off southern NSW in 2019 was offset by the reduced spawning area further south. The similarity in the estimates of spawning area from locations that sampled in both surveys re-enforces the findings of other studies that suggest spawning area is a good proxy for adult abundance of pelagic fishes (e.g. Mangel and Smith 1990; Gaughan et al. 2004; Ward et al. 2019). The similarity of the two estimates of spawning area (in locations sampled in both years) occurred despite the large differences in egg density and egg production recorded in the two surveys. This result emphasises the potential value of spawning area as a proxy for spawning biomass of Jack Mackerel off eastern Australia.

In terms of the effects on the estimate of spawning biomass, the increase in spawning area in 2019 compared to 2014 was largely offset by the reduction in egg production. The low estimate of egg production obtained in 2019 reflects, at least in part, a change in the model(s) used to estimate this parameter. Recent studies have shown that a GLM NB2 which has a negative binomial error distribution is suitable for estimating egg production in species such as Jack Mackerel. Unlike most other models (e.g. exponential model, various GLMs) estimates of egg production obtained using the GLM NB1 are not unduly influenced by a few samples with high egg densities (Ward et al. 2018a, b, 2019). The low estimate of egg production for 2019 also reflects the generally low egg densities observed in 2019 compared to 2014.

4.3 Adult parameters

The low egg densities observed in 2019 match the low estimate of spawning fraction obtained from the adult survey (see below). The low values of these two parameters may have occurred because the 2019 survey was conducted outside the main spawning season, or because 2019 was a year that for some reason (e.g. high SSTs) spawning rates were low. The high rates of atresia observed in ovaries of female Jack Mackerel collected in 2019, especially in the northern parts of the survey area, suggest that the peak of the spawning season may have occurred prior to the start of the survey (SARDI unpublished data).

The mean size of male and female Jack Mackerel collected in 2019 were lower than those collected in 2014. This size difference may reflect differences in the towing speed of the two different trawlers used in the two surveys. The *Western Alliance* which was used to collect samples in 2014, had a higher towing speed (consistently >4 knots) than the *Santa Rocco* (generally <4 knots) that was used in 2019. It is likely that large fast-swimming Jack Mackerel that were caught by the *Western Alliance* in 2014 were able to avoid capture by the *Santa Rocco* in 2019.

As was the case in 2014, only a small number of females with hydrated oocytes (4) were collected in 2019. This may be because trawls of adult samples were collected during the day, before most the ovaries of most females began to hydrate prior to spawning sometime after midnight (SARDI unpublished data). To address this issue, the relationship between female weight (W) and batch fecundity (F) was estimated from females with hydrated oocytes collected in both 2014 and 2019. The relationship obtained was similar to that obtained for *Trachurus trachurus* (e.g. Karlou-Riga and Economidis 1997).

The sensitivity analysis showed that estimates of F and W can vary substantially between years (e.g. 2014 and 2019), and that these differences can have a strong influence on the estimates of spawning biomass. F and W also contribute substantially to the overall uncertainty (e.g. 95% confidence intervals) of estimates of spawning biomass. Recent research on Sardine has shown that despite the high levels of variability in F and W observed between years, the estimates of \hat{F}/W obtained in individual years are remarkably similar (Ward et al. 2019, 2020). The low variability among years in \hat{F}/W means that inter-annual differences in this combined parameter have minimal influence on estimates of spawning biomass. The precision of the combined parameter is also higher than the precision of the two separate parameters. For these reasons, \hat{F}/W rather than F and W estimated separately should be used to calculate spawning biomass as this approach improves the overall precision of the DEPM (Ward et al. 2019, 2020).

4.4 Spawning biomass

This is the second dedicated application of the DEPM to Jack Mackerel in the East sub-area of the SPF. The estimate of spawning biomass for 2019 of 156,292 t (95% CI = 49,120–263,496) is similar to that obtained in 2014 (i.e. 157,805 t; Ward et al. 2015). The estimate of spawning biomass for 2019 is suitable for setting RBCs because it is based on robust and/or conservative estimates of all key parameters.

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Appendices

Appendix 1: Genetic identification of Jack Mackerel eggs

Molecular Identification

A molecular approach of Mitochondrial DNA (mtDNA) extraction, amplification, and sequencing for *Trachurus* spp. developed by Perry (2011) and refined by Neira et al. (2014) was employed to identify eggs of Jack Mackerel (*Trachurus declivis*). 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'ACTTCAGGGTGACCGAAGAA TCAGAA3'), 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 72 eggs were selected for mtDNA analysis; 29 identified morphologically as Jack Mackerel, 35 indeterminable, and 8 similar but morphologically different to Jack Mackerel (Tables A1-1). Indeterminable eggs consisted of early stage eggs whose morphological characteristics were masked by ethanol preservation, making morphological identification problematic.

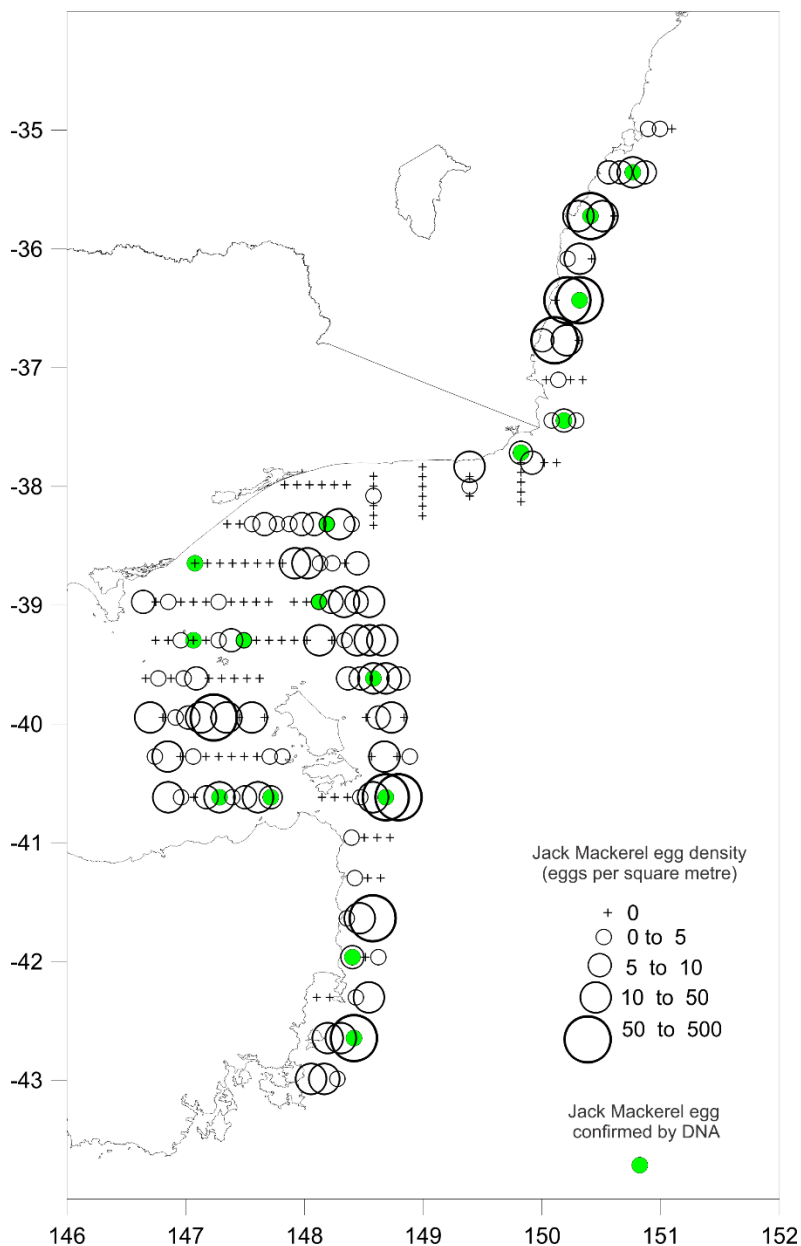
Molecular analyses successfully validated eggs identified as Jack Mackerel using species-specific morphological characters. The analysis further confirmed the presence of Jack Mackerel eggs where morphological identification was problematic in ethanol preserved samples, especially with early stage eggs.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

SPAWNING BIOMASS OF JACK MACKEREL EAST IN 2019

Morphological identification	n tested	Genetic Identification			Notes
		Jack Mackerel	Other	No DNA	
Jack Mackerel	29	28	1		Misidentified egg aligned with: <i>Neosebastes thetidis</i> (n = 1; 99%)
Possible Jack Mackerel: early stage eggs with limited characteristics for ID	35	25	9	1	Uncertain eggs aligned with: <i>Lepidotrigla mulhalli</i> (n = 4; 99%) <i>Lepidotrigla microptera</i> (n = 1; 91%) <i>Parapercis allporti</i> (n = 2; 99%) <i>Nemadactylus bergi</i> (n = 1; 99%) Other (n = 1)
Not Jack Mackerel, but possessing some similar characteristics	8	1	7		Sequences aligned with: <i>Lepidotrigla mulhalli</i> (n = 2; 95%) <i>Thysanophrys cirronasa</i> (n = 1; 99%) <i>Parapercis allporti</i> (n = 3; 99%) <i>Thyrsites atun</i> (n = 1; 99%)

Table A1-1. Molecular identifications of morphologically identified Jack Mackerel and similar eggs collected between south-eastern Tasmania and central New South Wales in summer 2019.

Figure A1–1. Distribution and densities (egg-m⁻²) of live Jack Mackerel eggs between south-eastern Tasmania and central New South Wales during January to February 2019. Green: Jack Mackerel eggs confirmed by genetic analysis.



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Appendix 2: Adult sampling locations for Jack Mackerel East DEPM

Trawl no.	Date	Start Time	Start Latitude	Start Longitude	Duration of trawl (h:m)	Depth (minimum, m)	Sample
1	17/01/19	9:10	-35°27.633	150°45.909	4:00		NA
2	19/01/19	8:45	-37°18.259	150°18.432	1:25	143	1
3	19/01/19	15:23	-37°42.676	150°04.444	0:15	130	NA
4	24/01/19	7:15	-37°46.352	150°05.384	2:09	139	2
5	24/01/19	10:40	-37°39.76	150°07.80	2:35	135	3
6	24/01/19	15:20	-37°59.992	149°54.147	2:10	138	NA
7	24/01/19	18:30	-37°56.93	149°40.36	2:30	121	NA
8	25/01/19	7:20	-38°12.211	149°12.473	2:15		NA
9	25/01/19	10:40	-38°18.287	148°55.11	2:40	133	NA
10	25/01/19	15:05	-38°20.57	148°31.42	2:37	169	4
11	25/01/19	18:45	-38°34.10	148°24.18	2:05	137	5
12	26/01/19	17:40	-38°27.320	148°25.665	3:40	141	6
13	01/02/19	15:40	-40°00.744	148°50.865	4:15	128	7
14	02/02/19	10:00	-40°05.88	148°50.48	2:50	124	NA
15	02/02/19	15:15	-40°09.67	148°51.50	4:05	121	8
16	03/02/19	6:30	-39°48.91	148°45.79	3:15	125	9
17	04/02/19	9:15	-40°57.82	148°42.16	3:45	129	10
18	04/02/19	13:40	-41°08.58	148°36.47	2:20	119	11
19	05/02/19	11:30	-41°36.88	148°34.19	4:10	117	12

Table A2-1: Date, time and locations of trawls off the *FV Santo Rocco* for Jack Mackerel during the 2019 DEPM survey.