Australian Government
Australian Fisheries Management Authority

Government of South Australia
Department of Primary Industries and Regions

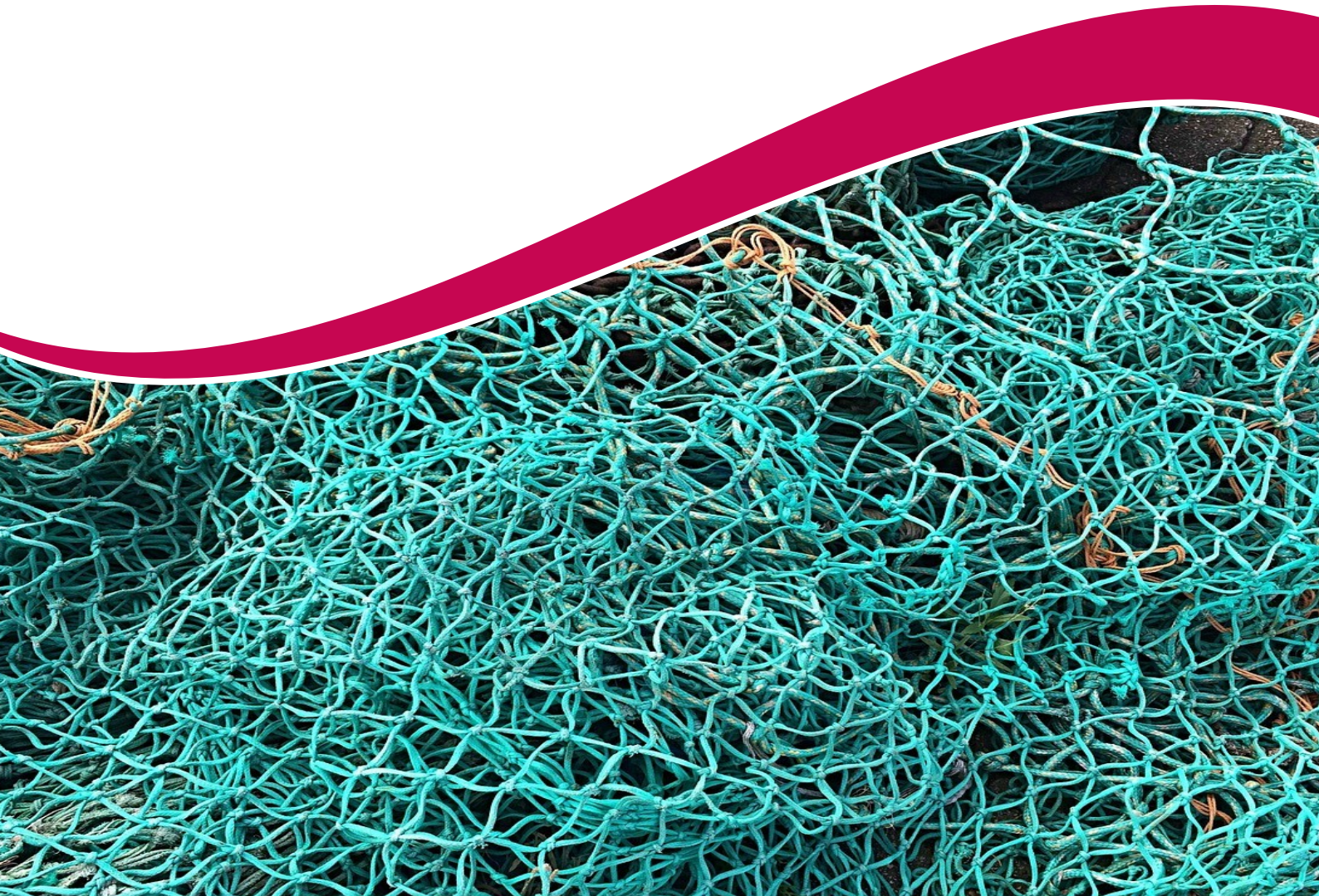
SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Spawning biomass of Redbait (*Emmelichthys nitidus*) in the East sub-area of the Small Pelagic Fishery during October 2020

Report to the Australian Fisheries Management Authority

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

RR2019/0853 June 2022



Contents

Figures

Tables

About this document

This publication may be cited as:
Grammer, G. L., Ward, T. M., Ivey, A. R. and Keane, J. P. (2022). Spawning biomass of Redbait (*Emmelichthys nitidus*) in the East sub-area of the Small Pelagic Fishery during October 2020. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2022/000081-1. SARDI Research Report Series No. 1131. 42pp.

AUTHOR AFFILIATIONS
Gretchen L. Grammer and Alex R. Ivey:
South Australian Research and Development Institute (Aquatic Sciences), 2 Hamra Ave., West Beach, SA 5024, Australia

Timothy M. Ward and John P. Keane:
Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Hobart, Tasmania 7001, Australia

DISCLAIMER

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

Author(s): G.L. Grammer, T.M. Ward, A. R. Ivey and J. Keane

Reviewer(s): J. Earl, R. McGarvey (SARDI) and H. Johnston (AFMA)

Approved by: S. Mayfield
Science Leader – Fisheries

Signed: 

Date: 8 June 2022

Distribution: AFMA, SARDI Aquatic Sciences, Parliamentary Library, State Library and National Library

Circulation: OFFICIAL

ALL ENQUIRIES
South Australian Research and Development Institute - SARDI Aquatic Sciences
2 Hamra Avenue West Beach SA 5024
PO Box 120 Henley Beach SA 5022
P: (08) 8207 5400 **F:** (08) 8207 5415 **E:** pirsa.sardiaquatics@sa.gov.au
W: http://www.pir.sa.gov.au/research

Acknowledgements

This application of the Daily Egg Production Method to Redbait (*Emmelichthys nitidus*) in the East sub-area of the Small Pelagic Fishery was funded by the Australian Fisheries Management Authority (AFMA). We are grateful to the owners, master, crew, and staff of the *FV Saxon Onward* for their professional and cheerful assistance during the egg and adult surveys. We thank Mr Lucas Kas for working diligently in the field to collect the samples and Dr Leonardo Durante and Ms Sharon Drabsch for processing the samples in the laboratory. This report was formally reviewed by Dr Jason Earl and Dr Richard McGarvey and approved for release by Dr Stephen Mayfield (SARDI Aquatic Sciences).

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 Small Pelagic Fishery (SPF). Estimates of *SB* 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 Redbait (*Emmelichthys nitidus*) in the East sub-area of the SPF in 2005 and 2006. Under the SPF Harvest Strategy guidelines, Redbait East would have reverted to Tier 3 in 2022/23 unless the DEPM was applied in 2020 to inform setting of RBCs in 2021. The reduction in the TAC associated with the decline to Tier 3 would have impeded the development of the fishing operation established off southern NSW in 2016/17.

Egg and adult surveys of Redbait East were conducted in continental shelf and inner slope waters between southern Tasmania and central New South Wales in October 2020. Previous studies have shown that this is the likely spawning area of Redbait in the East sub-area (Neira et al. 2008) and suggest the peak spawning season occurs in September-October (Ewing and Lyle 2009).

Objectives

The objectives of this study were to:

1. Estimate egg production, spawning area and adult reproductive parameters of Redbait from egg and adult surveys conducted in the East sub-area of the SPF during October 2020.
2. Estimate the spawning biomass of Redbait in the East sub-area in 2020.

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). Total daily egg production is the product of mean daily egg production (P_0) and spawning area (A). Mean daily fecundity is the product of mean sex ratio (R), mean spawning fraction (S) and mean relative fecundity (F') (Parker 1980, Ward et al. 2021).

To estimate total daily egg production, ichthyoplankton samples were collected from the *FV Saxon Onward* from 231 stations in shelf and inner slope waters between southern Tasmania and central New South Wales from 2–21 October 2020.

Redbait eggs were identified using standard laboratory procedures. Morphological identifications were validated using standard molecular techniques. Spawning area (*A*) was estimated using the Voronoi nearest neighbour method. Mean daily egg production (P_0) and mortality (z) were estimated using two models: (i) the linear version of the exponential egg mortality model with a bias correction factor (log-linear model) and a generalized linear model with a negative binomial error structure (GLM NB1). P_0 was also estimated using a method that assumed a fixed *z* (McGarvey and Kinloch 2001). The estimate of P_0 obtained using the log-linear model was used to estimate *SB*.

Modified demersal trawls for adult Redbait were undertaken from the *FV Saxon Onward* at 14 sites in shelf and inner slope waters between southern Tasmania and southern New South Wales during 2–22 October 2020. Redbait were caught in 13 of the 14 trawls; 12 of the trawls contained mature females. Estimates of *R* and *S* were calculated from samples collected in 2020. Estimates of *F'* were calculated from samples collected in the current survey and from samples collected in 2017 off western Tasmania (see Ward et al. 2021).

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

Results, Discussion and Implications

The total survey area was 57,486 km^2. Live Redbait eggs (n = 2,372) were collected at 79 of the sites (34.2%). The spawning area (*A*) was 19,715 km^2. Mean daily egg production (P_0, 95% CI) was 22.7 (12.3–42.5) eggs.day^{-1}.m^{-2}.

Trawl samples included 1,591 adult fish (781 males, 810 females); 145 fish with hydrated oocytes were collected in 2020, 105 were suitable for estimating batch fecundity. Data from these 105 fish were combined with data from 109 females with hydrated oocytes collected in 2017. A total of 214 hydrated females were used to estimate the relationship between batch fecundity and gonad-free female weight. Best estimates of adult parameters (95% CI) were: spawning fraction (*S*): 0.195 (0.151–0.239); sex ratio (*R*): 0.53 (0.46–0.60); and relative fecundity (*F'*): 82.6 (80.3–84.9) eggs.g^{-1}.

This is the first application of the DEPM to Redbait in the East sub-area of the SPF to cover the entire known spawning area. The estimate of *SB* for 2020 of 52,629 t (95% CI = 13,937–91,321) is similar to the preliminary estimates of *SB* obtained in 2005 and 2006 (i.e. 51,000 and 87,000 t; Neira et al. 2008). The estimate of *SB* for 2020 is suitable for setting RBCs, because it is based on robust and/or conservative estimates of key parameters.

Keywords: Redbait, *Emmelichthys nitidus*, East sub-area, Spawning Biomass, Small Pelagic Fishery, 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*). Species caught in the SPF go into the domestic markets for fishmeal, bait and human consumption (Patterson et al. 2021). 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 2019), and again in 2016/17 when a new fishing operation was established in the East sub-area off southern NSW. Since 2017/18, mid-water trawlers have taken catches of Blue Mackerel, Jack Mackerel and Redbait in the East sub-area of the SPF (Ward and Grammer 2021).

The SPF Harvest Strategy (the Harvest Strategy) and *SPF Management Plan 2009* were implemented in 2008/09. The Harvest Strategy was last revised in 2017 (AFMA 2008, 2009). 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 DEPM was originally developed for stock assessment of the Northern Anchovy, *Engraulis mordax* (Parker 1980, Lasker 1985). The method is applied to determinate or indeterminate spawning fishes that spawn multiple batches of pelagic eggs over an extended spawning season (Parker 1980, Ganias 2013). Globally, the DEPM has been used on more than 20 species of small to medium-sized pelagic fishes (e.g. Stratoudakis et al. 2006, Dimmlich et al. 2009, Neira et al. 2008, Ward and Grammer 2019).

The DEPM relies on the premise that *SB* can be calculated by dividing the mean number of pelagic eggs produced per day throughout the spawning area (i.e. total daily egg production) by the mean number of eggs produced per unit mass of adult fish (i.e. mean daily fecundity, Parker 1980, Lasker 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—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—sex ratio (*R*), spawning fraction (*S*) and mean relative fecundity (number of oocytes per gram of female weight, *F'*)—are estimated from adult samples obtained from 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). These assumptions are not always met in applications of the DEPM (see Bernal et al. 2012, Dickey-Collas et al. 2012, Ward et al. 2021).

Although the DEPM is widely used, a range of logistical and statistical challenges have been encountered. Estimates of *SB* are imprecise (e.g. Stratoudakis et al. 2006; Bernal et al. 2012, Dickey-Collas et al. 2012; Ward et al. 2018, 2021), and considerable uncertainties are associated with the estimation of several parameters (Fletcher et al. 1996, McGarvey and Kinloch 2001, Gaughan et al. 2004).

A recent review of the application of the method to Sardine off South Australia since 1995 showed that inter-annual variations in estimates of several parameters were low in comparison to statistical uncertainty (Ward et al. 2021). This meant that the precision of estimates of *SB* could be increased by estimating some parameters from historical data rather than annually (Ward et al. 2021). Increases in the precision of estimates of *SB* were 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—female weight and batch fecundity—as has been done in most recent applications of the DEPM (e.g. Stratoudakis et al. 2006).

For Sardine, uncertainties in the estimation of *S* mainly related to difficulties obtaining representative samples of the adult population (Ward et al. 2021). 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 spawning biomass (Stratoudakis et al. 2006). In some species, *S* may change with latitude (e.g. Jack Mackerel off south-eastern Australia; Sexton et al. 2017), which adds to the difficulty of estimating *S* reliably.

1.3 Redbait

Redbait (*Emmelichthys nitidus*, Richardson 1845) belongs to the family Emmelichthyidae, which contains three genera and 15 species (Nelson 2006). It is one of two species of emmelichthyid found off southern Australia, the other being the Rubyfish (*Plagiogeneion rubiginosum*) (Last et al. 1983, May and Maxwell 1986, Gomon et al. 2008).

Emmelichthyids are found throughout tropical and temperate waters world-wide. Generally, they are found in schools over continental shelf breaks, seamounts and submarine ridges (Markina and Boldyrev 1980, Meléndez and Céspedes 1986, Parin et al. 1997). They inhabit depths from the surface to >800 m, though are mostly recorded from mid-water trawls in 100–400 m water (Heemstra and Randall 1977, Smith and Heemstra 1986, Mel'nikov and Ivanin 1995). Within Australian waters, the range of Redbait extends from the mid coast of New South Wales to south-west Western Australia, including around Tasmania (Gomon et al. 2008). Ward et al. (2019) found Redbait to be widespread and relatively abundant in outer shelf and inner slope waters between Kangaroo Island and south-western Tasmania.

A large purse-seine fishery for small pelagic fishes developed off Tasmania in the mid-1980s (the basis for the SPF), and the majority of the catch was Jack Mackerel, with relatively small quantities of Redbait (~5%) and Blue Mackerel taken as by-product (Kailola et al. 1993, Pullen 1994, Ward and Grammer 2018). When mid-water trawling was trialled off Tasmania in 2001/02 to target sub-surface schools of Jack Mackerel, a total catch of over 5,000 t of small pelagic fishes was taken with 90% being Redbait (Welsford and Lyle 2003). The catch of Redbait peaked in 2003/04 at more than 7,000 t (e.g. Ward and Grammer 2021).

Effort and catch in the SPF declined through the 2000s, but increased again in 2014/15 when a factory trawler operated in both sub-areas (2014/15 to 2016/17; Ward and

Grammer 2019). Since 2017/18, mid-water trawlers have taken catches of Redbait from the East sub-area. More than 2,000 t of Rebait were taken in both 2019/20 and 2020/21, and in both years, Redbait was ~15% of the total catch taken by mid-water trawling off southern New South Wales (Ward and Grammer 2021; Patterson et al. 2020, Patterson et al. 2021).

The spawning habitat of Redbait has been described from egg, larval and environmental data collected between north-eastern Bass Strait and south-western Tasmania in 2005 and 2006 (Neira et al. 2008) and between eastern Kangaroo Island and south-western Tasmania in 2017 (Ward et al. 2019). Most spawning occurs along the continental shelf break and inner slope where water depths are approximately 200m and water temperatures are between 12 and 15.5°C (Neira et al. 2008, Ward et al. 2019).

Annual trends in Gonadosomatic Index (GSI) and macroscopic gonad stages indicated that peak spawning season of Redbait occurs off eastern Tasmania during September and October and off south-western Tasmania during October (Ewing and Lyle 2009).

Redbait is an asynchronous batch spawner with indeterminate fecundity and buoyant, pelagic eggs that make it suitable for the DEPM (e.g. Lasker 1985, Neira et al. 2008, Ward et al. 2019). Redbait eggs hatch in about 2.5–4.5 days after fertilisation depending on temperature (e.g. 13.1–16.5°C; Neira et al. 2008). Newly hatched yolk sac larvae range from 1.9–3.3 mm TL. Little is known about the early life history of Redbait. The distribution of eggs and larvae off south-eastern Australia have been described by Neira et al. (2008) and Ward et al. (2019). The DEPM was last applied to Redbait in the East sub-area of the SPF in 2005 and 2006 (Neira et al. 2008; Ewing and Lyle 2009; Neira and Lyle 2011).

1.4 Need

Under the Harvest Strategy guidelines, Redbait East would have reverted to Tier 3 in 2022/23 unless an application of the DEPM was completed in 2020 to inform the setting of RBCs in 2021. The reduction in the TAC associated with the decline to Tier 3 would have impeded the development of the fishing operation established off southern NSW in 2016/17.

1.5 Objectives

1. Estimate egg production, spawning area and adult reproductive parameters of Redbait from egg and adult surveys conducted in the East sub-area of the SPF during October 2020.
2. Estimate the *SB* of Redbait in the East sub-area in 2020.

2 Methods

2.1 Total Daily Egg Production

2.1.1 Ichthyoplankton surveys

During October of 2020, ichthyoplankton samples were collected from the *FV Saxon Onward* at 231 sites on 44 transects in shelf waters between south-eastern Tasmania and central New South Wales (Figure 2–1, Appendix 2). The survey was undertaken from 2-21 October 2020. An additional sample was taken at every second site for genetic validation of Redbait eggs (n = 115). There were interruptions during the survey due to a mechanical failure (3-6 October 2020) and weather delays (7-10 October and 14-16 October 2020). The survey was restarted from the southern-most transect after the weather delay over 14-16 October and continued north until the survey was finished on 21 October 2020 (Figure 2–1).

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 and depth were recorded using a Sensus Ultra™ data logger 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. Where flowmeter data was unavailable, the maximum depth recorded by the depth logger was used.

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 contents of 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 Redbait were identified using the morphological features in published descriptions for the species (Neira et al. 2008). Identifications of Redbait eggs preserved in ethanol were validated directly using the molecular techniques developed by Perry (2011) and refined by Neira et al. (2015). These results were used to confirm the morphological identification of the formalin preserved samples. This validation was done because

Redbait eggs have similar characteristics to other common species, especially Barracouta (*Thyrsites atun*) (See Appendix 1).

All eggs were staged following Ward et al. (2018, 2019) (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.

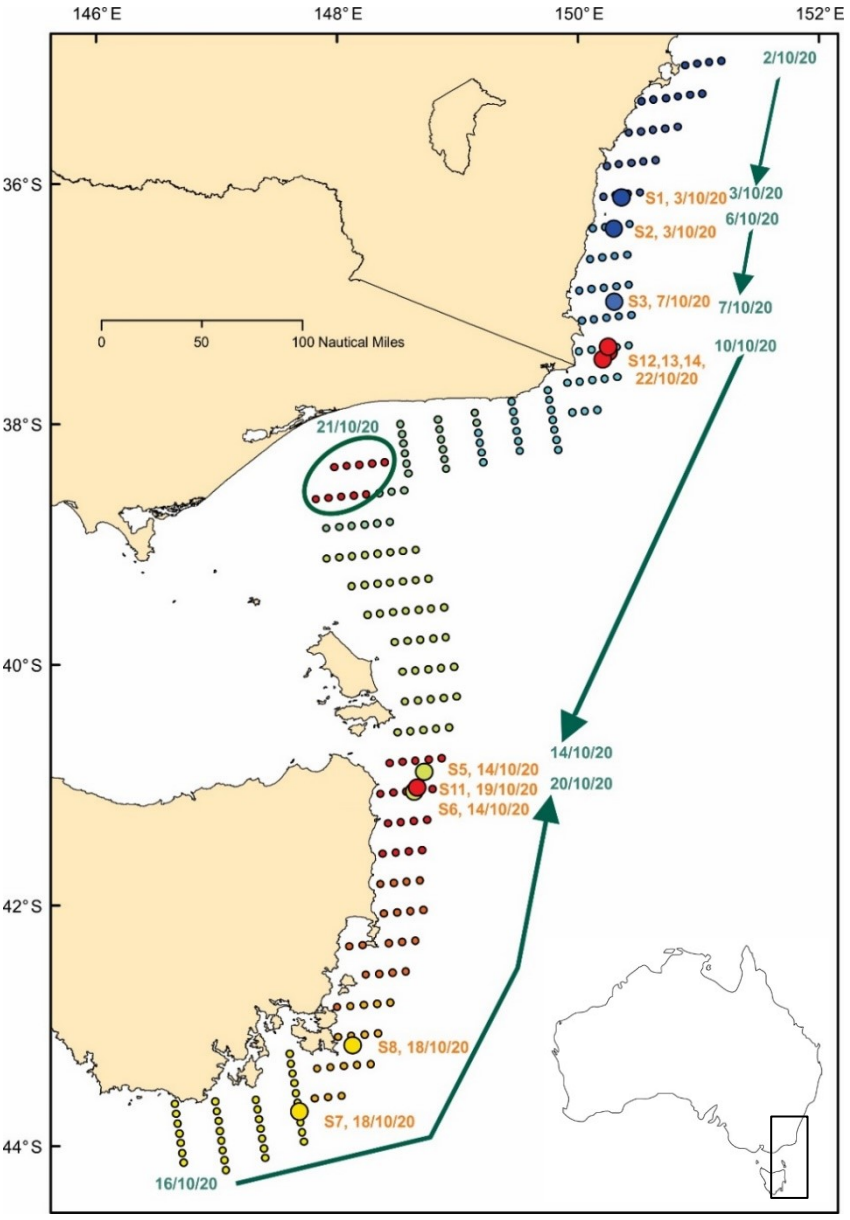


Figure 2–1. Area off south-eastern Tasmania to central New South Wales where the Daily Egg Production Method was applied to Redbait in October 2020. Locations shown are the egg sampling sites (small circles) and adult trawl sites (large circles). Colour ramp from blue to red indicates the chronological order from 2/10/20 to 22/10/20. Green indicates truncated legs of sampling with arrows indicates general direction of travel.

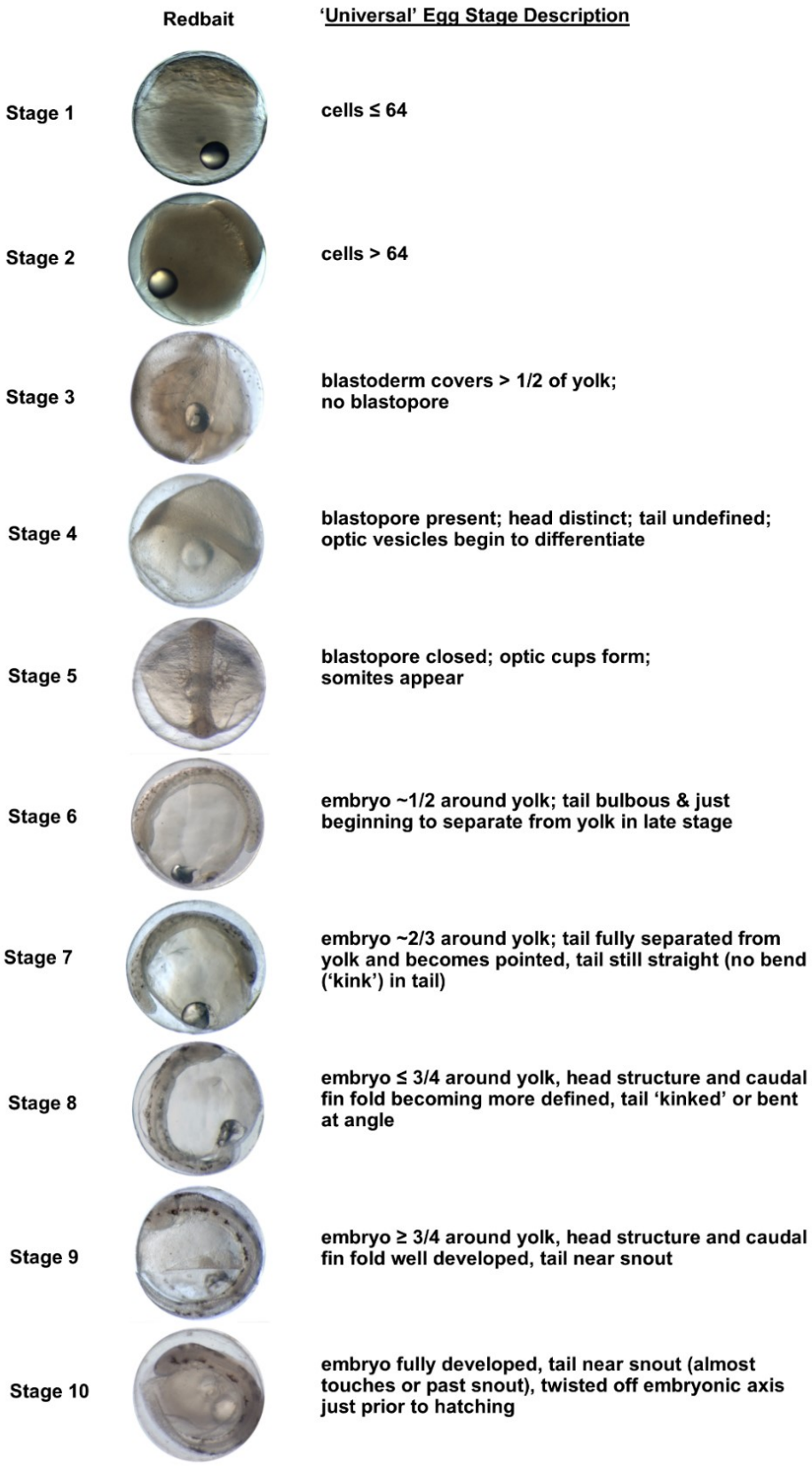


Figure 2–2. Egg stages of Redbait delineated by Ward et al. (2019) using the 'universal' egg stages of Ward et al. (2018).

2.1.4 Egg ageing, treatment of zero count egg samples and egg density

Based on water temperature data, egg samples were allocated to one of three temperature bands that covered the range of temperatures sampled during the survey (10–14°C, 14–18°C and 18–22°C). Published egg development rates reported for Redbait by Neira et al. (2008) were used to assign a mean age to each egg (Ward et al. 2019).

After the eggs were assigned an age, eggs in each sample were grouped into daily cohorts. This was done because a sample usually included eggs spawned on more than one night. The total number of eggs in each daily cohort was calculated by summing the number of eggs of each stage assigned to a spawning day (i.e. day 0, day 1, day 2, day 3, day 4). The age of a daily cohort was calculated from the average age of each stage within the daily cohort, weighted by the number of eggs in each stage.

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 (Ward et al. 2018). Samples with no eggs were excluded from the analyses and not considered part of the spawning area.

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

$$P_t = \frac{C.D}{V} \qquad \text{Equation 2}$$

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).

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 in the statistical program R (R Core Team 2021, 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 Redbait eggs were present in the plankton samples.

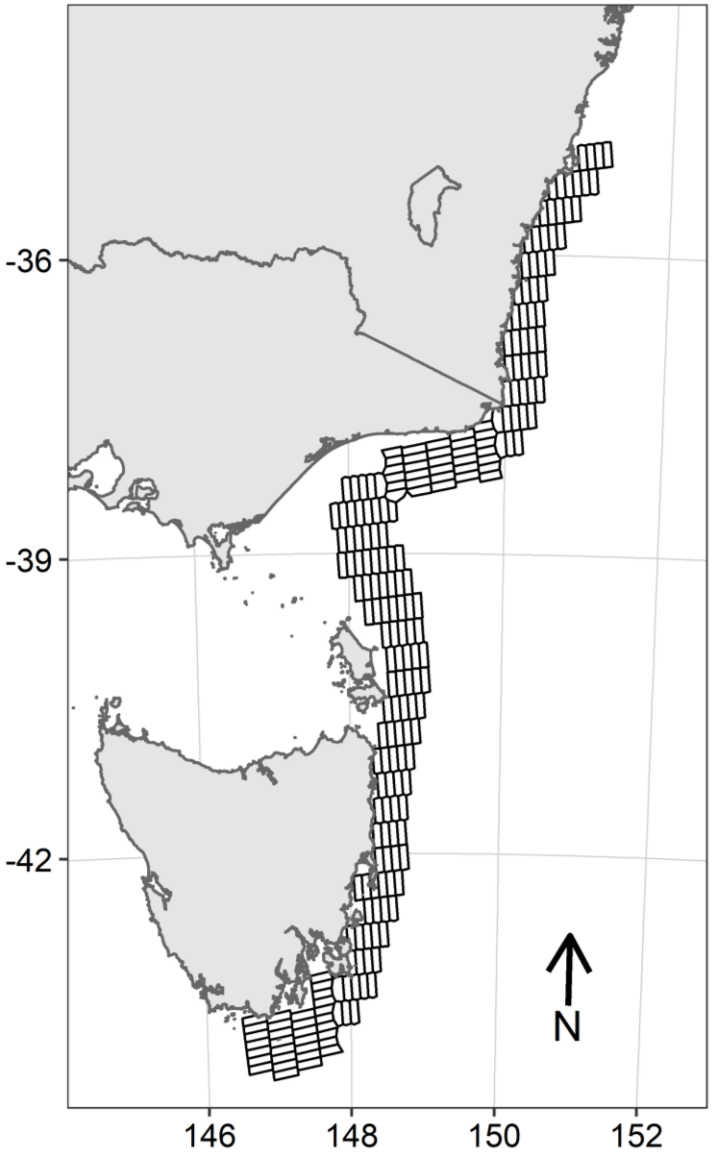


Figure 2–3. Polygons generated using the Voronoi natural neighbour method and used to estimate the spawning area of Redbait along eastern Tasmania to southern New South Wales in 2020.

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

The underlying model used to calculate P_0 was the exponential egg mortality model. A linear version of this model was fitted to estimates of egg age and density for each daily cohort at each site, because these data were not normally distributed and approximated a log-normal distribution (Picquelle and Stauffer 1985; Equation 3):

$$\ln(P_b) = \ln(P_{i,t} + 1) - Z_t \qquad \text{Equation 3}$$

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 Z is the instantaneous rate of egg mortality.

Because estimates of P_b from the log-linear version of the exponential mortality model have a negative bias, a bias correction factor was applied (Equation 4). This equation is hereafter referred to as the 'log-linear model'.

$$P_0 = e^{\left(\ln P_b + {\sigma^2}/{2}\right)} - 1 \qquad \text{Equation 4}$$

where, σ^2 is the variance of the estimate of biased mean daily egg production (P_b).

A generalised linear model (GLM) with a negative binomial error structure (NB1), was also used to estimate P_0 (Equation 5). The negative binomial error structure is considered suitable for over-dispersed data, such as egg density by age (e.g. Ward et al. 2011, 2018, 2021).

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

where E [P_0] is the expected value of P_0, g^{-1} is the inverse-link function, z_t 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 6).

$$\sigma = \mu*(1+ \mu +\varphi) \qquad \text{Equation 6}$$

where μ is the model estimate, σ is the model variance and φ is the overdispersion parameter. The GLM used a log-link function (Wood 2006) and was fit using the glmmTMB 'R' package (Brooks et al. 2017).

Young eggs are commonly under-represented in plankton samples, which may be due to sampling bias (e.g. Ward et al. 2018). To account for this under-representation, young eggs are often excluded from modelling to estimate P_0 (Stratoudakis et al. 2006, Bernal et al. 2012, Dickey-Collas et al. 2012, Ward et al. 2018). Following the methods used by Ward et al. (2019) for Redbait, all eggs aged ≤24 hours were excluded ('Day 1 removed') from calculations of P_0 to account for site specific differences in temperature and developmental rates that stretch over four days in cooler temperatures.

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. Two different datasets were used: 'Day 1 removed' and 'all Day cohorts included'. An assumed z of 0.3 day^{-1}·m^{-2} was applied to the mean total egg density for each dataset (Eq. 2, Eq. 4; McGarvey and Kinloch 2001). A mortality rate of 0.3 day^{-1}·m^{-2} is a standard value reported for similar pelagic species (Bunn et al. 2000, Ward

et al. 2019, Ward et al. 2021) and was comparable to the values obtained using the log-linear model and GLM NB1 in the current study.

2.2 Mean Daily Fecundity

2.2.1 Adult Sampling

Adult Redbait were sampled using a mid-water trawl net deployed from the *FV Saxon Onward* in shelf and inner slope waters between eastern Tasmania and southern New South Wales from 3–22 October (Figure 2–1). Samples of Redbait collected in trawls were dissected and sexed. The ovaries of mature females were removed, labelled and fixed in a 10% buffered formaldehyde seawater solution. Females, with ovaries removed, and mature males were labelled and frozen.

2.2.2 Female weight and Male weight

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

2.2.3 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:

$$R = \overline{\left[\frac{\overline{R_i}\, n_i}{N}\right]} \qquad \text{Equation 7}$$

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:

$$\overline{R_i} = \frac{F_i}{F_i + M_i} \qquad \text{Equation 8}$$

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

2.2.4 Relative Fecundity (*F'*)

Batch fecundity (*F*) 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 sub-sections counted. 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 linear relationship between female weight (ovaries

removed) and batch fecundity was estimated from females with hydrated oocytes using data from both the 2017 Redbait DEPM survey (Ward et al. 2019) and the current survey (see Ward et al. 2021).

A predicted batch fecundity ($\hat{F}$) was estimated for each mature fish collected that didn't have hydrated oocytes. This was done following the methods of Ward et al. (2021) where the linear relationship between gonad-free weight and *F* for all hydrated females (described above) was used as the predictor. Weight-dependent variance terms were incorporated to ensure that the variance for estimates of $\hat{F}$ was not under-estimated for larger fish (Ward et al. 2021). Predicted fecundities were estimated using the 'truncnorm' package (Mersmann et al. 2018) which prevented estimates of $\hat{F}$ falling below zero. Females <45 g were excluded (i.e. below the approximate size of 50% maturity; Neira et al. 2008, Ward et al. 2019).

Relative fecundity—the number of eggs produced per gram of total female weight—was calculated for each female by dividing batch fecundity (*F* or $\hat{F}$) by the total weight of the female (*W*). Then relative fecundity (*F'*) of the population was calculated:

$$F' = \bar{F}/\bar{W} \qquad \text{Equation 9}$$

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

The mean and variance of *F'* was calculated using a ratio estimator (Rice 1995)

$$Var(F') = \frac{1}{n_{fish}} \cdot \frac{1}{\bar{W}^2} \cdot \{(F')^2 \cdot \sigma_W^2 + \sigma_F^2 - 2 \cdot F' \cdot cov(F,W)\} \qquad \text{Equation 10}$$

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 aged according to the criteria developed by Hunter and Goldberg (1980) and Hunter and Macewicz (1985) and refined by Ganias (2012).

The spawning fraction of each sample was estimated as the mean proportion of females with hydrated oocytes (*d*0) (spawning will occur that night), <24 hour POFs (*d*1) (assumed

to have spawned on the night prior to capture), 24-48 hour POFs (*d*2) (assumed to have spawned two nights prior) and 48-72 hour POFs (*d*3) (assumed to have spawned three nights prior.

The mean spawning fraction of the population was then calculated from the average of sample means weighted by proportional sample size.

$$S = \overline{\left[\frac{\overline{S_i}\, n_i}{N}\right]} \qquad \text{Equation 11}$$

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:

$$\overline{S_i} = \frac{d0+d1+d2+d3}{4\, n_i} \qquad \text{Equation 12}$$

where, *d*0, *d*1, *d*2 and *d3* POFs are the number of mature females with hydrated oocytes and POFs aged <72 hours in each sample and n_i is the total number of females within a sample.

2.3 Spawning Biomass

Spawning biomass (*SB*) for Redbait was calculated according to Equation 1 using the estimate of P_0 obtained from the log-linear model and estimates of *A, R, F'* and *S* obtained during this study. The batch fecundity relationship used in the calculation of *F'* was obtained from adult samples collected in both 2017 and during the current study.

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 data collected during this study. A ratio estimator was used calculate the coefficients of variation (CVs) for *S*, *R*, and *F*' (see Rice 1995, Equation 10). 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 2021).

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. Information from previous studies was used to inform the range of values tested for each parameter.

Sensitivity analyses for the adult parameters used values from the surveys undertaken for Redbait along eastern Tasmania in 2005 and 2006 (Neira and Lyle 2011) and along western Tasmania in 2017 (Ward et al. 2019). Also included were 95% CIs from the current survey for F' and values of S that contained samples with a majority of atretic ovaries or did not include fish with hydrated oocytes (POFs only).

The minimum and maximum values used for A were ± 25% of the estimate from the current survey. Values of P_0 resulted from egg production models and mean egg density in the current survey and the estimate of P_0 from the 2017 survey (Ward et al. 2019).

3 Results

3.1 Total Daily Egg Production

3.1.1 Egg distribution and abundance

A total of 2,372 live Redbait eggs were collected at 79 of 231 (34.2%) sites on 44 transects between southern Tasmania and Jervis Bay, New South Wales (Figure 3-1). Eggs were found throughout the latitudinal extent of the survey, in areas of SST ranging from 10.5°C to 15.9°. These findings were confirmed by molecular confirmation (see Appendix 1) of Redbait eggs in ethanol preserved samples taken during the survey.

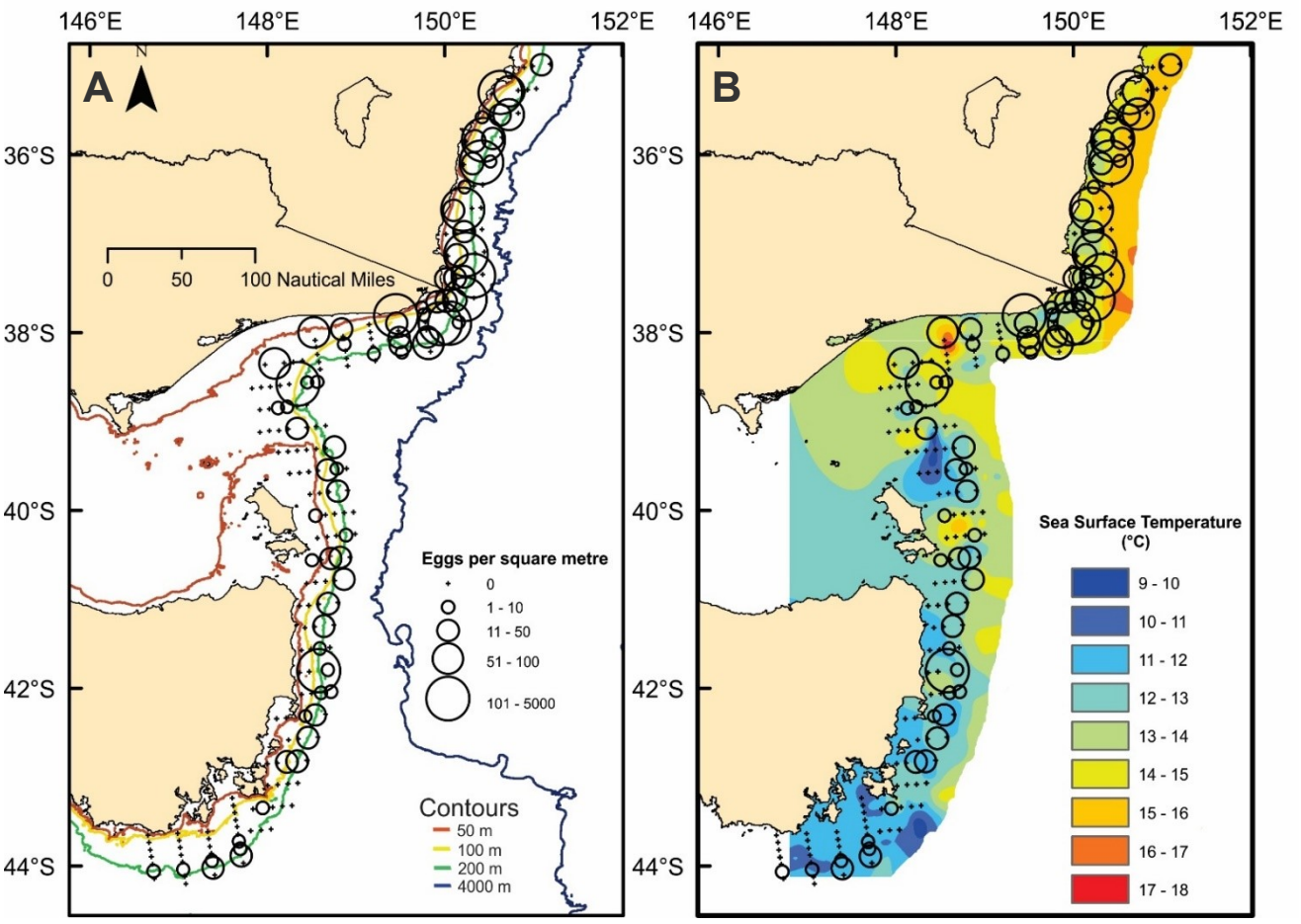


Figure 3–1. Distribution and densities (egg·m^{-2}) of live Redbait eggs between southern Tasmania and central New South Wales during October 2020. Densities are overlaid on bathymetry (left) sea surface temperatures (SST; °C, right) measured during the survey.

3.1.2 Egg density (P_t)

Egg densities were highest in mid to outer shelf waters between northern Bass Strait and southern New South Wales (NSW, Figure 3–1). Higher densities of eggs (P_t >10 eggs·m^{-2}) were collected where the depth was 48–950 m (mean: 177 m) and sea surface temperatures were 11.3–15.9°C (mean: 14.2°C). The highest four egg densities (>200 eggs.m^{-2}) were all off southern NSW (15.1–15.6°C SST).

3.1.3 Spawning area (A)

The estimated spawning area for Redbait was 19,715 km^2, comprising 34% of the total area sampled of 57,486 km^2.

3.1.4 Mean daily egg production (P_0)

The estimate of P_0 obtained using the log-linear model (Equation 4) was 22.7 (12.3–42.5) eggs·day^{-1}·m^{-2} and z was 0.338 day^{-1} (Table 3–1; Figures 3–2, 3–3). P_0 from the negative binomial GLM NB1 (Equation 5) was 43.7 (20.1–85.2) eggs·day^{-1}·m^{-2} and z was 0.374 day^{-1}. The method of McGarvey and Kinloch (2001), with z set at 0.3 and including all Day cohorts, produced an estimate of P_0 of 27.6 eggs·day^{-1}·m^{-2} (Table 3-1). Mean total egg density was 64.3 eggs·m^{-2} when all Day cohorts were included and 53.8 eggs·m^{-2} when Day 1 eggs were excluded (Day 1 removed).

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 Redbait in October 2020 generated by the two egg production models fits and by applying a fixed mortality of 0.3 to the mean total egg density.

Egg Production Model	P_0 eggs·day^{-1}·m^{-2} (95% CI)	z
Linear version of exponential model, corrected	22.7 (12.3–42.5)	0.338
GLM, Negative Binomial (NB1), log link	43.7 (20.1–85.2)	0.374
z w/ mean egg density (McGarvey and Kinloch 2001)	27.6 (-) all Day cohorts included 27.2 (-) Day 1 removed	0.300

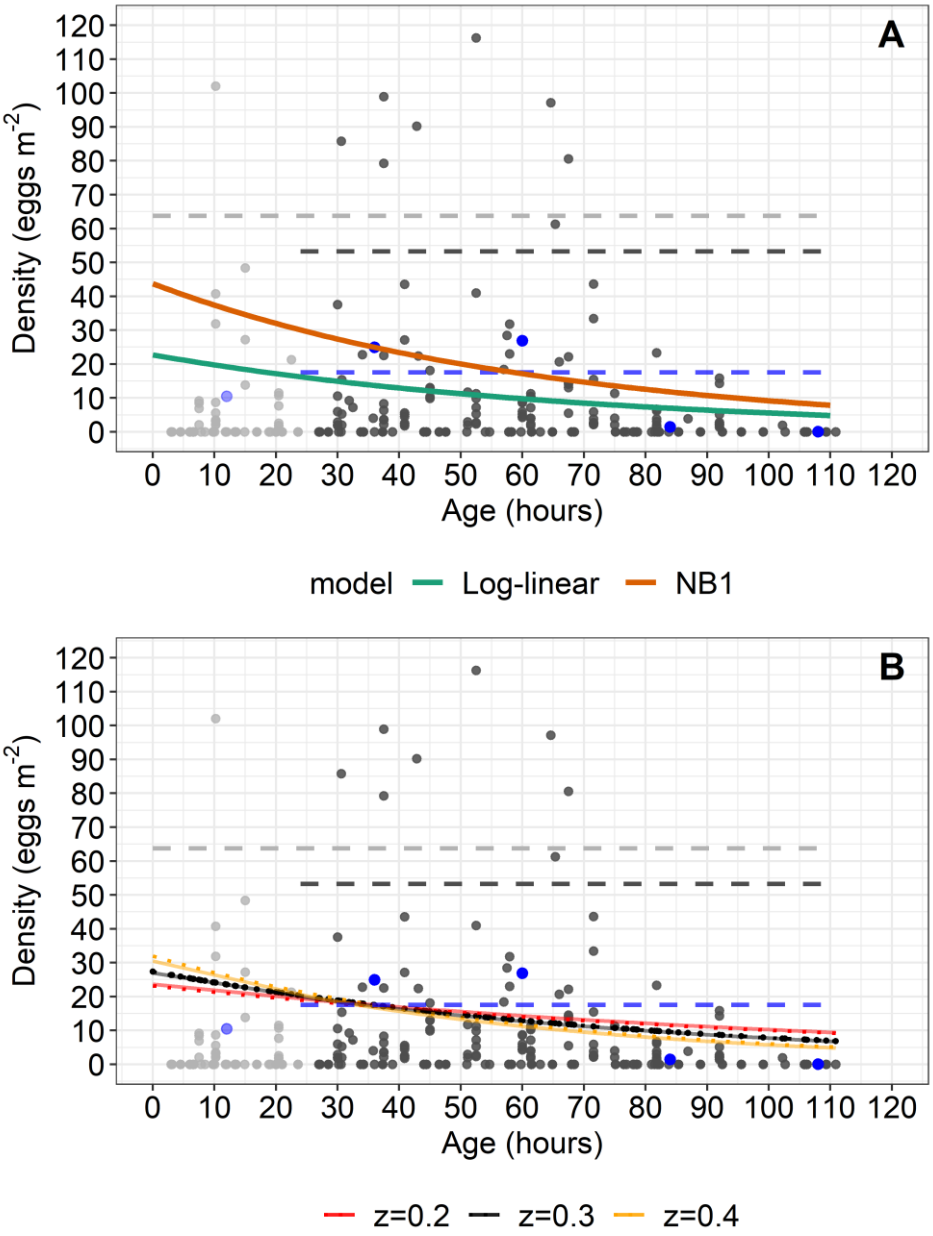


Figure 3–2. (A) Models fitted to egg densities (eggs.m^{-2}) and egg age (hours) of Redbait cohorts in October 2020. NB1: GLM with negative binomial error structure. (B) Set mortality (z = 0.2, 0.3 and 0.4) using the method of McGarvey and Kinloch (2001). Dashed light grey line: mean total egg density for all day cohorts; dashed dark grey line: mean total egg density with Day 1 eggs removed; dashed blue line: mean daily egg density for survey with Day 1 eggs removed; blue dots: mean egg density per day. Light grey dots are Day 1 eggs that were removed when fitting the log-linear model and GLM NB1.

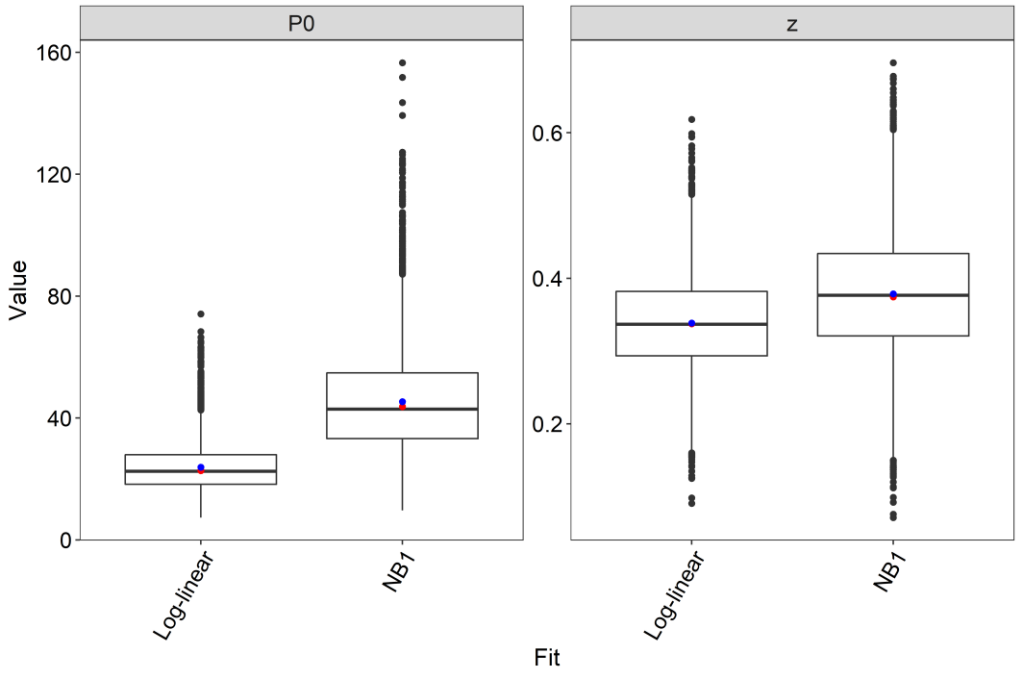


Figure 3–3. Mean daily egg production (P_0, eggs.day^{-1}.m^{-2}) and instantaneous daily mortality (z, day^{-1}) for Redbait from the two egg production models for data collected in October 2020. Horizontal black line is the median and box is the quartiles. Blue dot: bootstrapped mean; solid line: 99% Confidence Interval, black dots: outliers. NB1: GLM with negative binomial error structure.

3.2 Mean Daily Fecundity

Redbait were caught in 13 of the 14 trawls undertaken from the *FV Saxon Onward*, and 12 of the trawls contained mature females. A total of 1,591 mature Redbait were sampled across the 12 sites (Figure 2-1, Appendix 2).

3.2.1 Female weight and male weight

The mean weight of mature female Redbait in samples collected in 2020 ranged from 44.3 to 108.0 g, and the mean weight of mature males ranged from 42.2 to 99.9 g (Table 3-2). The weighted mean weight of mature females in 2020 was 80.8 g and mature males was 74.5g (Table 3-2).

3.2.2 Sex ratio (*R*)

The mean sex ratio by weight (*R*, 95% CI) calculated from all fish collected in 2020 was 0.53 (0.46–0.60) (Tables 3-2, 3-4). The total numbers of females and males collected were 810 (50.9% of fish) and 781 (49.1%), respectively. Estimates of *R* for individual samples ranged from 0.25 and 0.76 (Table 3-2).

Table 3-2. Number of males and females of Redbait in samples and estimates of mean female weight per sample and sex ratio (*R*, proportion of females by weight). Values in last row are sums (*) and weighted means (#).

Trawl	Male		Female		*R*
	n	Average weight (g)	n	Average weight (*W*, g)	
1	126	68.3	99	75.2	0.46
2	30	94.7	106	85.3	0.76
3	74	72.4	100	71.2	0.57
4	5	99.9	5	104.6	0.51
5	27	82.5	24	102.2	0.52
6	41	97.8	41	108.0	0.52
7	110	42.2	35	44.3	0.25
8	15	74.8	17	69.2	0.51
11	73	70.5	95	74.0	0.58
12	84	83.3	88	84.6	0.52
13	62	79.9	100	83.2	0.63
14	134	88.1	100	89.3	0.43
Total	**781***	**74.5#**	**810***	**80.8#**	**0.53#**

3.2.3 Relative Fecundity (*F'*)

In 2020, 145 females with hydrated oocytes were collected; of these, 105 females were used to estimate batch fecundity. Data from these fish were combined with data from hydrated females (n = 109) collected during the survey in 2017 (Ward et al. 2019) to calculate the batch fecundity relationship (Figure 3-5). A total of 214 hydrated females were included in the analysis. The fecundity-weight relationship estimated from these samples was: Batch Fecundity = 118.4 × Gonad Free Female Weight – 2441.6 (R^2 = 0.38, p < 0.001).

The estimate of relative fecundity (*F'*; eggs per gram of female weight) for 2020 was 82.6 (80.3–84.9) eggs·g^{-1} (Figure 3-6; Table 3-4). Relative fecundity (*F'*) is nearly constant across the range of weight (*W*) of mature females collected in 2020 (Figure 3-6B).

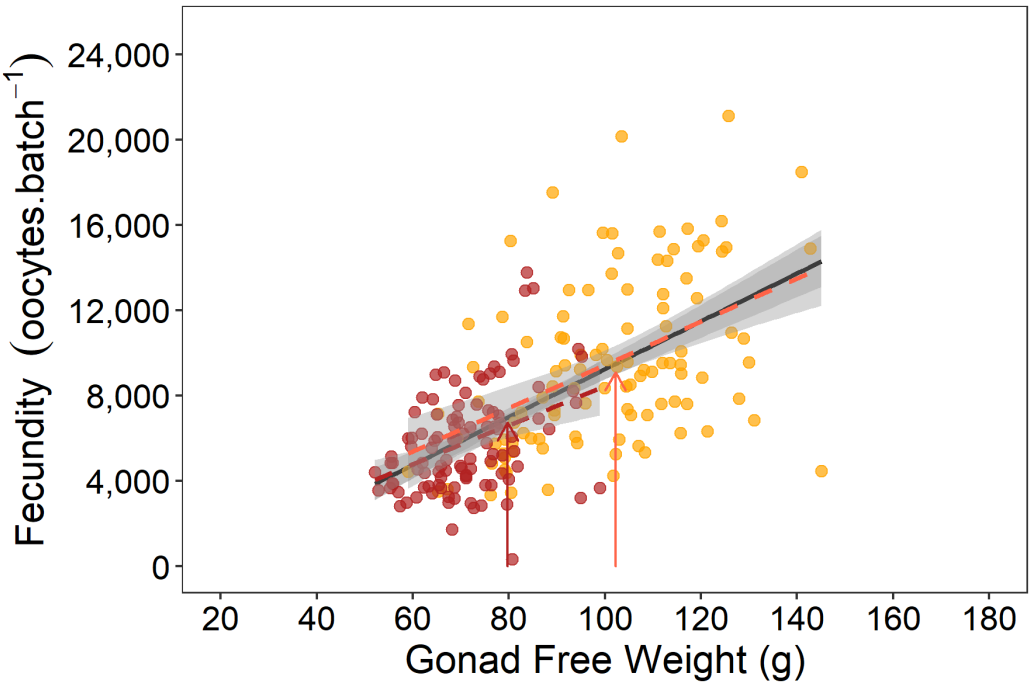


Figure 3-5. Relationship between gonad-free weight and batch fecundity (*F*, oocytes·batch^{-1}) for all hydrated Redbait collected in 2017 (orange), 2020 (red) and combined relationship (black). Batch Fecundity = 118.4 * Gonad Free Weight – 2441.6, shading = 95% CI. Arrows: mean gonad free weight for corresponding year.

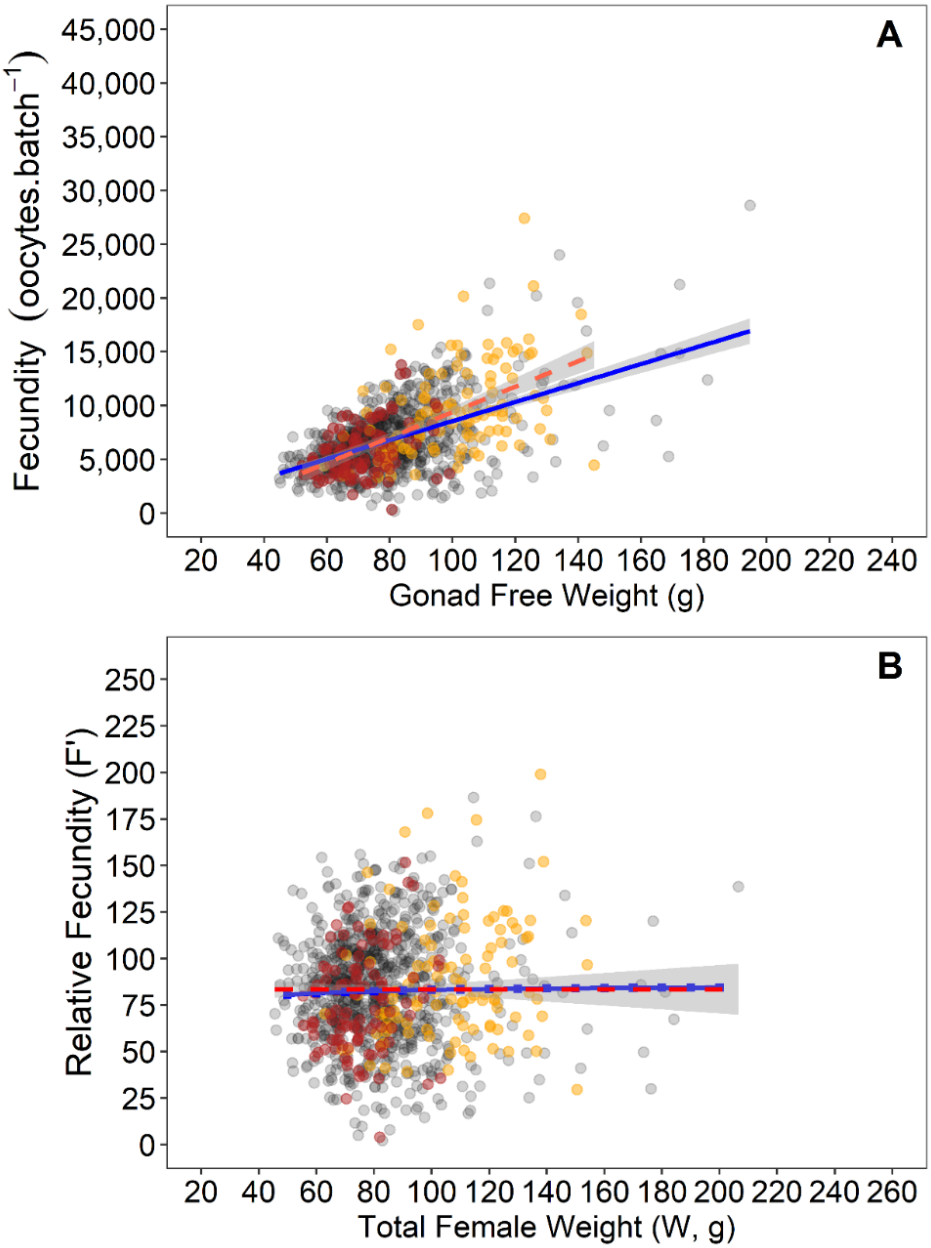


Figure 3-6. A) Batch fecundity and gonad-free weight for all Redbait use in estimate of relative fecundity (*F'*, egg.g^{-1}) in 2020. Red and orange circles: batch fecundity (*F*) from fish with hydrated oocytes collected in 2017 and 2020; grey circles: predicted fecundity ($\hat{F}$) for mature fish in 2020 with oocytes that were not hydrated. The regression slope for all mature females (blue) is shown in comparison with the batch fecundity relationship estimated from only hydrated females (dashed orange). B) Relationship between relative fecundity (*F'*) 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 and orange: 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.

3.2.4 Spawning fraction (*S*)

In 2020, a total of 810 ovaries were examined to estimate spawning fraction (*S*); 145 had hydrated oocytes, 114 had day-0 POFs, 142 had day-1 POFs and 119 day-2 POFs (Table 3-3). Samples 12–14 had high rates of atresia (42-57%; Figure 3-7; Table 3-3) and were removed from the final calculation of *S*. These samples were collected at the end of the survey (Figures 2-1, 3-7); high rates of atresia suggest that the spawning season was ending (Hunter and Macewicz 1985).

The spawning fraction (*S*, 95% CI) for 2020, calculated by removing the samples with high levels of atresia, was 0.195 (0.151–0.239); (Samples 12–14; Tables 3-3, 3-4). *S* calculated from all mature females collected in 2020 was 0.160 (0.114–0.207) (Table 3-3).

Table 3-3. Number of female Redbait per sample and estimates of spawning fraction (*S*) for fish collected between south-eastern Tasmania and central New South Wales in October 2020. Values in bottom rows are sums (*) and weighted mean (#). H: Hydrated; POF: Post ovulatory follicles.

Trawl	n	Hydrated	POF 0	POF 1	POF 2	Atresia	S H	S POF	S H + POF
1	99	66	9	14	9	1	0.67	0.11	0.25
2	106	10	25	35	31	4	0.09	0.28	0.23
3	100	24	22	29	17	7	0.24	0.23	0.23
4	5		2	1		3	0.00	0.20	0.15
5	24	1	5	4	3	9	0.04	0.17	0.14
6	41	3	8	9	10	12	0.07	0.22	0.18
7	35	1				4	0.03	0.00	0.01
8	17	2		1	1	3	0.12	0.04	0.06
11	95	12	18	18	16	14	0.12	0.18	0.17
12^	88	3	5	6	14	37	0.03	0.09	0.08
13^	100	12	10	9	9	57	0.12	0.09	0.10
14^	100	11	10	16	9	50	0.11	0.12	0.12
Total: all	810*	145*	114*	142*	119*		0.18#	0.15#	**0.16#**
Total: minus 12–14	517*	119*	87*	110*	87*		0.23#	0.18#	**0.19#**

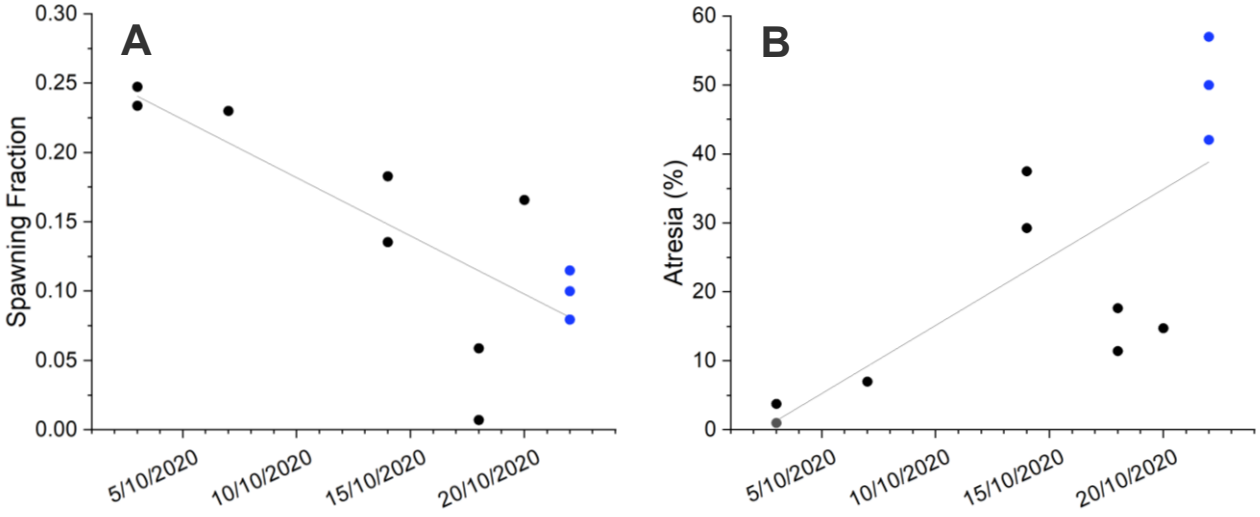


Figure 3-7. A) Relationship between *S* and date of capture; B) Relationship between % atresia found in each sample used to estimate *S* and date of capture. Blue: Samples 12, 13 and 14 collected late in the survey and had low estimates of S and high rates of atresia, which suggested that they may have been collected after the peak spawning season.

3.3 Spawning Biomass

The estimate of *SB* for Redbait East was 52,629 t (95% CI = 13,937–91,321). This value was calculated using the log-linear model to estimate P_0 and values of adult parameters estimated from the current survey (Table 3-4). The value of *S* for 2020 was calculated after removing samples with high rates of atresia (i.e. Samples 12-14).

Table 3-4. Estimates of adult parameters and bootstrapped 95% confidence intervals for Redbait sampled between southern Tasmania and central New South Wales during October 2020.

Parameter	Estimate	95% CI
Egg Production ($P_{0,}$ eggs·day^{-1}·m^{-2})	22.7	12.3–42.5
Spawning Area (*A*, km^2)	19,715	-
Sex Ratio (*R*)	0.53	0.46–0.60
Spawning Fraction (*S*)	0.195	0.151–0.239
Relative Fecundity (*F'*, eggs·g^{-1})	82.6	80.3–84.9

3.4 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 2020 (Table 3-4; Figure 3-8). The relationship between P_0 and SB was linear, and the sensitivity analysis demonstrates the strong influence that the model used to estimate P_0 has on estimates of SB (Figure 3-8).

SB increased linearly with A (Figure 3-8). The 2020 survey covered a greater total area than those conducted in 2005 and 2006 (Neira et al. 2008) and provided had a larger estimate of A. This spawning area included waters off southern NSW not included in previous surveys.

Estimates of SB increased as R decreased (Figure 3-8). The differences in R between the 2017 (0.43) and 2020 surveys are most likely due to the limitations of the previous adult sampling program rather than the relative abundance of sexes in the populations (e.g. Ward et al. 2021). The value of R (0.34) obtained from the trawl survey conducted by Neira et al. (2008) was biased towards males and produced a higher estimate of SB.

Variations in F' had a relatively small effect on SB (Figure 3-8). The estimate of F' for 2020 (82.6 eggs.g^{-1}) is similar to F' estimated for 2017 using the same methods and batch fecundity relationship as applied in the current study (86.3 eggs.g^{-1}; Figure 3-8). Fish collected during the current survey were smaller than those collected off western Tasmania in 2017 (Ward et al. 2019). Smaller fish were also collected off eastern Tasmania in 2005 and 2006 (Neira and Lyle 2011).

Estimates of SB increased as S decreased (Figure 3-8). The estimate of S obtained in this study (i.e. excluding samples with high rates of atresia) is similar to the estimate of S obtained during the 2017 survey (0.21; Ward et al. 2019). The value of S calculated from all samples (0.160, Figure 3-8, Table 3-3) was lower than the value obtained by excluding samples with high rates of atresia, and thus, provided a higher estimate of SB (Figure 3-8). The methods used by Neira et al. (2008) to estimate S were not clearly defined; therefore, that estimate of S (0.32) was not included in the sensitivity analysis.

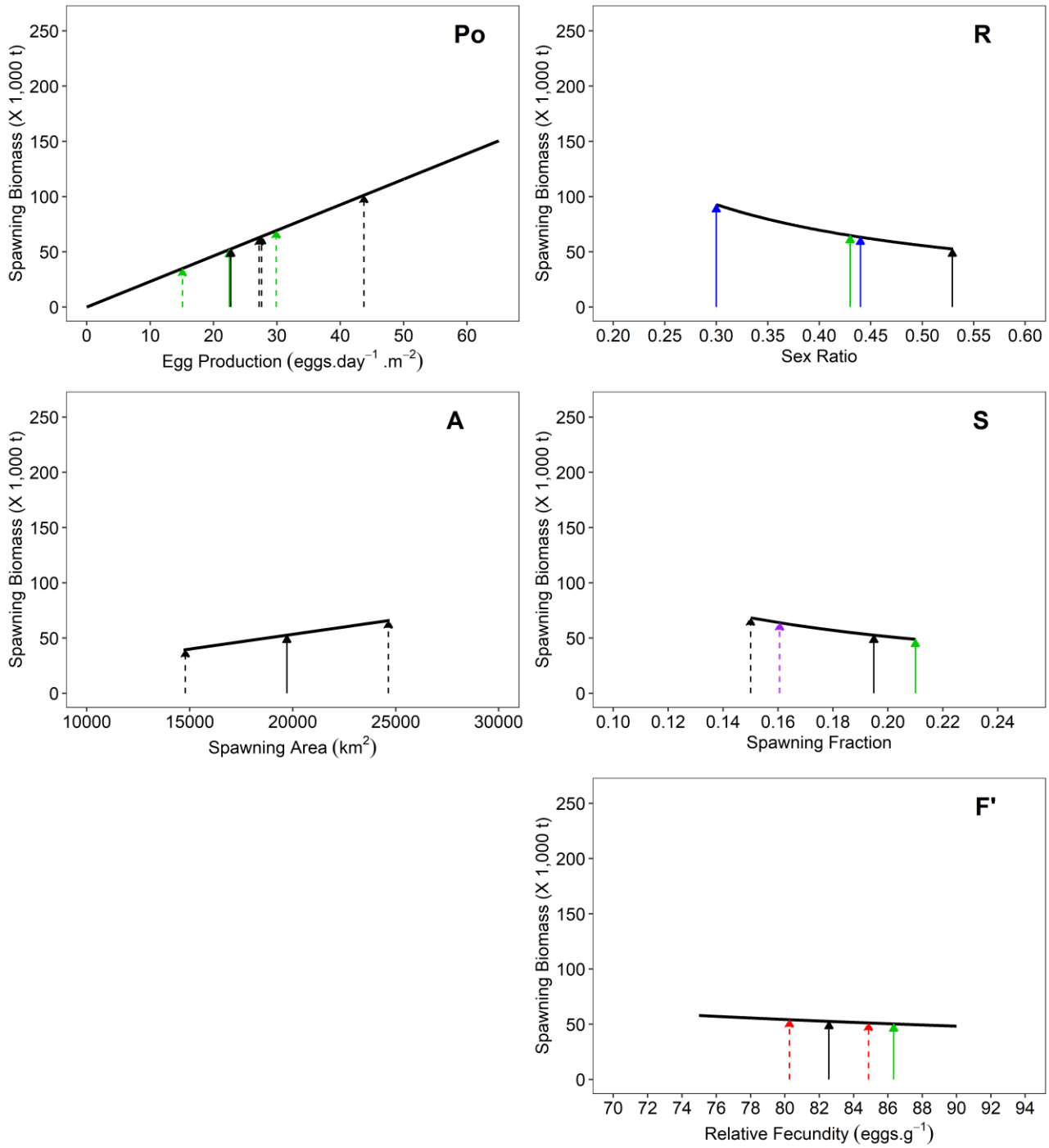


Figure 3-8. Sensitivity plots showing effects of variability in adult parameters and egg production on estimates of spawning biomass. Solid black arrows: parameters used for 2020 estimate. Dashed black: other plausible values of parameters calculated in 2020 (P_0: log-linear model, GLM NB1 (day 1 out) and mean total egg density (applied z of 0.3); *A*: ± 25%; *S*: POF only). Dashed purple: *S* calculated from all samples in 2020. Blue: parameters values from Neira et al. (2008). Solid green: parameter estimates from Redbait DEPM in 2017. Dashed green: alternate estimates of P_0 obtained in 2017. Dashed red: 95% CI for 2020 *F'*.

4 Discussion

4.1 Survey area, spawning area and egg distribution

This is the first application of the DEPM to Redbait in the East sub-area of the SPF since 2006 (Neira et al. 2008). It was designed to cover the entire spawning area of Redbait off eastern Australia, including waters off western Victoria and southern New South Wales that were not covered by the previous surveys. Surveying waters off southern New South Wales was important because Redbait catches have increased this area in recent years (Ward and Grammer 2021; Patterson et al. 2020, Patterson et al. 2021). The high abundance and wide distribution of Redbait eggs between Jervis Bay, NSW and Lakes Entrance, Victoria confirmed the importance of this previously unsurveyed region for spawning of Redbait. The increase in the survey area from less than 16,000 km^2 in 2005 and 2006 to over 57,000 km^2 in the present study largely explains the increase in spawning area from 13,220 km^2 in 2005 to 19,715 km^2 in 2020. The presence of eggs on transects in the northern part of the 2020 survey suggest that future surveys may need to extend even further north to ensure that the entire spawning habitat of Redbait in the East sub-area of the SPF is covered.

4.2 Mean daily egg production

Estimating P_0 is one of the key challenges of the DEPM (e.g. Stratadoukas et al. 2006; Dickey-Collas et al. 2012; Bernal et al. 2012; Ward et al. 2021). In Redbait, the typical challenges of estimating this parameter are exacerbated by the protracted period from spawning to hatching (i.e. >110 hours) compared to most other species to which the DEPM is commonly applied (e.g. approximately 40 hours for Sardine, Ward et al. 2021). This slow egg development rate is caused by the low water temperatures in which Redbait spawn (i.e. 10.5°C to 15.9°C in the present study) compared to species such as Sardine which typically spawn in waters around 18°C to 20°C (e.g. Ward et al. 2018).

Estimating egg production of Redbait is further complicated by the under-sampling of eggs less than 24 hours old. In this study, these problems were compounded by the relatively small number of sites at which eggs were collected (i.e. 79), which makes it challenging to estimate P_0 and z reliably (e.g. see Ward et al. 2021).

For this study, we used two models to estimate P_0 and z (the log-linear model and GLM NB1) and also estimated P_0 using a method (McGarvey and Kinloch 2001) that assumes a fixed z. The estimate of P_0 obtained using the log-linear model was chosen to estimate SB because this method has been rigorously tested on other species of small pelagic fishes and is known to provide conservative estimates of P_0 (e.g. Ward et al. 2018, Ward et al. 2019, Ward et al. 2021). Future studies should investigate methods used to estimate P_0 for

species, such as Redbait, in which young eggs are under-sampled and/or have long hatch times.

4.3 Mean Daily Fecundity

Estimates of both sex ratio and relative fecundity obtained in this study are robust and plausible. There is no evidence to suggest that the sex ratio by numbers of Redbait, or any other well studied species of small to medium sized pelagic fish in Australia (e.g. Sardine, Jack Mackerel, Blue Mackerel) is skewed towards either males or females. Therefore, an estimate of sex ratio by weight of 0.53 that is slightly skewed towards females (which, during the spawning season, are slightly heavier than males at a given length) is logically sound. The estimate of relative fecundity obtained in this study is also robust because it is based on a relatively large number of females with hydrated oocytes (i.e. 214).

The main uncertainty associated with the estimation of mean daily fecundity relates to the estimation of spawning fraction. The low estimates of spawning fraction from samples obtained near the end of the survey, combined with the prevalence of atresia in some females, suggest that these samples may have been collected after the peak spawning season. For this reason, samples that were collected after ichthyoplankton sampling had finished were not used to estimate *S*. The estimate of *S* obtained using the selected samples (i.e. 0.195) was similar to the estimate obtained for Redbait West (0.210; Ward et al. 2019) but considerably lower than the estimate of 0.32 obtained by Neira et al. (2008). We consider the estimate obtained by Neira et al. (2008) to be a preliminary value because it was based on "the presence of hydrated oocytes and/or fresh POFs" rather than the rigorous method of staging POFs (Ganias 2012) used in the present study. Prior to the next DEPM survey of Redbait in the East sub-area, it will be important to further investigate the spawning seasonality of this species in the region to ensure that the next survey coincides with the peak spawning season.

4.4 Spawning Biomass

This is the first DEPM survey of Redbait to cover the entire known spawning area east of longitude 146°30'E. The estimate of *SB* for Redbait East in 2020 of 52,629 t (95% CI = 13,937–91,321) is suitable for setting RBCs because it is based on robust and/or conservative estimates of all key parameters.

References

AFMA. 2008. Small Pelagic Fishery Harvest Strategy (last revised April 2017). Australian Fisheries Management Authority, Canberra. 9 pp.

AFMA. 2009. Small Pelagic Fishery Management Plan 2009. Australian Fisheries Management Authority, Canberra, Federal Register of Legislative Instruments F2010L00081. 51 pp.

Alheit, J. 1993. Use of the Daily Egg-Production Method for Estimating Biomass of Clupeoid Fishes - a Review and Evaluation. Bulletin of Marine Science 53:750-767.

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

Brooks, M. E., K. Kristensen, K. J. v. Benthem, A. Magnusson, C. W. Berg, A. Nielsen, and B. M. Bolker. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal 9:378-400.

Bunn, N. A., C. J. Fox, and T. Webb. 2000. A literature review of studies on fish egg mortality: implications for the estimation of spawning stock biomass by the annual egg production method. 111, Centre for Environment, Fisheries and Aquaculture Science.

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

Dimmlich, W. F., T. M. Ward, and B. W.G. 2009. Spawning dynamics and biomass estimates of Australian anchovy (*Engraulis australis*) in gulf and shelf waters of South Australia. Journal of Fish Biology 75:1560-1576.

Ewing, G. P., and J. M. Lyle. 2009. Reproductive dynamics of redbait, *Emmelichthys nitidus* (Emmelichthyidae), from south-eastern Australia. Fisheries Research 97:206-215.

Fletcher, W. J., N. C. H. Lo, E. A. Hayes, R. J. Tregonning, and S. J. Blight. 1996. Use of the daily egg production method to estimate the stock size of western Australian sardines (*Sardinops sagax*). Marine and Freshwater Research 47:819-825.

Ganias, K. 2012. Thirty years of using the postovulatory follicles method: Overview, problems and alternatives. Fisheries Research 117–118:63-74.

Ganias, K. 2013. Determining the indeterminate: Evolving concepts and methods on the assessment of the fecundity pattern of fishes. Fisheries Research 138:23-30.

Gaughan, D. J., T. I. Leary, R. W. Mitchell, and I. W. Wright. 2004. A sudden collapse in distribution of Pacific sardine (*Sardinops sagax*) off southwestern Australia enables an objective re-assessment of biomass estimates. Fishery Bulletin 102:617-633.

Gomon, M., D. Bray, and R. H. Kuiter. 2008. Fishes of the Australia's South Coast. Museum of Victoria. New Holland Publishers (Australia).

Heemstra, P. C., and J. E. Randall. 1977. A revision of the Emmelichthyidae (Pisces: Perciformes). Marine and Freshwater Research 28:361-396.

Hunter, J. R., and S. R. Goldberg. 1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. Fisheries Bulletin 77:641-652.

Hunter, J. R., and N. C. H. Lo. 1997. The daily egg production method of biomass estimation: Some problems and potential improvements. Ozeanografika 2:41-69.

Hunter, J. R., and B. J. Macewicz. 1985. Measurement of spawning frequency in multiple spawning fishes. National Marine Fisheries Services. National Oceanic and Atmospheric Administration Technical Report.

Kailola, P. J., M. J. Williams, P. C. Stewart, A. M. Reichelt, and C. Grieve. 1993. Australian Fisheries Resourses. Bureau of Resource Sciences, Department of Primary Industries and Energy and the Fisheries Research and Development Corporation, Canberra.

Last, P. R., E. O. G. Scott, and F. H. Talbot. 1983. Fishes of Tasmania. Tasmanian Fisheries Development Authority. 563 pp.

Lasker, R. 1985. An egg production method for estimating spawning biomass of pelagic fish: Application to the Northern Anchovy, *Engraulis mordax*. National Marine Fisheries Services. National Oceanic and Atmospheric Administration Technical Report.

Markina, N. P., and V. Z. Boldyrev. 1980. Feeding of the redbait on underwater elevations of the southwest Pacific. Biologiya Morya (Vladivostok) 4:40-45.

May, J. L., and J. G. H. Maxwell. 1986. Field guide to trawl fish from temperate waters of Australia. CSIRO Division of Fisheries Research.

McGarvey, R., and M. A. Kinloch. 2001. An analysis of the sensitivity of stock biomass estimates derived from the daily egg production method (DEPM) to uncertainty in egg mortality rates. Fisheries Research 49:303-307.

McGarvey, R., M. A. Steer, J. M. Matthews, and T. M. Ward. 2018. A stage-based estimator of daily egg production. ICES Journal of Marine Science 75:1638-1646.

Mel'nikov, Y., and N. A. Ivanin. 1995. Age-size composition and mortality of the rubyfish *Plagiogeneion rubiginosum* (Emmelichthyidae) in West Indian Submarine Ridge. Journal of Ichthyology 35:20-27.

Meléndez, C. R., and M. R. Céspedes. 1986. *Emmelichthys nitidus cyanescens* (Guichenot, 1848) in the Chilean Southern fisheries (Perciformes, Emmelichthyidae). Investigacion Pesquera 33:111-114.

Mersmann, O., H. Trautmann, D. Steuer, and B. Bornkamp. 2018. truncnorm: Truncated Normal Distribution. R package version 1.0-8. https://CRAN.R-project.org/package=truncnorm.

Moore, A., and M. Skirtun. 2012. The Small Pelagic Fishery. In J. Woodhams, S. Vieira and J. Stobutzki (Eds), Fishery Status Reports 2011, Australian Bureau of Agricultural and Resources Economics and Sciences, Canberra, 2012, 93 pp.

Neira, F. J., and J. M. Lyle. 2011. DEPM-based spawning biomass of *Emmelichthys nitidus* (Emmelichthyidae) to underpin a developing mid-water trawl fishery in south-eastern Australia. *Fisheries Research* 110:236-243.

Neira, F. J., J. M. Lyle, G. P. Ewing, J. P. Keane, and S. R. Tracey. 2008. Evaluation of egg production as a method of estimating spawning biomass of redbait off the east coast of Tasmania. Final report, FRDC project no. 2004/039. Tasmanian Aquaculture and Fisheries Institute, Hobart.

Neira, F. J., R. A. Perry, C. P. Burridge, J. M. Lyle, and J. P. Keane. 2015. Molecular discrimination of shelf-spawned eggs of two co-occurring *Trachurus* spp. (Carangidae) in southeastern Australia: a key step to future egg-based biomass estimates. ICES Journal of Marine Science: Journal du Conseil 72:614-624.

Nelson, J. S. 2006. Fishes of the World. Fourth Edition. John Wiley and Sons, Inc. Hoboken, New Jersey. 601 pp.

Parin, N. V., A. N. Mironov, and K. N. Nesis. 1997. Biology of the Nazca and Sala y Gomez submarine ridges, an outpost of the Indo-West Pacific Fauna in the Eastern Pacific Ocean: composition and distribution of the fauna, its communities and history. Advances in Marine Biology 32:145-242.

Parker, K. 1980. A direct method for estimating northern anchovy, *Engraulis mordax*, spawning biomass. Fisheries Bulletin 84:541-544.

Patterson, H., J. Larcombe, J.Woodhams and R. Curtotti. 2020. Fishery Status Reports 2020. Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra. CC BY 4.0.

Patterson, H., D. Bromhead, D. Galeano, J. Larcombe, J. Woodhams and R. Curtotti. 2021. Fishery Status Reports 2021. Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra. CC BY 4.0.

Perry, R. 2011. Spawning habitat of two commercial mackerel species (*Trachurus* spp.) in south-eastern Australia based on distribution of eggs identified using molecular techniques. Honors Thesis, University of Tasmania.

Picquelle, S., and G. Stauffer. 1985. Parameter estimation for an egg production method of anchovy biomass assessment. National Marine Fisheries Services. National Oceanic and Atmospheric Administration Technical Report.

Pullen, G. 1994. Fishery status report: Purse-seine (The Tasmanian jack mackerel fishery). Department of Primary Industry and Fisheries, Tasmania. Internal Report 13. 49pp.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rice, J. A. 1995. Mathematical Statistics and Data Analysis. Duxbury Press, Belmont, California.

Sexton, S. C., T. M. Ward, and C. Huveneers. 2017. Characterising the spawning patterns of Jack Mackerel (*Trachurus declivis*) off eastern Australia to optimise future survey design. Fisheries Research 186:223-236.

Smith, M. M., and P. C. Heemstra. 1986. Smith's Sea Fishes. MacMillan, Johannesburg. 1047 pp.

Smith, P. E., and S. L. Richardson. 1977. Standard techniques for pelagic fish egg and larva surveys. Food and Agriculture Organisation. Fisheries Technical Paper No. 175. 100 pp.

Somarakis, S., I. Palomera, A. Garcia, L. Quintanilla, C. Koutsikopoulos, A. Uriarte, and L. Motos. 2004. Daily Egg Production of anchovy in European waters. ICES Journal of Marine Science 61:944-958.

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

Turner, R. 2015. deldir: Delaunay Triangulation and Dirichlet (Voronoi) Tessellation. R package version 0.1-9. http://CRAN.R-project.org/package=deldir.

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

Ward, T. M., J. Carroll, G. L. Grammer, C. James, R. McGarvey, J. Smart, and A. R. Ivey. 2018. Improving the precision of estimates of egg production and spawning biomass obtained using the Daily Egg Production Method. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. FRDC project No. 2014/026. 90 pp.

Ward, T. M., and G. L. Grammer. 2018. Commonwealth Small Pelagic Fishery: Fishery Assessment Report 2017. Report to the Australian Fisheries Management Authority. South

Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2010/000270-9. SARDI Research Report Series No. 982. 114 pp.

Ward, T. M., and G. L. Grammer. 2019. Commonwealth Small Pelagic Fishery: Fishery Assessment Report 2018. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2010/000270-10. SARDI Research Report Series No. 1021. 132 pp.

Ward, T. M., and G. L. Grammer. 2021. Commonwealth Small Pelagic Fishery: Fishery Assessment Report 2020. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2020/000209-2. SARDI Research Report Series No. 1085. 8 pp.

Ward, T. M., G. L. Grammer, A. R. Ivey, and J. P. Keane. 2019. Spawning biomass of Redbait (*Emmelichthys nitidus*) between western Kangaroo Island, South Australia and south-western Tasmania in October 2017. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2019/000053-1. SARDI Research Report Series No. 1011. 38 pp.

Ward, T. M., G. L. Grammer, A. R. Ivey, J. J. Smart, and R. McGarvey. 2021. Increasing the precision of the daily egg production method; 2020's remix of a 1980's classic. ICES Journal of Marine Science 78:1177-1195.

Watson, D. F. 1981. Computing the n-dimensional Delaunay tessellation with application to Voronoi polytopes. Pages 167-172 The Computer Journal.

Welsford, D. C., and J. M. Lyle. 2003. Redbait (*Emmelichthys nitidus*): A synopsis of fishery and biological data. TAFI Technical Report Series, no. 20 20

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

Appendices

Appendix 1: Genetic identification of Redbait eggs

Molecular Identification

A molecular approach of Mitochondrial DNA (mtDNA) extraction, amplification, and sequencing established by Ward *et el*. (2005) was employed to genetically identify ethanol preserved fish eggs. DNA extractions from eggs 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). The primers FishF2 (5′TCGACTAATCATAAAGATATCGGCAC3′) and FishR2 (5′ACTTCAGGGTGACCGAAGAATCAGAA3′) were used in the PCRs to amplify an approximately 655 bp fragment from the 5′ region of the *cox1* gene. Sequences were aligned to reference data in the Fish Barcode of Life Database (BOLD) using Macrogen BLAST service.

The 25µl PCR reaction mixes included 8.5µl of ultrapure water, 1 µl of each primer (0.01mM), 8.5µl of MyTaq™ HS Mix (Meridian Bioscience), and 2.0µl of DNA. Amplifications were performed using a Mastercycler® Eppendorf gradient thermal cycler (Brinkmann Instruments, Inc.). The thermal regime consisted of an initial step of 3min at 95°C followed by 35 cycles of 0.5min at 95°C, 0.5min at 54°C, and 1min at 72°C, followed in turn by 10min at 72°C. and then held at 4°C.

A total of 71 eggs were selected for mtDNA analysis; 42 identified morphologically as Redbait, 28 indeterminable, and 1 similar but morphologically different to Redbait (Tables A1-1). Indeterminable eggs consisted of early stage eggs whose morphological characteristics were less definitive, making morphological identification problematic.

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

Table A1-1. Molecular identifications of morphologically identified Redbait and similar eggs collected between southern Tasmania and central New South Wales in October 2020.

Morphological identification	n tested	Genetic Identification		
		Redbait	Other	No DNA
Redbait	42	32	0	10
Possible Redbait: early stage eggs with limited characteristics for ID	28	19	2	7
Unlikely Redbait, but possessing some similar characteristics	2	1	1	0

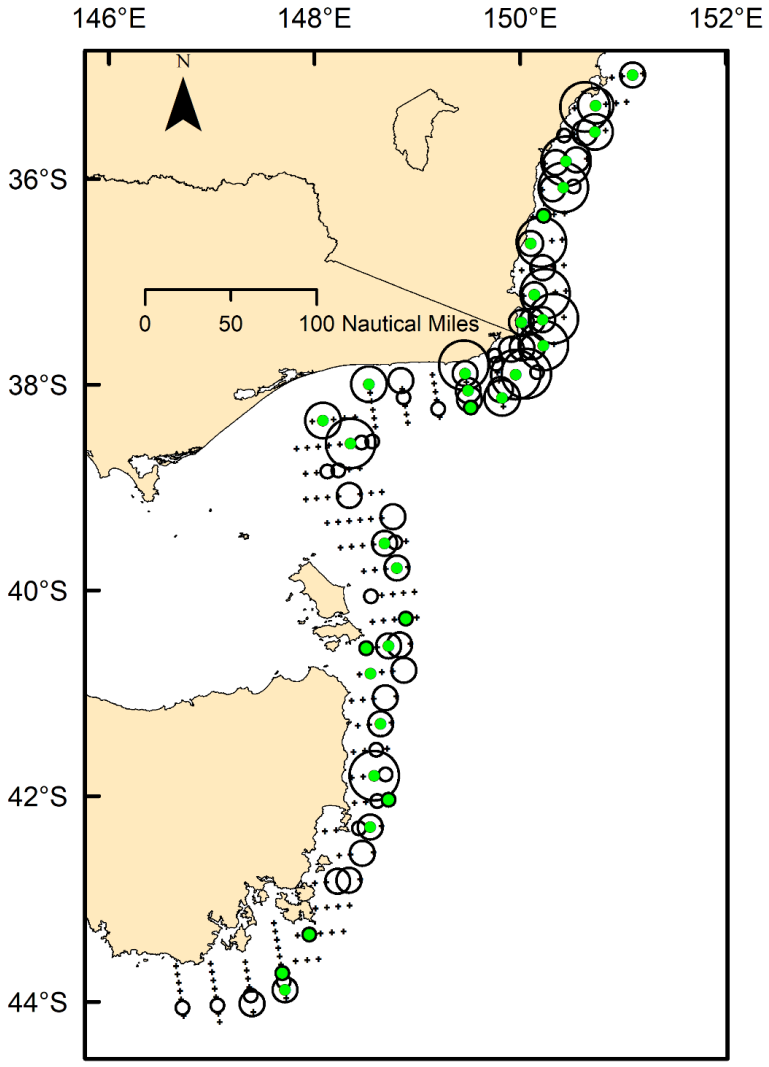


Figure A1–1. Distribution and relative densities of live Redbait eggs between southern Tasmania and central New South Wales during October 2020. Green: Redbait eggs confirmed by genetic analysis.

References

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D.N. (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences.* 360: 1847-1857.

Appendix 2: Adult sampling locations for Redbait East DEPM

Trawl No.	Date	Start Time	Start Latitude	Start Longitude	Duration of trawl h:m	Depth (start, m)	Redbait present	Adults sampled (n)
1	3/10/20	14:15	36°06’57” E	150°21’49” S	2:10	120	Y	225
2	3/10/20	19:10	36°22’26” E	150°18’10” S	50	140	Y	136
3	7/10/20	13:20	36°58’57” E	150°18’14” S	40	135	Y	174
4	13/10/20	10:50	39°17’26” E	148°38’12” S	30	126	Y	10
5	14/10/20	20:00	40°53’25” E	148°43’33” S	20	128	Y	51
6	14/10/20	21:30	41°03’16” E	148°38’33” S	25	128	Y	82
7	18/10/20	5:10	43°42’50” E	147°41’25” S	1:00	145	Y	145
8	18/10/20	15:40	43°09’47” E	148°07’58” S	45	115	Y	32
9	19/10/20	12:10	42°15’17” E	148°24’49” S	1:10	90	N	0
10	19/10/20	22:20	41°35’34” E	148°31’33” S	30	110	Y	0
11	20/10/20	10:30	41°01’24” E	148°40’02” S	1:00	128	Y	168
12	22/10/20	6:40	37°23’50” E	150°15’37” S	1:05	130	Y	172
13	22/10/20	8:30	37°27’26” E	150°12’30” S	1:25	125	Y	162
14	22/10/20	10:30	37°21’18” E	150°15’17” S	1:15	125	Y	234

Table A2-1. Date, time and locations of trawls off the *FV Saxon Onward* for Redbait during the 2020 DEPM survey.