Australian Government
Australian Fisheries Management Authority

RR2017/0805 March 2019

Spawning biomass of Redbait (*Emmelichthys nitidus*) between western Kangaroo Island, South Australia and south-western Tasmania in October 2017

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Report to the Australian Fisheries Management Authority

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SARDI Publication No. F2019/000053-1
SARDI Research Report Series No. 1011

March 2019

This publication may be cited as:
Ward, T. M., Grammer, G. L., Ivey, A. R., and Keane, P. (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. 38pp.

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SARDI Publication No. F2019/000053-1
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Date: 29 March 2019

Distribution: AFMA, SAASC Library, Parliamentary Library, State Library and National Library

Circulation: Public Domain

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ACKNOWLEDGEMENTS

This research was funded by the Australian Fisheries Management Authority (AFMA). We thank the masters, crews, owners and staff of the *RV Ngerin* and *FV Western Alliance* for their assistance. We are grateful to Mr David Guillot for his efforts to ensure the success of the adult surveys. We thank Mr Leonardo Mantilla, Mr Ian Moody, Mr Nat Navong, Mr Josh Nitschke, Ms Sonja Hoare and Mr Kriston Bott for diligently undertaking fieldwork and/or laboratory processing. Dr Jonathan Smart provided statistical advice. This report was formally reviewed by Dr Jonathan Smart and Dr Katherine Heldt and approved for release by Dr Stephen Mayfield (SARDI Aquatic Sciences).

ABBREVIATIONS

Abbreviation	Full Name
AFMA	Australian Fisheries Management Authority
CTD	Conductivity Temperature Depth
DEPM	Daily Egg Production Method
GIS	Geographic Information System
GLM	Generalised Linear Models
GSI	Gonadosomatic Index
NSW	New South Wales
PIRSA	Primary Industries and Regions South Australia
RBC	Recommended Biological Catch
SA	South Australia
SARDI	South Australian Research and Development Institute
SPF	Commonwealth Small Pelagic Fishery
TAC	Total Allowable Catch
TAS	Tasmania
TL	Total Length

EXECUTIVE SUMMARY

Background and Need

Estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator in the Commonwealth Small Pelagic Fishery (SPF) and are used to set Recommended Biological Catches (RBCs) for target species.

Prior to this study, the DEPM had been applied to all target species in each sub-area of the SPF, except Redbait in the West sub-area. The need to address this knowledge gap increased in 2014/15, when a factory trawler began operating in the fishery.

In the West sub-area, the trawler operated between western Kangaroo Island, South Australia and south-western Tasmania. The DEPM was applied to Redbait in this area in October 2017.

Objectives

The objectives of this study were to:

1. Determine the distribution and abundance of Redbait eggs between western Kangaroo Island and south-western Tasmania in October 2017.
2. Estimate adult reproductive parameters of Redbait in this region during this period.
3. Estimate the spawning biomass of Redbait in the eastern portion of the West sub-area of the SPF during October 2017.

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).

Ichthyoplankton samples used to estimate total daily egg production were collected from the *RV Ngerin* at 308 sites in shelf waters between western Kangaroo Island, South Australia and south-western Tasmania in October 2017. Ichthyoplankton samples were also collected opportunistically from the *FV Western Alliance* at 20 sites in Bass Strait and along the coast of north-eastern Tasmania. Egg samples were taken at a total of 328 sites.

Redbait eggs were identified using standard laboratory procedures and confirmed using molecular techniques. Spawning area was estimated using the Voronoi nearest neighbour method. Five models were used to estimate egg production (P_0). The value of P_0 used to estimate spawning biomass was the mean of the linear version of the exponential model and quasi-poisson generalised linear model (GLM).

A modified demersal trawl for adult Redbait was undertaken from the *FV Western Alliance* at each of 14 sites in shelf and slope waters between Portland, Victoria and western Tasmania during 13-19 October 2017. Significant numbers of Redbait were caught in nine of the 14 trawls; all but one of these samples included mature Redbait. Adult reproductive parameters were estimated using standard procedures and relevant information from previous studies.

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

Results, Discussion and Implications

A total of 3,280 live Redbait eggs were collected from 113 of the 328 sites. Redbait eggs were widely distributed in outer shelf and upper slope waters between western Kangaroo Island and south-western Tasmania. The highest egg densities of Redbait occurred off western Victoria and the west coast of Tasmania. Most Redbait eggs were collected from sites located in depths of 100-200 m and sea surface temperatures (SSTs) of 12–15°C. The estimated spawning area was 28,365 km^2, comprising 36.1% of the total area surveyed (78,212 km^2). Mean daily egg production (P_0) was 22.5 (CI = 10.4–58.1) $eggs \cdot day^{-1} \cdot m^{-2}$.

Adult parameters estimated from the survey [mean (95% CI)] were: sex ratio (*R*): 0.43 (0.34–0.55), female weight (*W*): 106.1 (101.2–110.5) g, batch fecundity (*F*): 9,821 (8,883–10,945) eggs, and spawning fraction (*S*): 0.21 (0.16–0.24).

Based on parameters estimated from the survey (except for sex ratio *R*, which was set at 0.50), the spawning biomass of Redbait in waters between western Kangaroo Island and south-western Tasmania in October 2017 was estimated to be 66,767 t (CI = 28,797–190,392). This estimate of spawning biomass is considered suitable for setting RBCs for Redbait in West sub-area of the SPF, because it is based on robust and/or conservative estimate of key parameters.

Keywords: Redbait, *Emmelichthys nitidus*, Daily Egg Production Method, Spawning Biomass, Small Pelagic Fishery, south-eastern Australia, Tasmania, Bass Strait

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. Redbait rarely exceeded 5% of the total catch, annual catches averaged ~700 t from 1984/85 to 1989/90 (Kailola et al. 1993, Pullen 1994, Ward and Grammer 2017).

The Commonwealth Small Pelagic Fishery (SPF) was established in 2000. It is a purse-seine and mid-water trawl fishery managed by the Australian Fisheries Management Authority (AFMA) that operates in Commonwealth waters (3–200 nautical miles offshore) 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. A detailed history of the SPF is provided 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.

The SPF Harvest Strategy and Management Plan were implemented in 2008/09 (AFMA 2008, 2009). The SPF Harvest Strategy was last revised in 2017. It is used to set Total Allowable Catches (TACs) for each 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 and are used to set Recommended Biological Catches (RBCs) and TACs under guidelines outlined in the Harvest Strategy (AFMA 2008).

Mid-water trawling to target sub-surface schools of Jack Mackerel off Tasmania was trialled in 2001/02 (Welsford and Lyle 2003). Between December 2001 and April 2002, a total catch of over 5,000 t of small pelagic fishes was taken; 90% was Redbait. In late 2002, a multi-purpose 50 m mid-water trawler began targeting small pelagic species off Tasmania, particularly Redbait. The catch peaked in 2003/04, when more than 7,000 t of Redbait was taken. Small-scale purse-seine operations were temporarily resumed in the late 2000s in response to declining trawl effort and catch (Emery et al. 2015). Effort and catch in the SPF increased during 2014/15 to 2015/16 when a factory trawler operated in both sub-areas of the fishery (Ward and Grammer 2017).

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, Ganias 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. female weight (W), sex ratio (R), batch fecundity (F) and spawning fraction (S), can be sampled 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).

Table 1. The equation for the Daily Egg Production Method (DEPM) used to calculate the spawning biomass (*SB*) of Redbait between western Tasmania and Kangaroo Island in October 2017.

Model Name	Equation	Eq. No.	Parameters	Reference
Daily Egg Production Method	$SB = \frac{P_0\, A\, W}{R\, F\, S}$	(1)	*SB:* spawning iomass P_0: mean daily egg production *A*: total spawning area *W*: mean female weight *R*: mean sex ratio *F*: mean batch fecundity *S*: mean spawning fraction	Parker 1985

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).

Estimates of spawning biomass obtained using the DEPM are imprecise (e.g. Alheit 1993, Hunter and Lo 1997, Stratoudakis et al. 2006, Bernal et al. 2012, Dickey-Collas et al. 2012). Interannual variations in estimates of spawning biomass are mainly driven by three parameters: P_0, A and S. There are considerable uncertainties associated with estimation of both P_0 and S (Fletcher et al. 1996, McGarvey and Kinloch 2001, Ward et al. 2001a, 2001b,

Gaughan et al. 2004, McGarvey et al. 2018). Confidence limits surrounding estimates of P_0 are usually high. A recent study evaluated the use of a variety of statistical approaches for estimating P_0, and identified options for reducing imprecision (Ward et al. 2018a). One of the options identified was to use the most precise method, a log-linear egg production model, which had a likely negative bias and reduced the potential for over-estimation of stock size (see Ward et al. 2018a). 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).

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. 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).

Redbait is widely distributed throughout the southern hemisphere, with the species reported from Tristan da Cunha in the southern Atlantic, the south-western coast of South Africa, St Paul and Amsterdam Islands, mid-oceanic ridges and seamounts through the Indian Ocean, Australia, New Zealand, submarine ridges in the south-eastern Pacific, and the southern coast of Chile (Markina and Boldyrev 1980, Meléndez and Céspedes 1986, Parin et al. 1997). Within Australian waters, the range of Redbait extends from mid New South Wales to south-west Western Australia, including Tasmania (Gomon et al. 2008).

Redbait is an asynchronous batch spawner with indeterminate fecundity. Annual trends in Gonadosomatic Index (GSI) and macroscopic gonad stages indicated that Redbait from eastern Tasmania spawn between September and November, with a peak in activity during September and October (Ewing and Lyle 2009). A similar pattern was evident for south-western Tasmania, although the peak occurred one month later during October and November

(Ewing and Lyle 2009). Spawning occurs along a 2.5 nautical mile (nm) corridor either side of the continental shelf break when mid-water temperatures are between 12 and 15.2°C (Neira et al. 2008).

Redbait eggs are positively buoyant and hatch about 2–4 days after fertilisation depending on temperature (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 post-hatching, although the distribution of eggs and larvae off eastern Australia have been described by Neira et al. (2008).

The spawning habitat of Redbait was described from egg, larval and environmental data collected over shelf waters between north-eastern Bass Strait and lower south-western Tasmania in 2005 and 2006 (Neira et al. 2008). The DEPM was subsequently used to estimate the spawning biomass of Redbait East in these years (Neira et al. 2008, Neira and Lyle 2011).

1.4 Need

The SPF Harvest Strategy specifies that estimates of spawning biomass obtained using the DEPM are used to set RBCs and TACs for each species and sub-area. Prior to this study, the DEPM had been applied to all SPF target species in the East sub-area, and to Blue Mackerel and Jack Mackerel in the West Sub-area, but not to Redbait in the West sub-area.

The need to apply the DEPM to Redbait in the West sub-area increased when a factory trawler entered the SPF in 2014/15 and began operating in both sub-areas (Ward and Grammer 2017). As the factory-trawler fished in the portion of the West sub-area of the SPF between western Kangaroo Island, South Australia and south-western Tasmania, the DEPM was applied to Redbait in this region.

1.5 Objectives

1. Determine the distribution and abundance of Redbait eggs between western Kangaroo Island and south-western Tasmania in October 2017.
2. Estimate adult reproductive parameters of Redbait in this region during this period.
3. Estimate the spawning biomass of Redbait in the eastern portion of the West sub-area of the SPF during October 2017.

2 METHODS

2.1 Total Daily Egg Production

2.1.1 Ichthyoplankton surveys

During October 2017, ichthyoplankton samples were collected from the *RV Ngerin* at 308 sites on 44 transects in shelf waters between western Kangaroo Island, South Australia and south-western Tasmania (Figure 1). These sites comprised the 'main survey'.

During October 2017, ichthyoplankton samples were also collected opportunistically from the *FV Western Alliance* in Bass Strait and along the coast of north-eastern Tasmania (Figure 1). These 20 exploratory sites were sampled to determine if Redbait spawns in Bass Strait during summer.

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 $m \cdot s^{-1}$. Location, sampling date/time, and depth were recorded for each plankton sample. Water temperature profiles were recorded with a Sea-Bird™ Conductivity-Temperature-Depth (CTD) attached to the nets (main survey only). 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 on every second transect, a duplicate sample was preserved in 95% ethanol for genetic validation. Exploratory samples collected in Bass Strait were fixed in formalin only.

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).

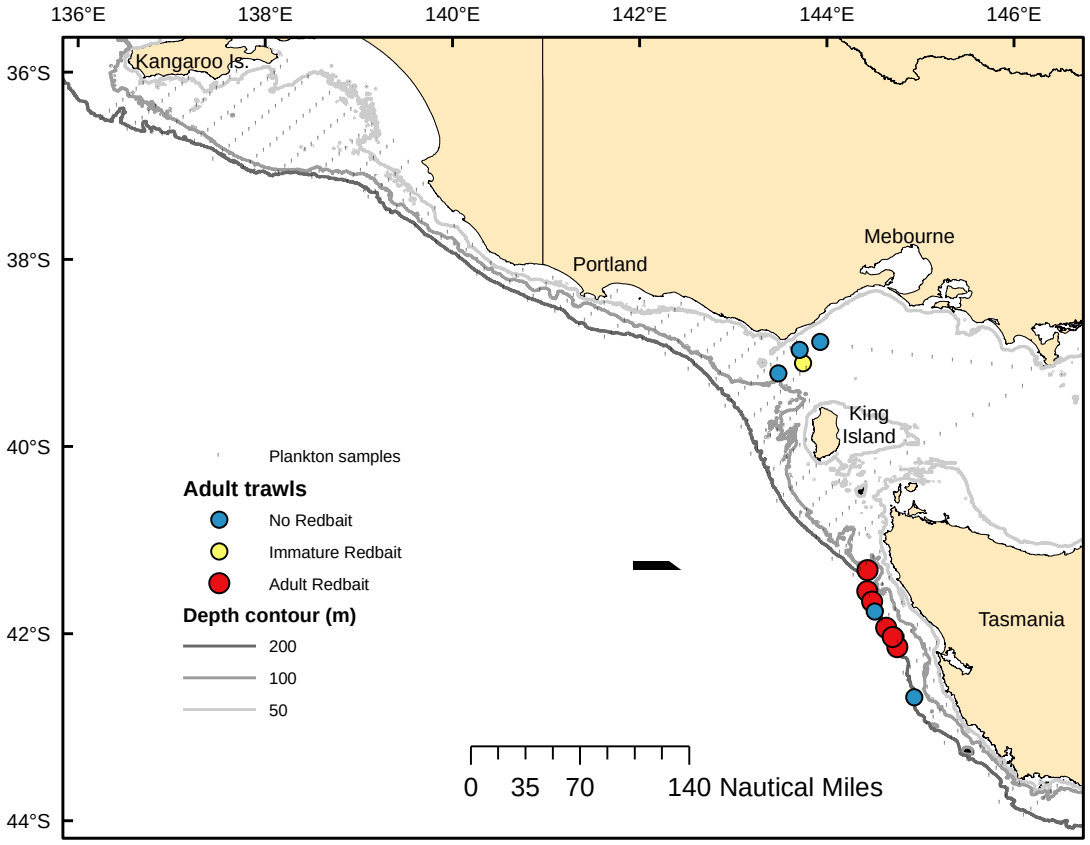

Figure 1: Area between western Tasmania and Kangaroo Island where the Daily Egg Production Method was applied to Redbait in October 2017. Locations shown are the main egg sampling sites, opportunistic egg sampling sites in Bass Strait and adult trawl sites.

All eggs were staged using the 'universal' egg staging method described by Ward et al. (2018a) (Figure 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.

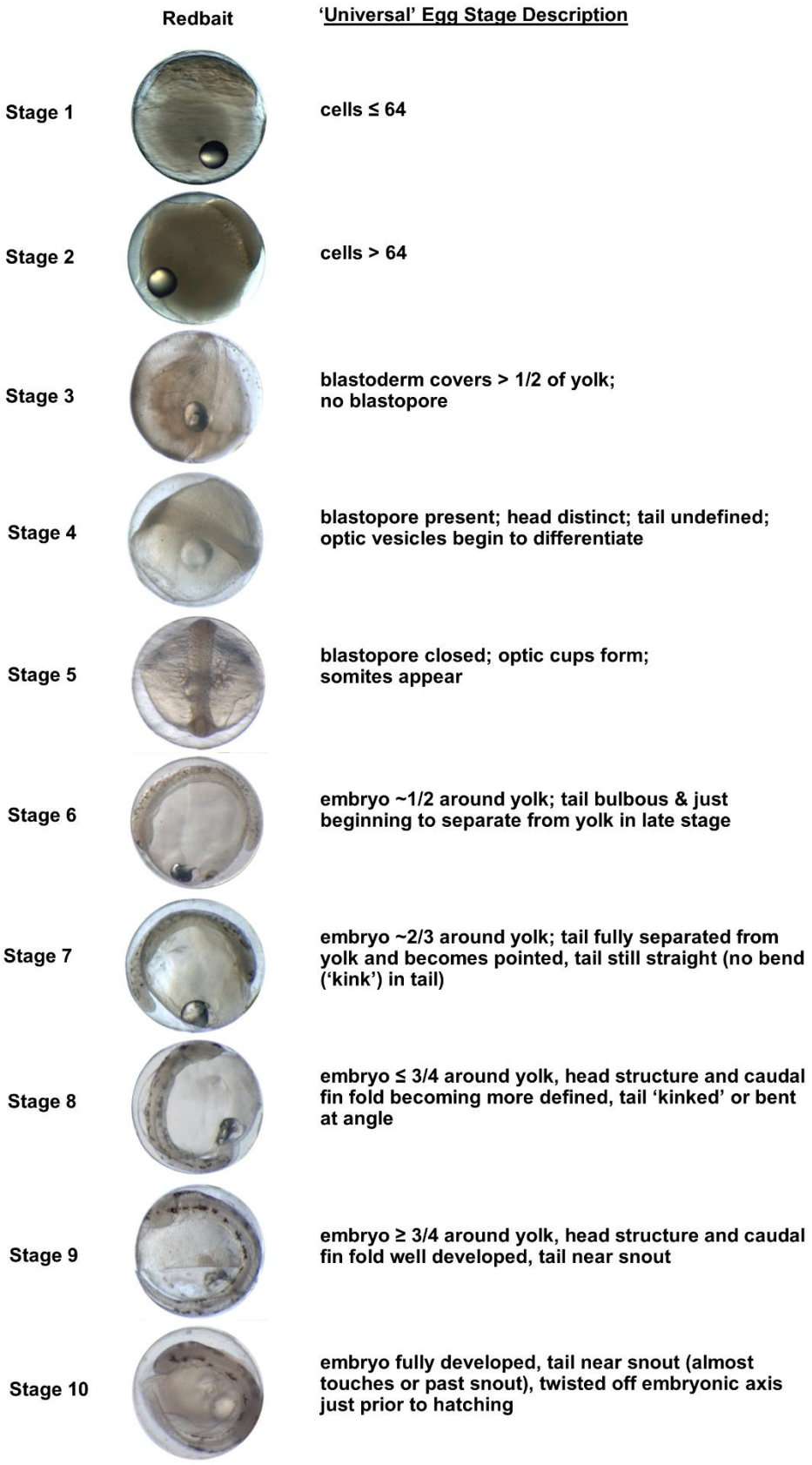

Figure 2. Egg stages of Redbait defined using the 'universal' egg stages of Ward et al. (2018a).

2.1.4 Egg ageing and treatment of zero count egg samples

Based on CTD 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. 2018a).

As samples often includes eggs spawned on more than one night, eggs in each sample were aggregated into daily cohorts. The total egg count and average age for each daily cohort were calculated by assigning each live egg stage to a day of spawning (e.g. day 0, day 1, day 2, etc.) and summing the number of eggs. The age assigned to each cohort was the weighted average of the number of eggs observed in each stage.

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

2.1.5 Egg density (P_s and P_t)

The density of eggs under one square metre was estimated for each sample (P_s) and each daily cohort (P_t) (Equation 2, Table 2).

2.1.6 Spawning area (A)

The Voronoi natural neighbour (VNN) method (Watson 1981) was applied using the ‘deldir’ function in the R package deldir (Turner 2015; R 3.5.1) and used to generate a polygon around each sampling site with the boundary as the midpoint equidistant between each sampling site (Figure 3). The area represented by each site (km^2) was determined using the 'areaPolygon’ function in the geosphere R package (Hijmans 2015). The spawning area (A) was defined as the total area of grids where live Redbait eggs were collected.

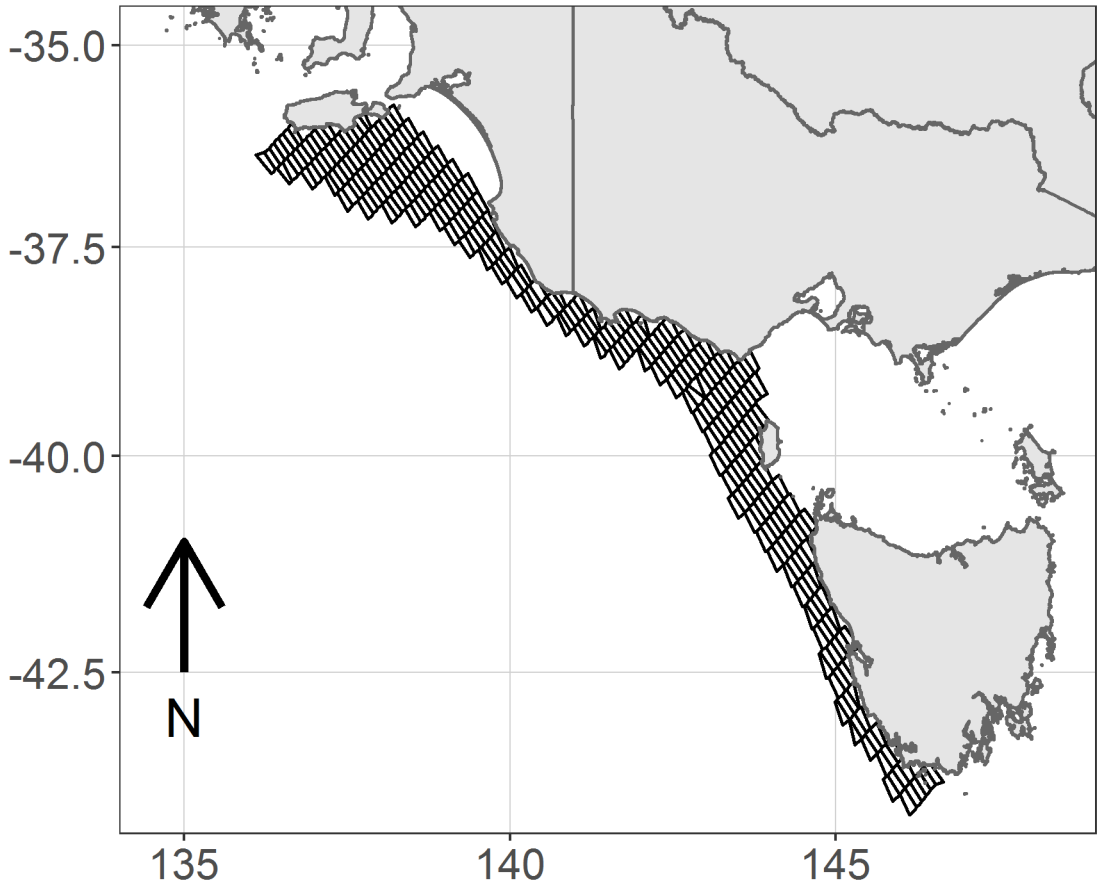

Figure 3. Voronoi natural neighbour polygons used to estimate the spawning area of Redbait between western Tasmania and Kangaroo Island in October 2017.

Table 2. Equations used to estimate egg density, mean daily egg production (P_0) and instantaneous egg mortality rate (z) for Redbait between western Tasmania and Kangaroo Island in 2017.

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

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

P_0 is the mean daily density of eggs produced per unit area (eggs·m^{-2}·day^{-1}) within the spawning area. Prior to estimating P_0, total egg density for each daily cohort was weighted by the relative size of each sampling area (i.e. area of a site in the VNN tessellation). P_0 and z were then estimated from the egg densities and average ages of daily cohorts.

P_0 and z are difficult to estimate precisely (e.g. Stratoudakis et al. 2006, Bernal et al. 2012, Dickey-Collas et al. 2012, Ward et al. 2018a). Based on the findings of Ward et al. (2018a), five different models were fitted to estimate P_0 and instantaneous egg mortality rate (z, day^{-1}). The distributions of daily cohort egg densities vary among species and surveys; different models appear may be suitable for different datasets (Ward et al. 2011, 2018a).

The underlying model used to calculate mean daily egg production (P_0) was the exponential egg mortality model (Equation 3a, Table 2). The model was applied in several ways. Non-linear least squares regression was used to fit Equation 3a and establish Equation 3b (Table 2). A linear version of the exponential egg mortality model (the 'log-linear model', Equation 4a) with a bias correction factor (Equation 4b, Table 2) was also used (Picquelle and Stauffer 1985). Data were fitted using three generalised linear models (GLMs, Equation 5, Table 2) with three different error structures: negative binomial, quasi and quasi-poisson. Instantaneous egg mortality rate was estimated as a free parameter in each of the models (Table 2). The mean value of egg production calculated from the log-linear (bias corrected) and quasi-poisson GLM models was used to estimate spawning biomass as recent studies have shown that these models perform reliably in most situations (Ward et al. 2018a, b).

Young eggs are commonly under-represented in plankton samples (e.g. Ward et al. 2018a). 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. 2018a). The egg production models (described above) were applied to six different datasets that increasingly removed egg stages up to Stage 4: 'All eggs stages', 'Stage 1 removed', 'Stages 1-2 removed', 'Stages 1-3 removed', 'Stages 1-4 removed', and 'Day 1 removed'. The dataset 'Day 1 removed' excluded all eggs aged ≤24 hours to account for site specific differences in temperature and developmental rates. The 'Day 1 removed' dataset was used to estimate spawning biomass as this approach accounts for differences in the rates of development of eggs through the early stages at sites with different water temperatures.

2.2 Adult Reproductive Parameters

2.2.1 Sampling methods

Adult Redbait were sampled using a modified demersal trawl net deployed from the *FV Western Alliance* in shelf and slope waters between Portland, Victoria and western Tasmania during 13-19 October 2017 (Figure 1). Nine of the 14 trawls caught substantial numbers of adult Redbait (>100 females), but in one catch most specimens were small and immature.

2.2.2 Parameter estimation methods

The ovaries of mature female Redbait were removed, labelled and fixed in a 10% formalin-seawater solution. Females (without ovaries) and mature males were labelled and frozen for laboratory processing.

Female weight (*W*)

In the laboratory, mature females and males from each sample were thawed and weighed (±0.01 g). Preserved ovaries were weighed before preparation for histological analysis. Fixation in formalin has a negligible effect on fish weight (Lasker 1985). The mean weight of mature females in the population was calculated from the average of sample means weighted by proportional sample size (Equation 6, Table 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 and used to estimate the mean batch fecundities of mature females in all samples.

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 (Equation 7a and 7b, Table 3).

Table 3. Equations used to estimate the female weight (*W*), sex ratio (*R*), and spawning fraction (*S*) of Redbait between western Tasmania and Kangaroo Island in October 2017.

Adult Parameter	Equation	Eq. No.	Parameters	Reference
Female Weight	$W = \overline{\left[\frac{\overline{W_i}\, n_i}{N}\right]}$	(6)	$\overline{W_i}$: mean female weight of each sample *i*; *n*: number of fish in each sample *N*: total number of fish collected in all samples	Lasker (1985)
Sex Ratio: sample	$\overline{R_i} = \frac{F_i}{F_i + M_i}$	(7a)	F_i: total weight of mature females in each sample *i* M_i: total weight of mature males in each sample *i*	Lasker (1985)
Sex Ratio: population	$R = \overline{\left[\frac{\overline{R_i}\, n_i}{N}\right]}$	(7b)	$\overline{R_i}$: mean sex ratio of each sample *n*: number of fish in each sample *N*: total number of fish collected in all samples and	Lasker (1985)
Spawning Fraction: sample	$\overline{S_i} = \frac{d0 + d1 + d2 + d3}{4\, n_i}$	(8a)	*d*0, *d*1, *d*2 and *d*3: the number of mature females with hydrated oocytes and POFs aged <72 hours in each sample n_i: is the total number of females within a sample.	Lasker (1985)
Spawning Fraction: population	$S = \overline{\left[\frac{\overline{S_i}\, n_i}{N}\right]}$	(8b)	$\overline{S_i}$: mean spawning fraction of each sample *n*: number of fish in each sample *i* *N*: total number of fish collected in all samples	Lasker (1985)

Spawning fraction (*S*)

Ovaries of mature females were processed using standard histological procedures and stained with haematoxylin and eosin. 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 calculated as the mean proportion of females with hydrated oocytes (*d*0) (i.e. spawning will occur that night), <24 hour POFs (*d*1) (assumed to have spawned on the night prior to capture), 24-48 hour POFs (*d2*) (assumed to have spawned two nights prior) and 48-72 hour POFs (*d*3) (assumed to have spawned three nights prior) (Equation 8a, Table 3). The mean spawning fraction of the population was calculated from the average of sample means weighted by proportional sample size (Equation 8b, Table 3).

2.3 Spawning Biomass (*SB*)

Spawning biomass for Redbait was calculated according to Equation 1 (Table 1) using the mean P_0 values obtained from the log-linear and quasi-poisson GLMs, spawning area (*A*), and adult parameters for *F, S,* and *W* estimated from the current survey. *R* was set at 0.50 to account for the skewed estimate of sex ratio from the survey.

2.4 Sensitivity Analysis

Sensitivity analyses were conducted to assess the effects of variation in individual parameters on the estimate of spawning biomass. 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 the 95% CIs from the current survey. A sex ratio of 0.50 was included for reference.

The minimum and maximum values used for spawning area were the mean spawning area from Neira and Lyle (2011) and the current survey *A* doubled. Values used for egg production were the 95% CIs of the mean P_0 of the log-linear and quasi-poisson GLM models, P_0 values from other models that have been used estimate egg production model (Ward et al. 2018 a, c) and P_0 values from the surveys of Neira and Lyle (2011).

3 RESULTS

3.1 Egg Distribution

A total of 3,280 live Redbait eggs were collected at 113 of 308 sites in the main survey. No Redbait eggs were collected at the 20 exploratory sites in Bass Strait. Bottom depths where live eggs were collected ranged from 38–850 m (mean: 138.1 m), and SSTs were 12.1–15.4°C (mean 13.9°C). These findings were confirmed by molecular identification of Redbait eggs in ethanol preserved samples taken during the main survey (Appendix 1).

3.1.1 Egg density (P_t)

Egg densities were highest in outer shelf waters off Portland (Victoria), and the west coast of Tasmania (Figure 4). Lower concentrations of eggs were also present at sites with similar depths south of Kangaroo Island and west of King Island. The highest densities of eggs (P_t >10 eggs·m^{-2}) were collected where the depth was 75–473 m (mean: 148.2 m). The highest egg density was off the north-west corner of Tasmania (2,026 eggs·m^{-2}). Redbait eggs were collected at sites with SSTs ranging from 12.2–15.4°C (mean 13.9°C). The mean SST for sites with eggs west of Tasmania was 13.2°C; off Kangaroo Island it was 14.7°C.

3.1.2 Spawning area (A)

The estimated spawning area for Redbait was 28,365 km^2, comprising 36.1% of the total area sampled (78,212 km^2, Table 4).

Table 4. Spawning Area (*A*) and total area surveyed for Redbait between western Tasmania and Kangaroo Island in 2017.

Survey Area (km^2)	Spawning Area (A)	Area with Eggs (%)
78,212	28,365	36.1

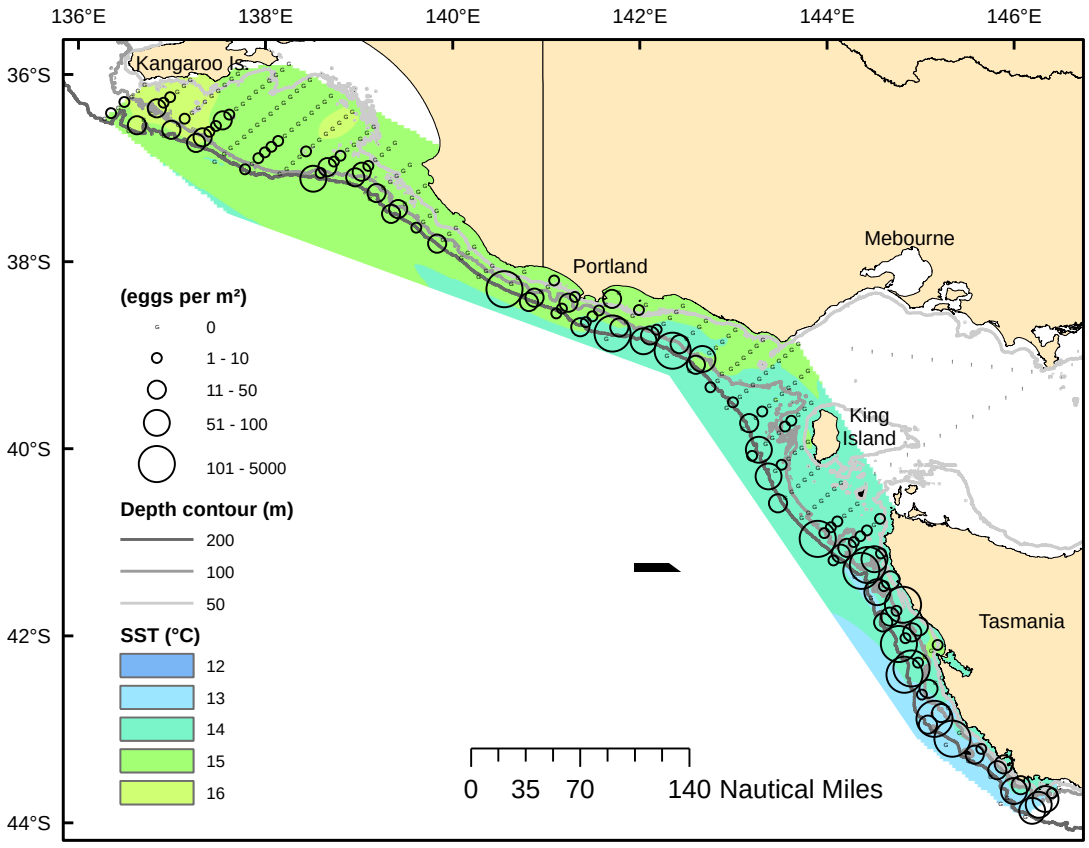

Figure 4. Distribution and abundance of Redbait eggs between western Tasmania and Kangaroo Island in October 2017.

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

The estimate of P_0 obtained using the 'Day 1 removed' dataset and averaging the results from the log-linear and quasi-poisson GLM models was 22.5 eggs·day^{-1}·m^{-2} (CI: 10.4–58.1) (Table 6, Figure 5). The confidence intervals of the model averaged P_0 are the lower 95% CI from the log-linear model and the upper 95% CI from the quasi-poisson GLM. This combination of dataset and models was used to estimate P_0 because it best incorporated site-specific differences in temperature eand gg-development rates, produced plausible estimates of z (0.21-0.29; Table 6, Figures 5 and 6) and reflects the findings of recent studies comparing the performance of different models (e.g. Ward et al 2018 a, c). The non-linear least square model fit was not included in the model averaging, because previous studies have shown that it is strongly influenced by samples with very large egg densities and often produces unrealistically high estimates of P_0 and z (e.g. Ward et al. 2018a).

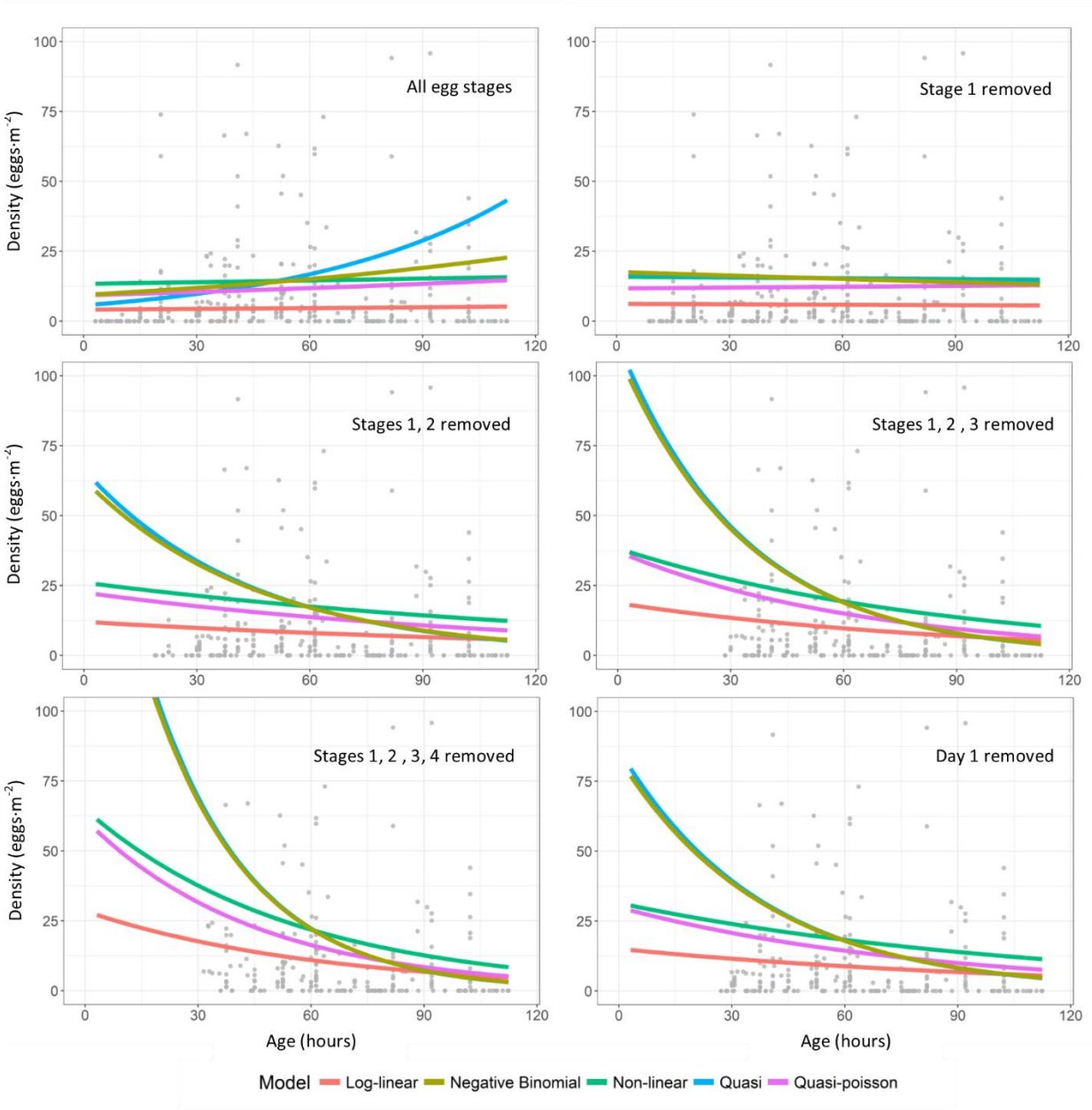

Figure 5. Egg production models (coloured lines) fitted to cohort egg densities (eggs·m^{-2}) and egg age (hours) of Redbait (grey circles) between western Tasmania and Kangaroo Island in October 2017. Six different datasets were used to assess the effects of under-representation of young eggs in samples. Egg stages up to Stage 4 were removed sequentially from each dataset: 'All eggs stages', 'Stage 1 removed', 'Stages 1-2 removed', 'Stages 1-3 removed', 'Stages 1-4 removed', and 'Day 1 removed' (eggs aged ≤24 hours removed). Note: The y-axis has been truncated and does not show the highest egg densities.

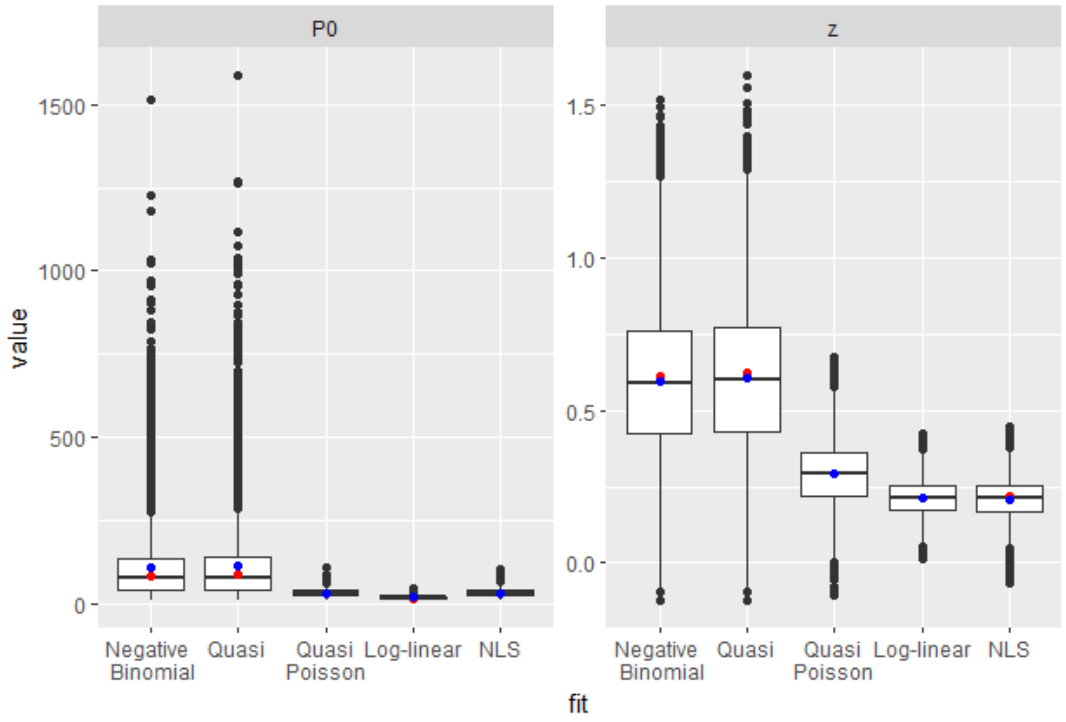

Figure 6. Estimates of mean daily egg production (P_0, eggs·day^{-1}·m^{-2}) and instantaneous daily mortality (z, day^{-1}) for Redbait, excluding day one eggs, from the five egg production models. NLS: Non-linear Least Squares. Red dot: mean estimate from field data; blue dot: mean estimate from bootstrapped data.

Table 5. Estimates of mean daily egg production (P_0, eggs·day^{-1}·m^{-2}) and instantaneous daily mortality (z, day^{-1}) for Redbait in October 2017, excluding day one eggs, generated by the five egg production models. Model averaged P_0 CIs: lower 95% CI of log-linear model and upper 95% CI of quasi-poisson GLM.

Egg Production Model	P_0 eggs·day^{-1}·m^{-2} (95% CI)	z day^{-1}
Log-linear	15.1 (10.4–27.0)	0.213
Non-linear least squares	31.4 (13.4–60.9)	0.217
Quasi GLM	86.3 (16.8–409.0)	0.627
Quasi-poisson GLM	29.9 (13.5–58.1)	0.293
Negative binomial GLM	83.2 (16.7–390.0)	0.614
Mean of log-linear and quasi-poisson GLM model fits	**22.5 (10.4–58.1)**	**0.253**

3.2 Adult Sampling

Nine of the 12 trawls undertaken from the *FV Western Alliance* caught substantial numbers of Redbait. Except for Trawl 12, which contained predominately immature fish, all of the other

nine samples contained mature, reproductively active Redbait. A total of 1,161 mature Redbait were sampled across the nine sites (Table 6). Estimates of the adult reproductive parameters used in calculations of spawning biomass are provided in Tables 6, 7 and 8. The means and bootstrapped 95% confidence intervals are shown in Table 8.

3.2.1 Female weight (*W*)

The mean weight of mature female Redbait in samples collected in 2017 ranged from 82.8 to 115.8 g (Table 6). The weighted mean weight of mature females in 2017 was 106.1 g (95% CI 101.2–110.5, Table 6, 8).

Table 6. Number of males and females of Redbait 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 (#).

Trawl	Male	Female	Mean male weight (g)	Mean female weight (g, *W*)	Sex ratio by weight (*R*)
1	314	102	96.9	98.4	0.25
2	213	102	96.5	96.9	0.32
3	179	101	95.5	102.6	0.38
4	149	103	100.7	106.1	0.42
5	62	102	110.0	110.5	0.62
7	61	102	105.6	115.8	0.65
8	75	102	101.1	113.8	0.60
9	104	102	105.6	106.7	0.50
12	4	10	80.2	82.8	0.72
Total	1161*	826*	99.2#	106.1#	0.43#

3.2.2 Sex ratio (*R*)

The sex ratio calculated from the 2017 survey was 0.43 (95% CI = 0.34–0.55, Table 6, 8). The mean sex ratio of samples ranged between 0.25 and 0.72 (Table 6). However, a sex ratio of 0.5 was used in the final spawning biomass calculation because males appear to be over-represented in demersal trawl samples taken during daylight hours.

3.2.3 Batch fecundity (*F*)

One hundred and nine female Redbait with hydrated oocytes were collected and used to determine batch fecundity in 2017. Based on the relationship between fecundity and female weight (Batch Fecundity = 111 × Gonad Free Female Weight – 1,510, $R^2 = 0.22$) for all females with hydrated oocytes collected in 2017 (Figure 7) and the mean gonad free female weight for all samples collected in 2017 (102.2 g), mean batch fecundity was 9,821 oocytes per batch (95% CI = 8,883–10,945; Table 8).

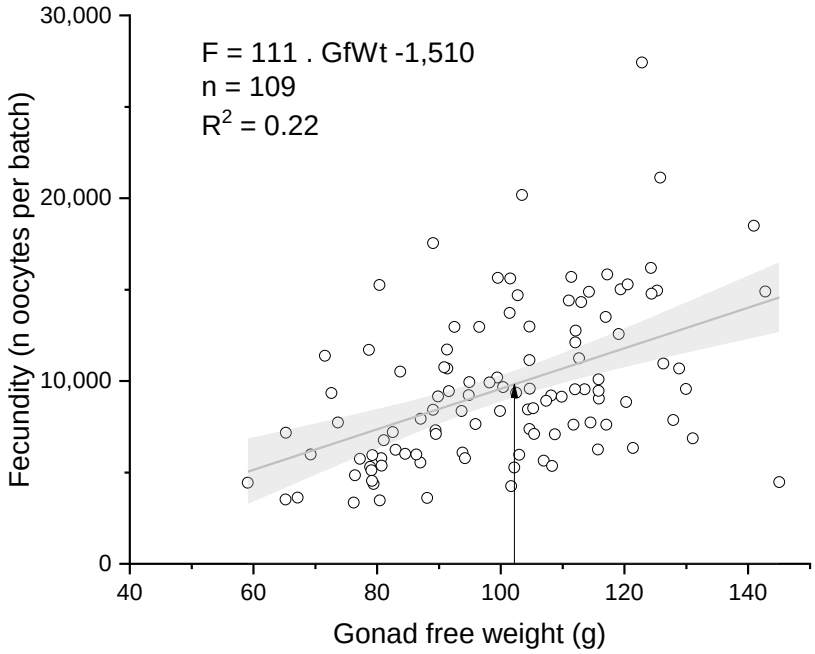

Figure 7. Relationship between gonad-free weight and batch fecundity for female Redbait with hydrated oocytes collected between western Tasmania and Kangaroo Island in October 2017 (open black circles, shaded area = 95% CI). The vertical arrow is the mean gonad free weight for 2017 (102.2 g).

3.2.4 Spawning fraction (S)

Of the 826 ovaries examined, 173 had hydrated oocytes, 172 had day-1 POFs, 203 day-2 POFs and 134 day-3 POFs (Table 7). The spawning fraction of females in each sample ranged from 0.17 to 0.25. The weighted mean spawning fraction for all mature female Redbait in 2017 was 0.21 (95% CI = 0.16–0.24, Table 8).

Table 7. Number of females in samples and estimates of spawning fraction, *S* of Redbait collected between western Tasmania and Kangaroo Island in October 2017 Values in bottom row are sums (*) and weighted means (#). POF: Post ovulatory follicles; H: Hydrated oocytes.

Trawl	Hydrated	Day 1 POF	Day 2 POF	Day 3 POF	Total females	H only	H+1	H+1+2	H+1+2+3
1	12	10	26	20	102	0.12	0.11	0.16	0.17
2	28	10	25	18	102	0.27	0.19	0.21	0.20
3	25	17	19	24	101	0.25	0.21	0.20	0.21
4	32	28	20	7	103	0.31	0.29	0.26	0.21
5	16	30	30	17	102	0.16	0.23	0.25	0.23
7	20	26	26	14	102	0.20	0.23	0.24	0.21
8	21	17	28	20	102	0.21	0.19	0.22	0.21
9	13	33	27	13	102	0.13	0.23	0.24	0.21
12	6	1	2	1	10	0.60	0.35	0.30	0.25
	173*	**172***	**203***	**134***	**826***	**0.21#**	**0.21#**	**0.22#**	**0.21#**

Table 8. Estimates of adult parameters and bootstrapped 95% confidence intervals for Redbait sampled between western Tasmania and Kangaroo Island in October 2017.

Reproductive Parameter	Estimate (95% CI)
Female Weight (*W*, g)	106.1 (101.2–110.5)
Batch Fecundity (*F*, eggs·female^{-1})	9,821 (8,883–10,945)
Sex Ratio (*R*)	0.43 (0.34–0.55)
Spawning Fraction (*S*)	0.21 (0.16–0.24)

3.3 Spawning Biomass (*SB*)

The estimate of spawning biomass for Redbait was 66,767 t (CI = 28,797–190,392). This value was calculated using the mean of the log-linear and quasi-poisson model fits to estimate P_0 (Table 5) and values of adult parameters from the current survey with the exception of *R*, which was set at 0.50 due to the skewed estimate obtained from the adult survey (Table 8). The model averaged CIs on the estimate of spawning biomass were the lower 95% CI of the log-linear model and upper 95% CI of the quasi-poisson GLM. The P_0 value from the log-linear model produced a spawning biomass estimate of 44,701 t (95% CI = 28,797–89,860); theestimate obtained using the P_0 value from the quasi-poisson model was 88,833 (95% CI = 38,352–190,392).

3.4 Sensitivity Analysis

The sensitivity analysis showed that variations in estimates of all parameters corresponding to known and potential uncertainties have strong influences on the estimates of spawning biomass (Figure 8). 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*; doubling the spawning area doubled the spawning biomass. *A* may have been under-estimated in this study because: 1) Redbait is known to occur in parts of the West sub-area not covered by the survey and 2) the presence of eggs in the most offshore sites on several transects suggests that spawning may have occurred in waters offshore from the survey. If *A* was under-estimated, it would have had a corresponding (conservative) effect on the estimate of spawning biomass.

The relationship between P_0 and spawning biomass was also linear. The sensitivity analysis showed the strong influence that the model used to estimate P_0 has on estimates of spawning

biomass (Figure 8). 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 quasi-poisson GLM (Ward et al. 2018c; SARDI unpublished data). 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 very high estimates of P_0. Using the mean P_0 value from the log-linear and quasi-poisson GLM model produced an estimate of spawning biomass lower than obtained using other models.

Estimates of spawning biomass increased as *S* decreased. The estimate of *S* obtained in the present study was based on a large number of samples; it is considered to be reliable and unbiased because similar estimates were obtained using females with hydrated oocytes, Day 1, Day 2 and Day 3 POFs. The estimate of *S* obtained in this study produced a higher estimate of spawning biomass than the estimate of *S* obtained by Neira and Lyle (2011).

Estimates of spawning biomass also increased as *R* decreased. Adjusting *R* to 0.50 produced a more conservative estimate of spawning biomass than the skewed value of 0.43 obtained from the trawl survey (Figure 8). The value of *R* (0.34) obtained from the trawl survey conducted by Neira and Lyle (2011) was also biased towards males and produced a higher estimate of spawning biomass than were obtained by adjusting R to 0.50.

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 larger and relatively less fecund than those collected off eastern Tasmania in 2005 and 2006 (Neira and Lyle 2011). Using the relationship between ovary free weight of females and batch fecundity reported by Neira and Lyle (2011) to estimate *F* produced a lower the estimate of spawning biomass than using the value of *F* estimated in the present study (Figure 8).

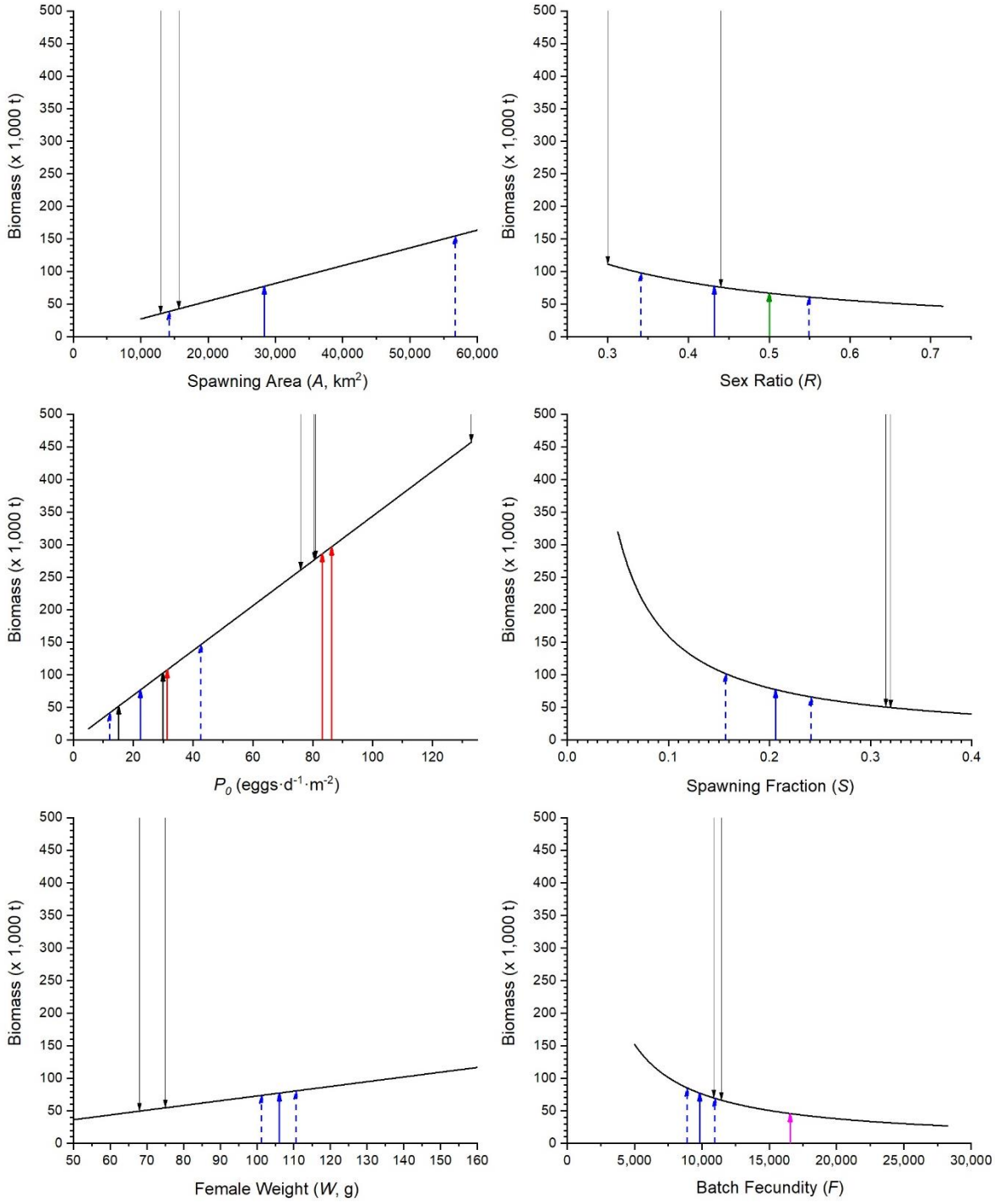

Figure 8. Sensitivity analysis of the effects of each parameter on estimates of spawning biomass of Redbait between western Tasmania and Kangaroo Island in October 2017. Blue solid: parameters used in the current DEPM calculations to estimate spawning biomass; Blue dashed: 95% CI of current DEPM parameters except *A* (*A* min: mean *A* from Neira and Lyle 2011, *A* max: current survey *A* doubled); Black arrows (upward): P_0 estimates from the log-linear and quasi-poisson GLM model fits; Black arrows (downward): Parameter estimates for Redbait East DEPM survey from Neira and Lyle 2011; Red: P_0 estimates from the remaining egg production model fits; Green: *R* set to 0.50; Magenta: *F* estimated using mean ovary free weight of current survey with the linear relationship reported by Neira and Lyle 2011.

4 DISCUSSION

4.1 Egg Distribution and Implications for Stock Structure

This study demonstrates that Redbait is widespread and relatively abundant in outer shelf and upper slope waters in the eastern portion of the West sub-area of the SPF. This finding contrasts with the distribution of Redbait catches by SPF vessels in the West sub-area, which have been taken from only a few locations; mainly a small area south-west of Kangaroo Island (Ward and Grammer 2018). The results of this survey do not suggest the presence of a discontinuity in the spawning habitat of Redbait off the Bonney Coast, which was observed for Australian Sardine and Jack Mackerel in previous surveys (e.g. Ward et al. 2018b).

The ranges of depths and SSTs at which Redbait eggs were collected during the present survey (38–850 m, 12.1–15.4°C) were similar to those in which Redbait eggs have been collected in the East sub-area of the SPF (25–1025 m, 11.5–15.5°C; Neira et al. 2008, 2009, Neira and Lyle 2011). No Redbait eggs were collected from Bass Strait. In combination with previous studies, the results of this survey suggest that Redbait occurs continuously at the shelf break between southern NSW and south-western Kangaroo Island, although Neira et al. (2009) showed that egg densities were relatively low south of Tasmania. It remains unclear whether Redbait occurring in the East and West sub-areas of the SPF comprise two separate stocks.

4.2 Spawning Biomass and Uncertainty

The sensitivity analyses conducted in this study showed that variations in all parameters corresponding to known and potential uncertainties in the values have strong effects on the estimate of spawning biomass. The effects of this uncertainty were managed by choosing parameter values that were considered to be robust and/or produced conservative estimates of spawning biomass.

The spawning area of Redbait in waters between western Kangaroo Island and south-western Tasmania (28,365 km^2) was more than double the largest estimate of spawning area obtained for the east coast (13,220 km^2; Neira and Lyle 2011). As spawning biomass is strongly correlated with spawning area (Mangel and Smith 1990, Gaughan et al. 2004; Ward et al. 2017), this finding suggests the spawning biomass of Redbait in the West sub-area of the SPF is substatially larger than in the East sub-area. However, it should be noted that Neira and Lyle (2011) excluded some parts of the East sub-area where eggs were present in low densities from the data-set used to estimate spawning area (i.e. south of 43.5°S). It should

also be noted, that the estimate of spawning area used to calculate spawning biomass in the present study is considered to be conservative because: 1) Redbait eggs were collected from the most offshore sites on several transects, suggesting spawning may also have occurred in waters not covered by the survey, and 2) Redbait is known to occur in parts of the West sub-area (e.g. the Great Australian Bight; Gomon et al. 2008) that were not sampled in the present study.

The uncertainties associated with estimation of P_0 are well known (Fletcher et al. 1996, McGarvey and Kinloch 2001, Ward et al. 2001a, 2001b, Gaughan et al. 2004, McGarvey et al. 2018). The estimate of P_0 obtained in the present study (22.5 eggs·m^{-2}·day^{-1}) is considered to be conservative because rates of misidentification of Redbait eggs appear to be low (Appendix 1) and the models used to estimate P_0 have been shown in previous studies to produce lower estimates of this parameter than other models. The estimate of P_0 used to estimate spawning biomass in the present study is also much lower than the values presented by Neira and Lyle (2011) for the East sub-area of ~80 eggs·m^{-2}·day^{-1} (presented as ~4 eggs·0.05 m^{-2}·day^{-1}). The model comparisons conducted in the present study suggest that the high estimates of P_0 obtained by Neira and Lyle (2011) may reflect their use of a GLM with a negative binomial error distribution. In the present study, the GLM with a negative binomial error distribution produced an estimate of P_0 of 83.2 eggs·m^{-2}·day^{-1}, which is similar to the estimate of Neira and Lyle (2011) and more than twice as high as the estimates obtained using the log-linear model (15.07 eggs·m^{-2}·day^{-1}) and the quasi poisson GLM (29.9 eggs·m^{-2}·day^{-1}). Although in the present study, the non-linear least squares model produced an estimate of P_0 similar to the quasi-poisson GLM, it was excluded from the model averaging process because previous studies have shown that a few samples with very high egg densities can strongly influence estimates of P_0 obtained using this model (Ward et al. 2018a).

The estimate of S used to estimate spawning biomass in the present study (0.21) is lower than the estimates of S (i.e. 0.32) obtained by Neira and Lyle (2011). Estimates of S obtained in both studies were based on relatively large numbers of fish. The difference in S between the two studies may reflect real spatial or temporal variations in spawning rates. The estimate of S obtained in the present study is considered to be reliable and was used to estimate spawning biomass because similar estimates were obtained using females with hydrated oocytes, Day 1, Day 2 and Day 3 POFs, suggesting that samples were not biased towards females that were (or were not) spawning on a particular night (Stratoudakis et al. 2006).

The estimates of sex ratio obtained in the present study (0.43) and reported by Neira and Lyle (2011) for the East sub-area (0.32 in 2005 and 0.44 in 2006) suggest that males are over-represented in trawl samples. In the present study, we used the predicted R of 0.5 to estimate

spawning biomass because it is more plausible than the estimates from the trawl surveys and provides a more conservative estimate of spawning biomass.

The relationship between *W* and *F* established in the present study was obtained from over 100 females with hydrated oocytes and considered robust. Females collected in the present study were larger and relatively less fecund than those sampled by Neira and Lyle (2011). Samples taken by Neira and Lyle (2011) were taken by mid-water trawling at night, whereas samples obtained in the present study were taken in demersal trawls during the day. The apparent difference in the relationship between batch fecundity and female size in the East and West sub-areas warrants further investigation.

Like the estimates of spawning biomass obtained in other studies (see Stratoudakis et al. 2006, Bernal et al. 2012, Dickey-Collas et al. 2012), the estimate of spawning biomass obtained in the present study is imprecise, i.e. 66,767 t (CI = 28,797–190,392). As has been done in other applications of the DEPM to the SPF and the South Australian Sardine Fishery, this inherent uncertainty has been managed by ensuring methods used to estimate the key parameters are robust and/or conservative. For this reason, the estimate of spawning biomass presented in this report provides a suitable basis for setting RBCs and TACs for Redbait in the West sub-area in the SPF.

5 SUMMARY AND CONCLUSIONS

This study was the first dedicated application of the DEPM to Redbait in the West sub-area of the SPF. It was undertaken in the region between western Kangaroo Island, South Australia and south-western Tasmania, because that was where fishing was undertaken in the SPF in the period immediately preceding the survey. The results showed that Redbait is widely distributed and relatively abundant in this eastern portion of the West sub-area. The estimate of spawning biomass 66,767 t (CI = 28,797–190,392) is considered suitable for setting RBCs, because it is based on robust and/or conservative estimates of key parameters.

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APPENDICES

APPENDIX 1: Genetic identification of Redbait (*Emmelichthys nitidus*) eggs

J P. Keane

Introduction

Morphological identification of planktonic fish eggs to species is complex given the similarity of morphological characters that a vast number of species spawning at any one time may possess. There are over 5000 fish species in Australian waters, and it is estimated that 70% of eggs are less than 1.5mm, 60% have a single oil globule and most have a smooth chorion (Ahlstrom and Moser 1980). Identification is further complicated by the complex developmental changes from fertilisation through to hatching.

Ichthyoplankton samples are typically fixed in formalin, as it results in good preservation of morphological characters (Steedman, 1976). However, formaldehyde interacts with DNA making genetic identification problematic (Karaiskou *et al.,* 2007; Goodsir *et al.,* 2008). In contrast, ethanol is a reliable preservative for DNA but causes fish eggs to shrink and become opaque, leading to difficulties in visually identifying or assigning developmental stages to eggs (Goodsir *et al.,* 2008). As such there is no preservation method that produces good samples for both molecular and morphological identification.

In this study, we employed a molecular approach to identify and validate ethanol preserved eggs of Redbait (*Emmelichthys nitidus*), as well as to identify eggs that possess similar morphological characteristics to this species.

Methods

Samples

Replicate ichthyoplankton samples to the main DEPM survey were completed at approximately every second station; the sample was immediately drained of excess seawater and preserved in 96% ethanol. Ichthyoplankton samples were collected using a bongo sampler equipped with 500 um mesh, two 3 m long plankton nets enclosed in a purpose built, weighted stainless steel frame to facilitate vertical drops. The mouth of each net (0.6 m diameter) was fitted with a General Oceanics flowmeter to estimate total volume of water filtered during each vertical haul. The net was lowered to within 10 m of the seabed or to a maximum of 200 m. Haul speed was around 0.3 ms^{-1}.

Redbait eggs were identified using morphological features described by Neira et al. (2008) by scientists at the South Australian Research and Development Institute (SARDI). A subset of eggs identified as Redbait (n = 48) and morphologically similar eggs (n = 15) were sent to the Institute for Marine and Antarctic Studies (Hobart, Tasmania) for molecular identification.

Molecular identification

A molecular approach of Mitochondrial DNA (mtDNA) extraction, amplification, and sequencing established by Ward et al. (2005) was employed to genetically identify ethanol preserved fish eggs. DNA extractions from the 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′ACTTCAGGGTGACCGAAGAA TCAGAA3′) were used in the PCRs to amplify a fragment (~ 655 bp) from the 5′ region of the *cox1* gene. Sequences were aligned to reference data in the Fish Barcode of Life Database (BOLD) using BioEdit biological sequence alignment editor.

The 25µl PCR reaction mixes included 18.75 µl of ultrapure water, 2.25µl of 10× PCR buffer, 1.25µl of $MgCl_2$ (50mM), 0.25µl of each primer (0.01mM), 0.125µl of each dNTP (0.05mM), 0.625U of *Taq* polymerase, and 0.5–2.0µl of DNA template. Amplifications were performed using a Mastercycler® Eppendorf gradient thermal cycler (Brinkmann Instruments, Inc.). The thermal regime consisted of an initial step of 2 min at 95°C followed by 35 cycles of 0.5min at 94°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.

Results and Discussion

Of the 63 eggs subjected to mtDNA analysis, 60 (95%) yielded quality DNA to enable sequencing (Table 1). Molecular identification via alignment of the *cox1* sequences revealed a total of 39 eggs, 87%, matching that of initial morphological identifications. The 6 eggs suspected to be Redbait that aligned with other species were all early to mid stage eggs (Stage 3–6). From the 15 suspected non-Redbait eggs subjected to molecular analyses, all yielded quality mtDNA to enable sequencing. All but one sequences aligned to species other than Redbait indicating minimal misidentification of non-Redbait eggs (Table 1). The single miss-identified egg was at developmental Stage 3 where minimal morphological features are present.

Table 1. *Cox1*-based specific identifications of morphologically identified Redbait and similar eggs.

		Genetic Identification			
Morphological identification	**n tested**	**Redbait**	**Other**	**No DNA**	**Notes**
Redbait	48	39	6	3	Misidentified eggs aligned with: *Caprodon longimanus* (n=2; 93%) *Thyrsites atun* (n=1; 99%) *Coelorinchus simorhynchus* (n=1; 92%) *Centroberyx lineatus* (n=1; 87%) *Achoerodus viridis* (n=1; 97%)
Not Redbait, but possessing some similar characteristics	15	1	14		Sequences aligned with: *Thyrsites atun* (n=7; 99-100%) *Caprodon longimanus* (n=4 92-93%) Other (n=3)

Molecular identification confirmed the morphometric identification of Redbait and non-redbait eggs, with very low levels of misidentifications in early to mid stage eggs. Misidentifications in ethanol preserved eggs is not uncommon given ethanol causes fish eggs to shrink and become opaque, thus masking key morphological characters (Goodsir et al., 2008). As such, morphological identifications from formalin preserved eggs within the main DEPM study are likely to be of higher accuracy than ethanol preserved eggs used for validation purposes. The difficulty in visually identifying or assigning developmental stages to ethanol preserved eggs infers that ethanol should not be used as a primary fixative when morphological identification and staging is required. Rather ethanol fixation should complement formalin fixed samples to facilitate genetic validation of formalin fixed samples as has been done in this study. Formalin fixation results in good preservation of morphological characters, however, formaldehyde interacts with DNA making genetic identification problematic (Steedman, 1976; Karaiskou et al., 2007; Goodsir et al., 2008). Uncertainties in the identification of Redbait would be resolved by developing a species-specific *in situ* hybridisation probe for the species (see Oxley et al 2017).

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APPENDIX 2: Adult sampling locations for Jack Mackerel West DEPM

Table 1: Date, time and locations of trawls off the *FV Western Alliance* for Jack Mackerel during the 2016/17 DEPM survey.

Shot no.	Date	Start Time End Time	Start Latitude Longitude End Latitude Longitude		Temp. °C Surface: Bottom	Depth (m)
1	30/01/17	09:49 11:40	39°10.04 143°44.25	39°03.51 143°45.19	18.5:15.3	85-88
2	30/01/17	13:04 15:05	38°57.21 143°43.98	38°51.52 143°49.69	18.8:15.6	75-80
3	30/01/17	15:53 17:54	38°49.87 143°49.78	38°54.86 143°42.43	18.7:N/A	75
4	31/01/17	07:35 09:37	38°57.58 142°20.53	38°52.48 142°13.84	18.2:N/A	170-180
5	31/01/17	10:33 12:31	38°52.79 142°10.37	38°49.79 142°02.35	18.2:12.4	173-190
6	01/02/17	12:49 14:26	41°18.98 144°26.17	41°25.00 144°25.65	16.3:N/A	150-180
7	01/02/17	15:36 17:19	41°33.13 144°26.08	41°39.10 144°28.88	16.5:N/A	180-220
8	01/02/17	19:56 21:39	41°58.61 144°39.88	42°03.87 144°43.43	16.9:N/A	170-180
9	02/02/17	07:65 09:57	42°41.04 144°56.25	42°48.44 144°57.00	15.8:N/A	160-190
10	02/02/17	12:44 14:42	43°02.85 145°11.98	43°08.01 145°18.63	14.9:N/A	155-165
11	02/02/17	15:35 17:35	43°12.49 145°22.09	43°18.00 145°28.62	14.4:N/A	170-175
12	03/02/17	14:37 16:05	41°24.15 144°25.73	41°18.76 144°26.20	16.1:N/A	150-170