

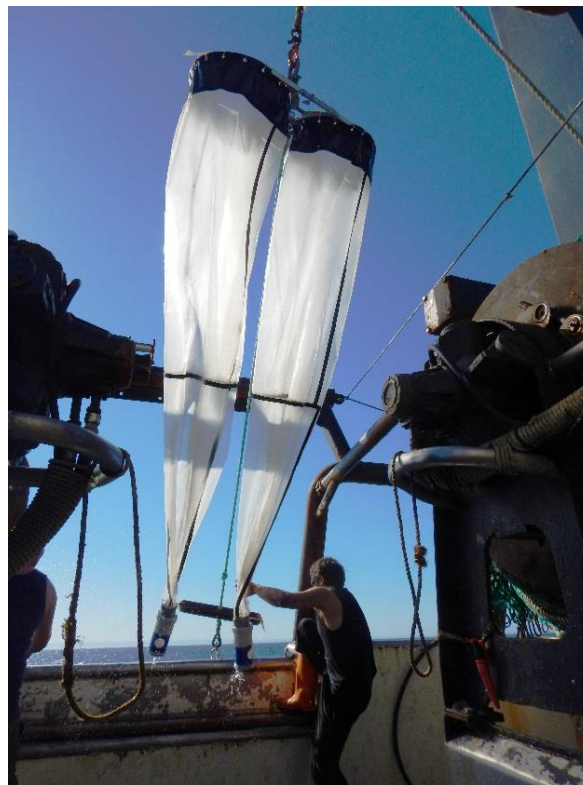


Australian Government
Australian Fisheries Management Authority

RR2016/0803 May 2018



Spawning biomass of Jack Mackerel (*Trachurus declivis*) and Sardine (*Sardinops sagax*) between western Kangaroo Island, South Australia and south-western Tasmania



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



www.afma.gov.au

 Protecting **our** fishing future

**Spawning biomass of Jack Mackerel
(*Trachurus declivis*) and Sardine (*Sardinops sagax*)
between western Kangaroo Island, South Australia and
south-western Tasmania**

Report to the Australian Fisheries Management Authority

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

**SARDI Publication No. F2018/000174-1
SARDI Research Report Series No. 983**

June 2018

This publication may be cited as:

Ward, T. M., Grammer, G. L., Ivey, A. R., Smart, J. J. and Keane, P. (2018). Spawning biomass of Jack Mackerel (*Trachurus declivis*) and Sardine (*Sardinops sagax*) between western Kangaroo Island, South Australia and south-western Tasmania. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2018/000174-1. SARDI Research Report Series No. 983. 51pp.

South Australian Research and Development Institute

SARDI Aquatic Sciences

2 Hamra Avenue

West Beach SA 5024

Telephone: (08) 8207 5400

Facsimile: (08) 8207 5415

<http://www.pir.sa.gov.au/research>

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 Chief, 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.

© 2018 SARDI

This work is copyright. Apart from any use as permitted under the *Copyright Act* 1968 (Cth), no part may be reproduced by any process, electronic or otherwise, without the specific written permission of the copyright owner. Neither may information be stored electronically in any form whatsoever without such permission.

SARDI Publication No. F2018/000174-1

SARDI Research Report Series No. 983

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

Reviewer(s): J. Tanner, O. Burnell (SARDI) and K. Bennetts (AFMA)

Approved by: S. Mayfield
Science Leader - Fisheries

Signed: 

Date: 4 June 2018

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

Circulation: Public Domain

TABLE OF CONTENTS

LIST OF FIGURES	V
LIST OF TABLES.....	VI
ACKNOWLEDGEMENTS	VII
ABBREVIATIONS	VIII
EXECUTIVE SUMMARY	1
INTRODUCTION	4
1.1 Background.....	4
1.2 The Daily Egg Production Method (DEPM)	5
1.3 Jack Mackerel.....	7
1.4 Australian Sardine.....	8
1.5 Need.....	9
1.6 Objectives.....	10
2 METHODS.....	11
2.1 Total Daily Egg Production.....	11
2.2 Adult Reproductive Parameters	19
2.3 Spawning Biomass (<i>SB</i>)	22
2.4 Sensitivity Analysis	22
3 RESULTS	23
3.1 Jack Mackerel DEPM.....	23
3.2 Sardine DEPM	29
4 DISCUSSION	35
4.1 Distribution and stock structure.....	35
4.2 Jack Mackerel.....	35
4.3 Australian Sardine.....	36
5 CONCLUSIONS.....	37
REFERENCES	38
APPENDICES.....	43

LIST OF FIGURES

Figure 1: DEPM survey area along the continental shelf of southeastern Australia..	12
Figure 2. Representative egg stages of Jack Mackerel and Sardine using the ‘universal’ egg stages of Ward et al. 2018.	14
Figure 3. Voronoi natural neighbour polygons used to estimate spawning area Dots: area estimated for exploratory sampling sites outside main survey area.....	17
Figure 4. Spatial patterns of Jack Mackerel egg distribution and abundance between December 2016 and February 2017.	24
Figure 5. Estimates of mean daily egg production (P_0 , eggs·day ⁻¹ ·m ⁻²) and instantaneous daily mortality (z , day ⁻¹) for Jack Mackerel.	25
Figure 6. Egg production model fits to cohort egg densities (eggs·m ⁻²) plotted by egg age (hours) for Jack Mackerel.....	26
Figure 7. Sensitivity analysis of the effects of each parameter on estimates of spawning biomass of Jack Mackerel.....	28
Figure 8: Spatial patterns of Sardine egg distribution and abundance between December 2016 and February 2017.....	30
Figure 9. Estimates of mean daily egg production (P_0 , eggs·day ⁻¹ ·m ⁻²) and instantaneous daily mortality (z , day ⁻¹) for Australian Sardine	31
Figure 10. Egg production model fits to cohort egg densities (eggs·m ⁻²) plotted by egg age (hours) for Sardine	32
Figure 11. Sensitivity analysis of the effects of each parameter on estimates of spawning biomass of Sardine.....	34

LIST OF TABLES

Table 1. The Daily Egg Production Method (DEPM) equation to calculate spawning biomass (<i>SB</i>) and details of other equations	6
Table 2. References used to identify the eggs of Jack Mackerel and Sardine and for species-specific egg temperature-development rates.....	13
Table 3. Details of the equations used for the adult parameters of Jack Mackerel and Australian Sardine to estimate spawning biomass.....	21
Table 4. Spawning Area (<i>A</i>) and total area surveyed for Jack Mackerel.	23
Table 5. Point estimates of mean daily egg production (P_0 , eggs·day ⁻¹ ·m ⁻²) and instantaneous daily mortality (z , day ⁻¹) for Jack Mackerel.....	25
Table 6. Mean, minimum and maximum adult parameters calculated for Jack Mackerel during the 2014 DEPM survey off eastern Tasmania and southern NSW.	26
Table 7. Spawning Area (<i>A</i>) and total area surveyed for Australian Sardine	29
Table 8. Point estimates of mean daily egg production (P_0 , eggs·day ⁻¹ ·m ⁻²) and instantaneous daily mortality (z , day ⁻¹) for Australian Sardine	31
Table 9. Mean, minimum and maximum adult parameters calculated for Australian Sardine from South Australian DEPM surveys between 1998 and 2016.....	33

ACKNOWLEDGEMENTS

This research was funded by the Australian Fisheries Management Authority (AFMA). We thank the masters, crews, owners and staff of the *RV Ngerin*, *FV Dell Richie II* and *FV Western Alliance* for their professional and cheerful assistance. The efforts of Mr Stuart Richie and Mr David Guillot to ensure the success of the surveys are recognised with gratitude. We thank Mr Stuart Sexton, Mr Leonardo Mantilla, Mr Ian Moody, Mr Josh Nitschke and Mr Nick Hill for working diligently in the field and laboratory to collect and process project samples. This report was formally reviewed by Dr Jason Tanner and Dr Owen Burnell 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
GAB	Great Australian Bight
GLM	Generalised Linear Models
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

EXECUTIVE SUMMARY

Background and Need

The Harvest Strategy for the Small Pelagic Fishery (SPF) specifies that estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the primary biological performance indicator for target species. Estimates of spawning biomass are used to set Recommended Biological Catches (RBCs) and Total Allowable Catches (TACs).

Prior to this study, the DEPM had been applied to all SPF target species in the East sub-area, and to Blue Mackerel in the West sub-area. The need to apply the DEPM to Jack Mackerel and Redbait in the West sub-area increased in 2014/15, when a factory trawler entered the SPF and began operating in both sub-areas. In the West sub-area, the factory trawler operated between western Kangaroo Island, South Australia and south-western Tasmania.

The DEPM was applied to Jack Mackerel in waters between western Kangaroo Island, South Australia and south-western Tasmania in December 2016 to February 2017. Exploratory egg sampling was also undertaken in Bass Strait and off north-eastern Tasmania.

Objectives

The objectives of this study were to:

1. Determine distribution and abundance of eggs of Jack Mackerel and Sardine between western Kangaroo Island, South Australia and south-western Tasmania.
2. Estimate adult reproductive parameters of Jack Mackerel during the peak spawning period between western Kangaroo Island and south-western Tasmania.
3. Estimate the spawning biomass of Jack Mackerel and Sardine between western Kangaroo Island and south-western Tasmania.

The original proposal for this study did not include application of the DEPM to Australian Sardine. This species was added to the objectives when it became evident that significant numbers of eggs of Australian Sardine were present in the plankton samples.

Methods

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

To estimate total daily egg production, ichthyoplankton samples were collected from the *RV Ngerin* from 306 sites in shelf waters between western Kangaroo Island, South Australia and

south-western Tasmania between 2 December 2016 and 6 February 2017. Ichthyoplankton samples were also collected opportunistically from the *FV Western Alliance* at 41 sites in Bass Strait and along the coast of north-eastern Tasmania. A total of 347 sites was sampled.

Jack Mackerel and Sardine eggs were identified using standard laboratory procedures. Egg identifications of Jack Mackerel were confirmed using molecular techniques. Spawning area was estimated using the Voronoi nearest neighbour method. Five models were used to estimate egg production (P_0). For Jack Mackerel, the value of P_0 used to estimate spawning biomass was the mean estimate from four models. For Sardine, the estimate of P_0 used to estimate spawning biomass was the value obtained from the log-linear model.

Modified demersal trawls for adult Jack Mackerel were undertaken from the *FV Western Alliance* at 12 sites in shelf and slope waters between Portland, Victoria and western Tasmania during 30 January to 3 February 2017. Few adult Jack Mackerel and no adult Sardine were collected. Adult parameters for Jack Mackerel used in this study were obtained from a trawl survey conducted off eastern Tasmania and Victoria during 2014. Adult parameters for Sardine were obtained from surveys conducted off South Australia between 1998 and 2016.

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 639 live Jack Mackerel eggs were collected from 55 of the 347 sites. The highest densities of eggs were recorded in waters north-west of King Island, south-east of Kangaroo Island and in Bass Strait. Most Jack Mackerel eggs were collected from sites with bottom depths of 48–97 m and sea surface temperatures (SSTs) of 16.0–19.6°C. The estimated spawning area from the extended survey was 13,898 km², comprising 15.9% of the total survey area (87,374 km²). Mean daily egg production (P_0) was 9.6 eggs·day⁻¹·m⁻². Adult parameters [mean (min–max)] from samples collected off eastern Tasmania and Victoria in 2014 were: sex ratio (R): 0.47 (0.38–0.56), female weight (W): 208.8 g (133.9–250.9), batch fecundity (F): 34,068 eggs (16,599–94,743), and spawning fraction (S): 0.056 (0.000–0.134). The estimate of spawning biomass from the extended survey of ~31,000 t is indicative of adult abundance between western Kangaroo Island and south-western Tasmania, but is an underestimate of the total abundance of Jack Mackerel in the West sub-area. Sensitivity analysis showed that, for this study, spawning area had a larger effect on estimates of spawning biomass than S or P_0 .

A total of 4,837 live Sardine eggs were collected from 105 of the 347 sites. The highest densities of Sardine eggs were collected south-east of Kangaroo Island, north of King Island

and in Bass Strait. Most Sardine eggs came from sites with bottom depths of 22–156 m and SSTs of 15.8–20.4°C. The estimate of spawning area from the extended survey was 26,366 km² (30.2% of the 87,374 km² total survey area). Mean daily egg production (P_0) was 33.2 eggs·day⁻¹·m⁻² (95% CI = 19.5–63.5). Adult parameters [mean (min–max)] from Sardine collected off South Australia during 1998–2016 were: sex ratio (R): 0.54 (0.36–0.68), female weight (W): 57.0 g (45.2–78.7), batch fecundity (F): 17,116 eggs (10,904–24,790), and spawning fraction (S): 0.114 (0.040–0.179). The estimate of spawning biomass from the extended survey of ~47,000 t is indicative of adult abundance between western Kangaroo Island and south-western Tasmania, but is an underestimate of the total abundance of Sardine in the West sub-area. Sensitivity analysis showed that the egg production model had a strong influence on estimates of P_0 and spawning biomass. For Sardine, the log-linear model usually provides estimates of P_0 that are more precise and lower than those from other models.

The estimates of spawning biomass of Jack Mackerel (31,069 t) and Sardine (47,283 t) for the portion of the West Sub-area between western Kangaroo Island and south western Tasmania, where a factory trawler operated in 2014/15 and 2015/16, are underestimates of the spawning biomass in the West sub-area. Exploratory ichthyoplankton sampling suggested that key spawning habitat in Bass Strait was not included in the survey. Both Jack Mackerel and Sardine also occur west of the survey area.

Our results suggest that distinct sub-populations of both Jack Mackerel and Sardine occur between the Bonney Coast and southern NSW, including waters off north-eastern Tasmania, and that Bass Strait is an important spawning area for both species. Future DEPM studies should be designed to cover this entire area.

Keywords: Jack Mackerel, *Trachurus declivis*, Australian Sardine, *Sardinops sagax*, Daily Egg Production Method, Spawning Biomass, Small Pelagic Fishery, 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. Catches of Jack Mackerel peaked at ~40,000 t in 1986/87, making it Australia's largest fishery by weight at that time (Kailola *et al.* 1993, Pullen 1994, Ward and Grammer 2017).

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

A detailed history of the SPF is described in Moore and Skirtun (2012). Catch and effort in the SPF have fluctuated over time, driven by a combination of social, economic and biological factors. Catch and effort increased in 2014/15 to 2015/16 when a factory trawler operated in both sub-areas (Ward and Grammer 2017).

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

The DEPM has previously been applied to Jack Mackerel in the East sub-area (Ward *et al.* 2015b, Ward *et al.* 2016). The present study is the first application of the DEPM in the West sub-area of the SPF. It was conducted in the area between western Kangaroo Island, South Australia and south-western Tasmania to focus on the portion of the West sub-area where the factory trawler was operating in 2014/15 and 2015/16.

1.2 Daily Egg Production Method (DEPM)

The difficulties associated with using fishery-dependent methods for stock assessment of schooling fishes have been recognised widely for many years (e.g. Walters and Maguire 1996, Barange *et al.* 1999, Gaertner and Dreyfus-Leon 2004). For example, over-estimation of stock size is a major risk when commercial catch-per-unit-effort (CPUE) is used as an index of abundance; this approach has contributed to the collapse of several major fisheries (e.g. Walters and Maguire 1996). The use of fishery-dependent data is particularly problematic in new and developing fisheries, such as the SPF, where fishing activity is often sporadic and focused on a small and unknown portion of the total stock, and fishing methods, including vessels, change rapidly (see Ward *et al.* 2017). The Daily Egg Production Method (DEPM) has been widely applied to small pelagic fish because it is often the most practical method available for fishery-independent stock assessment (Parker 1980, Lasker 1985, Ward and McLeay 1998, Stratoudakis *et al.* 2006, Ward *et al.* 2009, 2017). However, acoustic methods have replaced or been used in conjunction with the DEPM in situations where the schooling behaviour of the target species is well understood (e.g. Coetzee 2000), and the resources (e.g. research vessels and sampling equipment) needed to produce reliable acoustic-based estimates of relative or absolute abundance are available (e.g. Barange *et al.* 1999, Zwolinski *et al.* 2012).

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 Daily Egg Production Method (DEPM) equation to calculate spawning biomass (*SB*) for Jack Mackerel and Sardine.

Model Name	Equation	Eq. No.	Parameters	Reference
Daily Egg Production Method	$SB = \frac{P_0 A W}{R F S}$	(1)	<i>SB</i> : Spawning Biomass <i>P</i> ₀ : mean daily egg production <i>A</i> : total spawning area <i>W</i> : mean female weight <i>R</i> : mean sex ratio <i>F</i> : mean batch fecundity <i>S</i> : 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 driven mainly 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). In contrast, several authors have noted the advantages of presence-absence sampling used to estimate *A* and the high correlation of spawning area with spawning biomass (Mangel and Smith 1990, Gaughan *et al.* 2004). 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.* 2018). One of the options identified was to use the most precise method, which had a likely negative bias and reduced the potential for over-estimation of stock size (see Ward *et al.* 2018). 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 Jack Mackerel

Egg production methods have been applied to several trachurid species. For example, since 1995, annual egg production surveys have been applied to Horse Mackerel (*Trachurus trachurus*) off the Iberian Peninsula (Ward *et al.* 2015a). The DEPM was first applied to this stock in 2007. The DEPM has been successfully applied to Chilean Jack Mackerel (*Trachurus murphyi*) off the central coast of Chile (1999-2006; Ruiz *et al.* 2008) and also to Yellowtail Scad (*Trachurus novaezelandiae*) off eastern Australia in 2009 (Neira 2009).

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

Jack Mackerel is a serial spawner (Marshall *et al.* 1993, Neira 2011). It spawns in spring along the New South Wales (NSW) coast (Maxwell 1979, Keane 2009) and during summer further south off Tasmania and in the Great Australian Bight (Stevens *et al.* 1984, Jordan *et al.* 1995, Ward *et al.* 2016, Sexton *et al.* 2017). The main spawning area is thought to be located off south-eastern Australia in eastern Bass Strait and off eastern Tasmania, Victoria and southern NSW (Bulman *et al.* 2015). Off eastern Tasmania, spawning occurs continuously from December to February (Williams and Pullen 1986, Jordan 1994, Neira 2011). Limited information is available on the spawning patterns in the West sub-area (Stevens *et al.* 1984).

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

The first dedicated application of the DEPM to Jack Mackerel off the south-east coast of Australia was done in 2014 (Ward *et al.* 2015b). The estimate of spawning biomass of 157,805 t (95% CI) was based on reliable estimates of key adult parameters and considered robust. A preliminary study based on samples collected off south-eastern Australia in 2002–2004 provided estimates of spawning biomass in the range of 114,000–169,000 t (Neira 2011). Ecosystem modelling estimated the spawning biomass of Jack Mackerel of south-east Australia to be 130,000–170,000 t (Fulton 2013). Similar ecosystem modelling suggested the

biomass of Jack Mackerel west of Tasmania was approximately 60,000–110,000 t (Smith *et al.* 2015).

1.4 Australian Sardine

Sardines (*Sardinops*, *Sardina*, *Sardinella*) form the basis of some of the world's largest fisheries (Schwartzlose *et al.* 1999) and have been the focus of extensive research (e.g. Stratoudakis *et al.* 2006). The DEPM has been widely applied to Sardines both within Australia and overseas. For example, the DEPM has been used since 1994 to estimate the spawning biomass off California and is the longest fishery-independent biomass index for Pacific Sardine (*Sardinops sagax*) (Ward *et al.* 2015a). The DEPM has also been applied to Sardine populations off Peru (*S. sagax*), the Iberian Peninsula (*Sardina pilchardus*), and Brazil (*Sardinella brasiliensis*) (Alheit 1993, Ward *et al.* 2015a).

Sardine (*Sardinops sagax*) occurs throughout temperate Australian waters from Rockhampton (Queensland) to Shark Bay (Western Australia), including northern Tasmania (Gomon *et al.* 2008). Australian Sardine is structured as a meta-population where extensive mixing occurs among neighbouring sub-groups (Whittington *et al.* 2008, Izzo *et al.* 2017). An integrated analysis by Izzo *et al.* (2017) suggested there were at least four stocks: 1) south-western Australia; 2) South Australia; 3) south-eastern Australia; and 4) eastern Australia. There is also evidence that two separate spawning groups occur off eastern Australia: a northern group off southern Queensland and northern NSW, and a southern group off southern NSW to Tasmania (Sexton *et al.* in press).

Commercial fishing for Sardine in Australia began in the 1800s (Kailola *et al.* 1993), however combined national catches did not exceed 1,000 t until the 1970s. Purse-seine fisheries developed off south-western Western Australia and South Australia in the 1980s and 1990s, respectively (Kailola *et al.* 1993, Ward and Staunton-Smith 2002). Mass mortality events in 1995 and 1998/9 caused by a herpesvirus reduced the adult biomass of Australian Sardine populations by ~70% (Ward *et al.* 2001b, Gaughan *et al.* 2004). The fishery off South Australia recovered quickly and is now Australia's largest fishery by weight, with a total catch of over 40,000 t in 2017 (Ward *et al.* 2017). In contrast, the fishery off Western Australia has remained relatively small, with a total catch of approximately 1,500 t in 2014 (Fletcher and Santoro 2015). Off eastern Australia, the annual catch of Sardine increased rapidly during the early 2000s, peaking at almost 5,000 t in 2008/09, but since then has declined due to a variety of factors that have reduced the level of fishing effort (Izzo *et al.* 2017, Ward and Grammer 2017).

In Australia, Sardines spawn in open waters between the coast and shelf break (Blackburn 1950, Fletcher and Tregonning 1992, Fletcher *et al.* 1994). They are serial spawners with asynchronous oocyte development and indeterminate fecundity. Spawning peaks in winter–early spring in the northern part of its range, off southern Queensland and northern NSW and in summer further south (Sexton *et al.* in press).

The DEPM has been used to estimate the spawning biomass of Australian Sardine in South Australia since 1995 (Ward *et al.* 2017). During 2014, DEPM surveys for Sardine were undertaken off eastern Australia during both summer and winter/spring (Ward *et al.* 2015b, 2015c). The summer survey in January 2014 from southern NSW to central Tasmania estimated the spawning biomass to be approximately 11,000 t (Ward *et al.* 2015b). The winter/spring survey in August/September 2014 between southern Queensland and NSW estimated the spawning biomass to be approximately 50,000 t (Ward *et al.* 2015c).

1.5 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 in the West Sub-area, but not to Jack Mackerel or Redbait in the West sub-area.

The need to apply the DEPM to Jack Mackerel and Redbait in the West sub-area increased when a factory trawler entered the SPF in 2014/15 and began taking both species in both sub-areas (Ward and Grammer 2017). As the factory-trawler operated in the part of the West sub-area between western Kangaroo Island, South Australia and south-western Tasmania, this area was identified as the region in which the DEPM surveys of Jack Mackerel and Redbait should be conducted.

A DEPM survey for Jack Mackerel was conducted between western Kangaroo Island, South Australia and south-western Tasmania in December 2016 to February 2017. A DEPM survey for Redbait was conducted in this area in October 2017. The present report documents the findings of the survey for Jack Mackerel.

1.6 Objectives

1. Determine distribution and abundance of eggs and larvae of Jack Mackerel and Sardine between western Kangaroo Island, South Australia and south-western Tasmania.
2. Estimate adult reproductive parameters of Jack Mackerel during the peak spawning period between western Kangaroo Island and south-western Tasmania.
3. Estimate the spawning biomass of Jack Mackerel and Sardine between western Kangaroo Island, South Australia and south-western Tasmania

The original proposal for this study did not include application of the DEPM to Sardine. This species was added to the objectives when it became evident that significant numbers of eggs of Sardine were present in the plankton samples. Information on the distribution of eggs and larvae obtained in this study provides important insights into the stock structure of both Jack Mackerel and Sardine off southern Australia.

2 METHODS

2.1 Total Daily Egg Production

2.1.1 Ichthyoplankton surveys

During the summer of 2016/17, ichthyoplankton samples were collected from the *RV Ngerin* in shelf waters between western Kangaroo Island, South Australia and south-western Tasmania (Figure 1). The first leg of the survey was conducted during 2–12 December 2016 between western Kangaroo Island and Portland, Victoria. The second leg was conducted from 25 January to 6 February 2017 and covered the area between Portland and south-western Tasmania (Figure 1). These two legs are collectively called the ‘main survey’ and included 44 transects and 306 sites (Figure 1).

During 30 January to 3 February 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 41 exploratory sites were sampled to determine if Jack Mackerel and/or Sardine spawn in Bass Strait during summer. This opportunistic sampling was done because eggs and larvae of both species were collected from eastern Bass Strait and off eastern Tasmania during an ichthyoplankton survey in the summer of 2014 (Ward *et al.* 2015b). The main survey combined with the opportunistic sampling sites is referred to as the ‘extended survey’ and includes 347 sites (Figure 1). Estimates of spawning biomass presented in this report are based on the results of the extended survey.

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 $\sim 1 \text{ m}\cdot\text{s}^{-1}$. 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 collected for genetic validation; the paired cod-ends were combined and preserved in 95% ethanol. Exploratory samples collected in Bass Strait were fixed in formalin only. Location, sampling date/time, and depth were also recorded for each plankton sample.

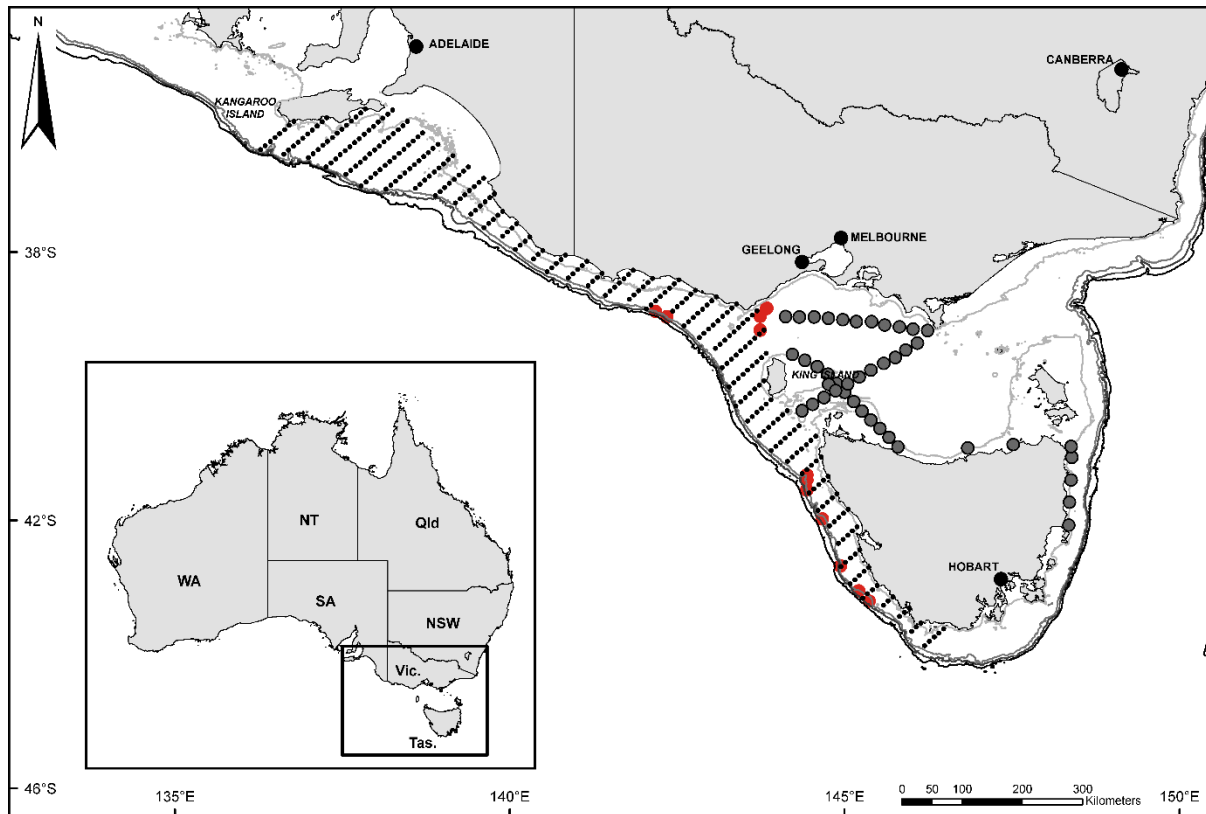


Figure 1: DEPM survey area along the continental shelf of south-eastern Australia. Locations are shown of the main survey sites (black dots), opportunistic sites in Bass Strait (grey dots), and adult trawl sites (red dots).

2.1.3 Egg identification and validation

Eggs of Jack Mackerel and Sardine were identified using the morphological features in published descriptions for the same or closely related species (Table 2). Identifications of Jack Mackerel eggs preserved in ethanol were validated using the molecular techniques developed by Perry (2011) and refined by Neira *et al.* (2015). These results were used to evaluate the morphological identification of the formalin preserved samples. This validation was done because Jack Mackerel eggs have similar characteristics to other common species, especially Yellowtail Scad (*Trachurus novaezelandiae*) (See Appendix 1).

All eggs were staged using the ‘universal’ egg staging method described by Ward *et al.* (2018) (Figure 2). The distinctive developmental characteristics of the ‘universal’ stages help to reduce staging errors in the laboratory. Stages also have a similar duration (Ward *et al.* 2018). Total counts of eggs per stage per sample were recorded.

Table 2. References used to identify the eggs of Jack Mackerel and Sardine and for species-specific egg temperature-development rates.

Species	Egg identification Reference: Species	Egg temperature-development rates Reference: Species
Jack Mackerel	Ahlstrom and Ball (1954): <i>Trachurus symmetricus</i> Crossland (1981): <i>T. declivis</i> Cunha <i>et al.</i> (2008): <i>Trachurus trachurus</i> Ward <i>et al.</i> (2015): <i>T. declivis</i>	Cunha <i>et al.</i> (2008): <i>T. trachurus</i>
Sardine	Lo <i>et al.</i> (1996): <i>Sardinops sagax</i> White and Fletcher (1998): <i>S. sagax</i> Neira <i>et al.</i> (1998): <i>S. sagax</i>	Lo <i>et al.</i> (1996): <i>S. sagax</i>

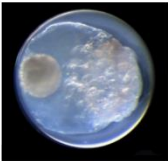
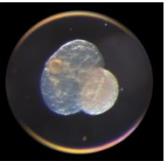
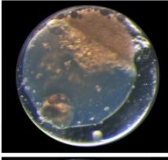
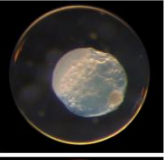
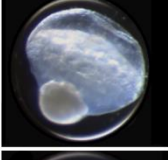
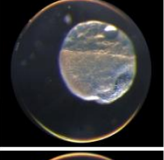
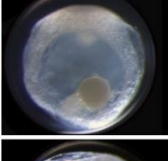
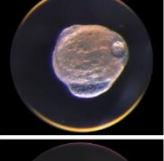
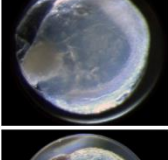
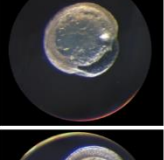
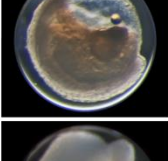
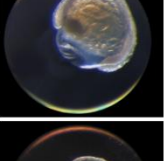
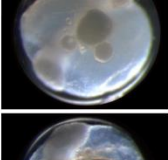
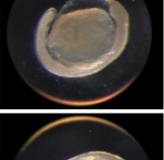
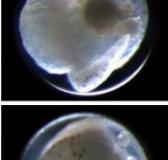
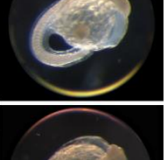
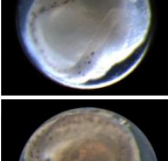
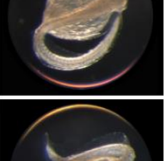
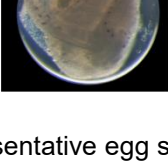
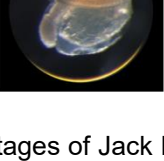
	Jack Mackerel	Australian Sardine	<u>'Universal' Egg Stage Description</u>
Stage 1			cells \leq 64
Stage 2			cells > 64
Stage 3			blastoderm covers > 1/2 of yolk; no blastopore
Stage 4			blastopore present; head distinct; tail undefined; optic vesicles begin to differentiate
Stage 5			blastopore closed;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

Figure 2. Representative egg stages of Jack Mackerel and Sardine using the 'universal' egg stages of Ward *et al.* 2018.

2.1.4 Egg ageing and treatment of zero count egg samples

Egg samples were binned into three temperature bands, based on the CTD temperature data (main survey) and a hull-mounted temperature sensor (opportunistic sites), that covered the range of temperatures typically sampled in DEPM surveys off eastern and southern Australia (14–18°C, 18–22°C, and 22–26°C). The temperature bins made the staged survey eggs comparable to published temperature egg development rates from the same or a closely related species (Table 2). These rates were used to assign a mean age to each egg (Ward *et al.* 2018). Generally, pelagic marine fish eggs of about 1 mm diameter hatch in about 48 hours at temperatures of 18–22°C, >48 hours in waters <18°C and <36 hours in waters >22°C (Pauly and Pullin 1988).

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

Samples were also identified where a zero count should (and should not) be allocated to one or more daily egg cohorts (Ward *et al.* 2018). Samples with no eggs were excluded from the analyses and were not considered part of the spawning area. Samples with eggs could contain several possible combinations of daily cohorts depending on the ambient water temperature, the 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, but not found, in the sample.

2.1.5 Egg density (P_s)

The density of eggs under one square metre of water (P_s) was estimated for each sample (Equation 2, Table 3).

Table 3. Equations used to estimate mean daily egg production (P_0) and instantaneous egg mortality rate (z) for Jack Mackerel and Sardine.

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	$E[P_0] = g^{-1}(-z t + \varepsilon)$	(5)	$E[P_0]$: expected value of P_0 g^{-1} : inverse-link function z: the instantaneous rate of daily egg mortality at age t ε : error term	Wood (2006), Ward <i>et al.</i> (2011, 2018)

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 3.4.1) 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 VNN tessellations could not be applied to the exploratory sampling sites due to their spacing. The mean area of individual main survey sites was used to calculate the additional spawning area of these exploratory sites. This was considered appropriate as the main sampling sites were spaced about $9 \text{ km} \times 28 \text{ km}$ apart and therefore provided a consistent spawning area per site. The spawning area (A) was defined as the total area of grids where live Jack Mackerel or Sardine eggs were collected.

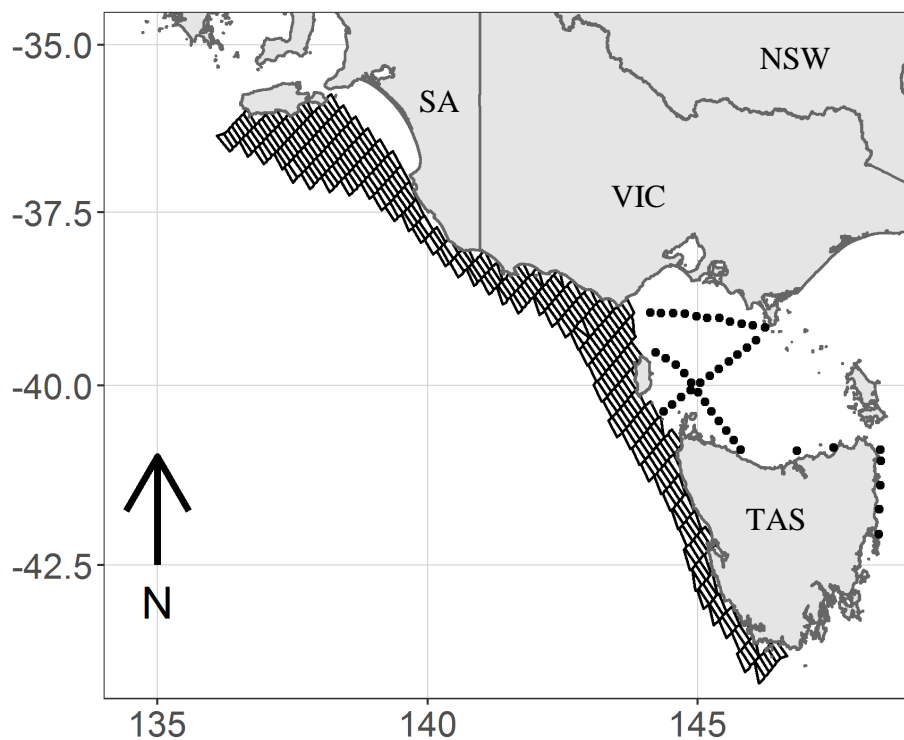


Figure 3. Voronoi natural neighbour polygons used to estimate spawning area within the main survey + exploratory sites. Dots: area estimated for exploratory sampling sites outside main survey area.

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 within the spawning area ($\text{eggs}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$). 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). Daily cohort egg densities and their average ages were used to estimate P_0 .

Based on the findings of Ward *et al.* (2018), five different models were fitted to estimate mean daily egg production (P_0) and instantaneous egg mortality rate (z , day^{-1}). 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.* 2018). The distributions of daily cohort egg densities may vary among species and surveys; different models may be suitable for different datasets (Ward *et al.* 2011, Ward *et al.* 2018).

The underlying model used to calculate mean daily egg production (P_0) was the exponential egg mortality model (Equation 3a, Table 3). The model was applied in several ways. Non-linear least squares regression was used to fit Equation 3a (Equation 3b, Table 3). A linear version of the exponential egg mortality model (Equation 4a) with a bias correction factor (Equation 4b, Table 3) was also used, which we refer to as the 'log-linear model'. The linear version of the exponential model requires bias correction as the uncorrected model has a strong negative bias (Picquelle and Stauffer 1985). For more complex error distributions, data were fitted using three general linear models (GLMs, Equation 5, Table 3). Models were fitted using three different error structures: negative binomial, quasi and quasi-Poisson. The different GLMs are referred to using the error structures as descriptors. Negative binomial and quasi error structures are considered suitable for over-dispersed data, such as egg density by age (e.g. Ward *et al.* 2011, 2018). Instantaneous egg mortality rate (z) was estimated as a free parameter in each of the models (Table 3).

All five of the models ('Log-Linear', 'Non-linear Least Squares', 'Negative Binomial GLM', 'Quasi GLM', 'Quasi-Poisson GLM') were fitted to the egg samples for both Jack Mackerel and Sardine. Model choice for each survey followed recommendations by Ward *et al.* (2018). The mean value of egg production calculated from four models that provided plausible estimates of z was used to estimate spawning biomass for Jack Mackerel, while the value of egg production from the log-linear model was used to estimate spawning biomass for Sardine.

2.2 Adult Reproductive Parameters

2.2.1 Sampling methods

Adult Jack Mackerel 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 from 30 January to 3 February 2017 (Figure 1). Four of the 12 trawls caught substantial amounts of Jack Mackerel, but most specimens were small and immature. Adult Sardine were not collected during the survey.

2.2.2 Parameter estimation methods

Too few mature Jack Mackerel were collected during the trawl survey to estimate adult reproductive parameters. Instead, adult reproductive parameters used to calculate spawning biomass were obtained from the survey of Jack Mackerel conducted off south-eastern Australian during the summer of 2014 (Ward *et al.* 2015b).

Values of adult reproductive parameters of Australian Sardine used to calculate spawning biomass were obtained from surveys conducted off South Australia between 1998 and 2016.

The following sections describe the methods used to estimate adult parameters in the 2014 Jack Mackerel survey and 1998-2016 South Australian Sardine surveys. When adult Jack Mackerel were collected, the ovaries of mature females were removed, labelled and fixed in a 10% formalin-seawater solution. Females (sans ovaries) and mature males were labelled and frozen for laboratory processing. A similar process has been used for Australian Sardine, however, mature females were preserved whole in the formalin solution rather than only the ovaries.

Female weight (W)

In the laboratory, mature females from each sample were removed from formalin or thawed and weighed (± 0.01 g). 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 4). Mature males in each sample were thawed and weighed (± 0.01 g).

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 4).

Spawning fraction (F)

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 plus day-0 POFs (d_0) (assumed to be spawning or have spawned on the night of capture), day-1 POFs (d_1) (assumed to have spawned the previous night) and day-2 POFs (d_2) (assumed to have spawned two nights prior) (Equation 8a, Table 4). The mean spawning fraction of the population was calculated from the average of sample means weighted by proportional sample size (Equation 8b, Table 4).

Table 4. Details of the equations used for the adult parameters of Jack Mackerel and Australian Sardine to estimate spawning biomass.

Adult Parameter	Equation	Eq. No.	Parameters	Reference
Female Weight	$W = \left[\frac{\overline{W_i n_i}}{N} \right]$	(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	$R = \left[\frac{\overline{R_i n_i}}{N} \right]$	(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	$\overline{S_i} = \frac{d0 + d1 + d2}{3 n_i}$	(8a)	$d0$, $d1$ and $d2$: the number of mature females with POFs aged day 0, 1 or 2 in each sample n_i : is the total number of females within a sample.	Lasker (1985)
Spawning Fraction: population	$S = \left[\frac{\overline{S_i n_i}}{N} \right]$	(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)

2.3 Spawning Biomass (**SB**)

Jack Mackerel

Spawning biomass for Jack Mackerel was calculated according to Equation 1 (Table 1) using the mean value of P_0 obtained from the four models, spawning area (A) of the extended survey and adult parameters for R , F , S , and W estimated from the 2014 Jack Mackerel survey in south-eastern Australia.

Sardine

Spawning biomass for Sardine was similarly calculated using the mean value of P_0 obtained from the log-linear model fit, spawning area (A) of the extended survey, and adult parameters for R , F , S , and W estimated from Australian Sardine DEPM surveys in South Australia from 1998–2016.

2.4 Sensitivity Analysis

Sensitivity analyses were conducted to assess the effects of varying the parameter values used to calculate spawning biomass on the estimate of spawning biomass. The sensitivity analysis focuses on the parameters of the extended survey. Each parameter in Equation 1 was varied in turn, while keeping all other variables constant. Estimates of adult parameters for the sensitivity analyses were minimum and maximum values taken from the 2014 Jack Mackerel survey off south-eastern Australia and from Sardine surveys in South Australia between 1998 and 2016. The minimum value used for spawning area (A) was the A of the main survey in our current study. The maximum A value an estimate of the total spawning area of the SPF West sub-area from 146°30'E to Kangaroo Island. This was calculated by estimating the unsampled area of Bass Strait west of 146°30'E (~35,400 km²) and multiplying that by the percentage of opportunistic sites containing live eggs for each species (54% for Jack Mackerel and 78% for Sardine). Values of egg production (P_0) resulted from egg production models for each species in the current extended survey. The P_0 value from the 2014 DEPM off south-east Australian was added as an additional comparison for Jack Mackerel.

3 RESULTS

3.1 Jack Mackerel

A total of 639 live Jack Mackerel eggs were collected at 55 of 347 sites in the extended survey. Within the main survey, 508 live eggs were collected at 33 sites. At the exploratory sites, 131 live eggs were collected at 22 sites. Bottom depths where live eggs were collected ranged from 38–179 m (mean: 75.1 m), and SSTs were 16.0–20.4°C (mean 17.6°C). Molecular identification of Jack Mackerel eggs from ethanol preserved samples in the main survey confirmed these findings (Appendix 1).

3.1.1 Egg density (P_t)

Egg densities were highest north-west of King Island (TAS), south-east of Kangaroo Island and in Bass Strait (Figure 4). The majority of eggs ($P_t > 10$ eggs·m⁻²) were collected at sites where the bottom depth was 48–97 m (mean: 73.6 m). The highest density of eggs was north-west of King Island (154 eggs·m⁻²). Most Jack Mackerel eggs were collected at sites with SST ranging from 16.0–19.6°C (mean 17.5 °C). The mean SST for sites with eggs north-west of King Island was 17.9 °C; the mean SST where eggs occurred off Kangaroo Island was 16.6 °C. In Bass Strait, the mean temperature where eggs were collected was 17.4 °C.

3.1.2 Spawning area (A)

The estimated spawning area for Jack Mackerel in the extended survey was 13,898 km², comprising 15.9% of the total area sampled (87,374 km², Table 5). The main survey covered 77,051 km² with a spawning area of 8,358 km² (10.8%, Table 5). The spawning area around Kangaroo Island was 2,789 km² of the extended survey spawning area (20%; A excluding Kangaroo Island: 11,109 km²).

Table 5. Spawning Area (A) and total area surveyed for Jack Mackerel in the main survey and with the addition of the Bass Strait exploratory sites (extended survey).

Region	Survey Area (km ²)	Spawning Area (A)	Area with Eggs (%)
Extended Survey	87,374	13,898	15.9
Main Survey	77,051	8,358	10.8

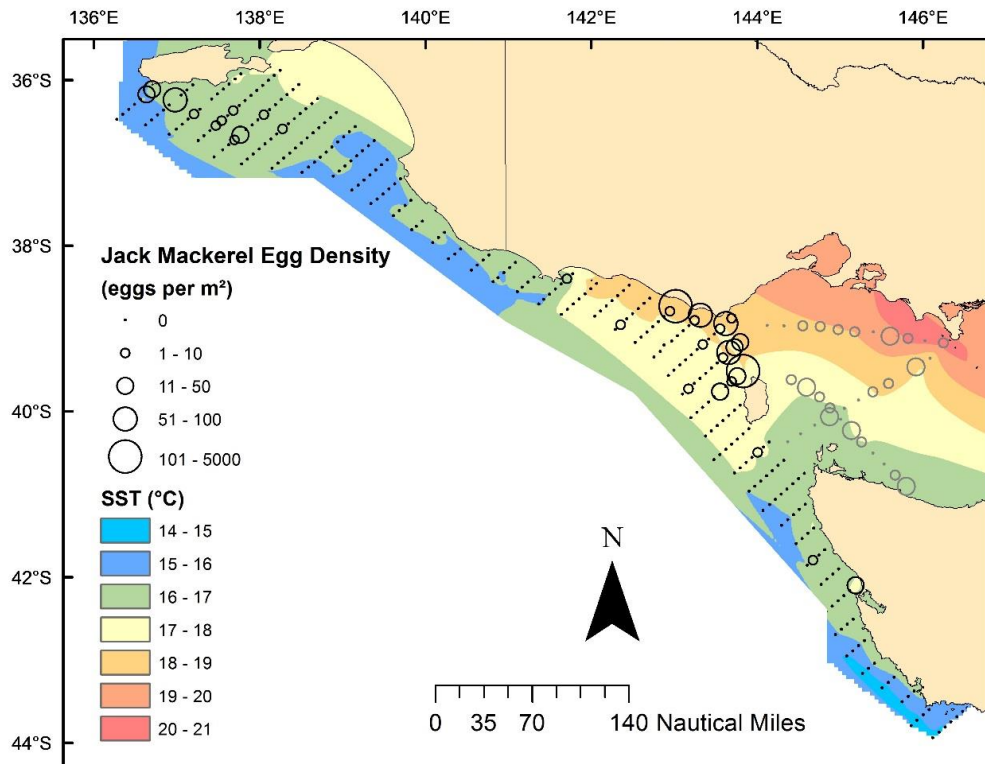


Figure 4. Spatial patterns of Jack Mackerel egg distribution and abundance between December 2016 and February 2017. Black circles are egg density (eggs·m⁻²) in main survey area. Grey circles are egg density from exploratory sites in Bass Strait. Sea surface temperature (SST) data in Bass Strait for the exploratory sites a hull-mounted temperature sensor.

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

The estimate of mean daily egg production (P_0) for the extended survey was 9.6 eggs·day⁻¹·m⁻² (95% CI: 3.7–21.2) and instantaneous daily egg mortality (z , day⁻¹) was 0.18 (Table 6, Figure 5). These values were calculated by averaging the results from the non-linear least squares and the three GLM model fits, since the log-linear model produced a biologically unrealistic mortality estimate (-0.01; Table 6, Figures 5 and 6).

Table 6. Point estimates of mean daily egg production (P_0 , eggs·day⁻¹·m⁻²) and instantaneous daily mortality (z , day⁻¹) for Jack Mackerel in the extended survey generated by the five egg production models fits.

Egg Production Model	Extended Survey		Main Survey	
	P_0 eggs·day ⁻¹ ·m ⁻² (95% CI)	z day ⁻¹	P_0 eggs·day ⁻¹ ·m ⁻²	z day ⁻¹
Log-Linear	4.7 (3.1–10.3)	-0.005	6.6	0.029
Non-linear Least Squares	8.8 (4.0–16.0)	0.124	12.2	0.182
Quasi GLM	10.4 (3.5–25.7)	0.228	15.5	0.336
Quasi-Poisson GLM	8.9 (3.6–18.1)	0.156	12.5	0.228
Negative Binomial GLM	10.3 (3.5–25.1)	0.221	15.3	0.329
Mean of all model fits excluding Log-Linear	9.6 (3.7–21.2)	0.182	13.9	0.269

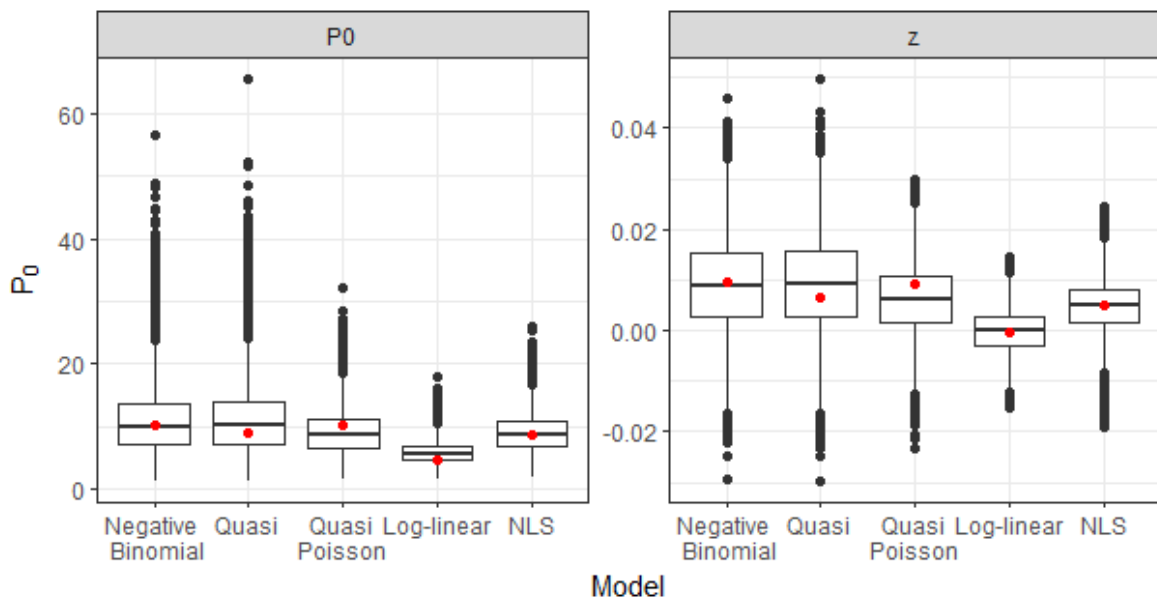


Figure 5. Estimates of mean daily egg production (P_0 , eggs·day⁻¹·m⁻²) and instantaneous daily mortality (z , day⁻¹) for Jack Mackerel in the extended survey from the five egg production models fits. NLS: Non-linear Least Squares.

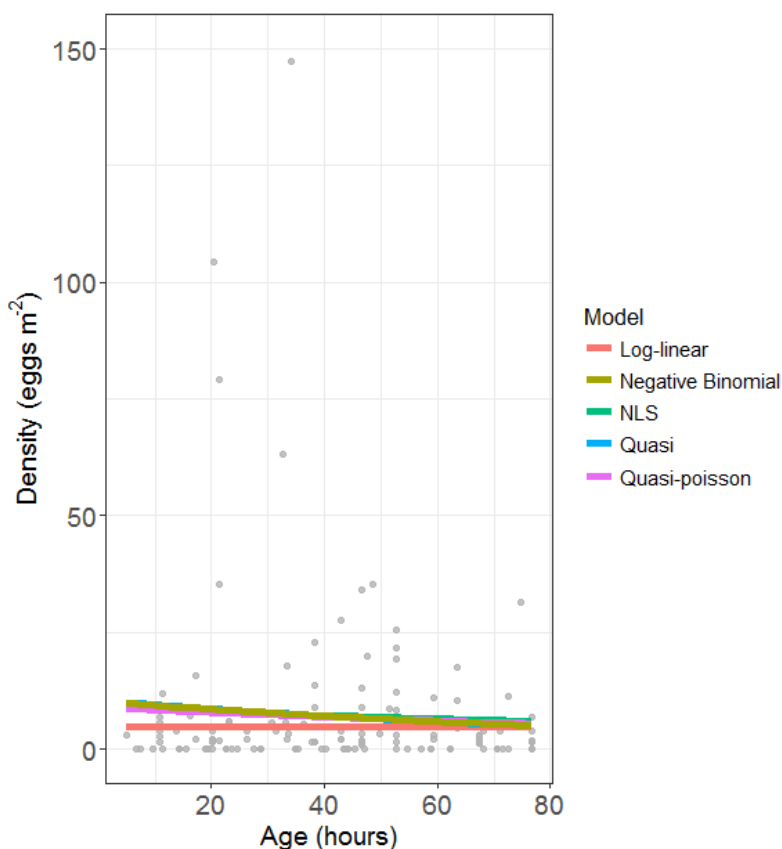


Figure 6. Egg production model fits to cohort egg densities (eggs·m⁻²) plotted by egg age (hours) for Jack Mackerel. NLS: Non-linear Least Squares.

3.1.4 Female weight (*W*)

Four of the 12 trawls undertaken from the *FV Western Alliance* caught significant numbers of Jack Mackerel. Most Jack Mackerel caught were small and juvenile; the catch during the only shot with large adults was small. Data from the south-eastern Jack Mackerel DEPM survey in 2014 (Ward et al. 2015b) were used to estimate the reproductive parameters of adult Jack Mackerel (Table 7). The mean weight of mature females collected during the Jack Mackerel survey off south-eastern Australia in 2014 was 208.8 g (Table 7).

Table 7. Mean, minimum and maximum adult parameters calculated for Jack Mackerel during the 2014 DEPM survey off eastern Tasmania and southern NSW.

Reproductive Parameter	2014 Mean (min–max)
Female Weight (<i>W</i> , g)	208.8 (133.9–250.9)
Batch Fecundity (<i>F</i> , eggs·female ⁻¹)	34,068 (16,599–94,743)
Sex Ratio (<i>R</i>)	0.47 (0.38–0.56)
Spawning Fraction (<i>S</i>)	0.056 (0.000–0.134)

3.1.5 Batch fecundity (F)

The mean batch fecundity for Jack Mackerel from south-eastern Australia in 2014 was 34,068 eggs·female⁻¹ (Table 7).

3.1.6 Sex ratio (R)

The mean sex ratio for Jack Mackerel from south-eastern Australia in 2014 was 0.47 (Table 7).

3.1.7 Spawning fraction (S)

The mean spawning fraction for Jack Mackerel from south-eastern Australia in 2014 was 0.056 (Table 7).

3.1.8 Spawning Biomass (SB)

The estimate of spawning biomass for Jack Mackerel from the extended survey was 31,069 t. This value was calculated using the mean of all model fits, excluding the log-linear model, to estimate P_0 (Table 6) and the mean values of adult parameters from south-eastern Australia in 2014 (Table 7).

3.1.9 Sensitivity Analysis

The three parameters with the strongest influence on spawning biomass are spawning area, A , mean daily egg production P_0 , and spawning fraction S (Figure 7). The presence of Jack Mackerel eggs in 54% of the exploratory samples in Bass Strait suggested that the survey did not cover the entire spawning area and that spawning biomass was under-estimated. If spawning occurred in 54% of the area that was not surveyed, the estimate of spawning biomass for Jack Mackerel in the West sub-area (146°30'E to Kangaroo Island) would have been ~74,000 t (Figure 7). The effects of S and P_0 on estimates of spawning biomass of Jack Mackerel in the current survey are small compared to the effect of spawning area.

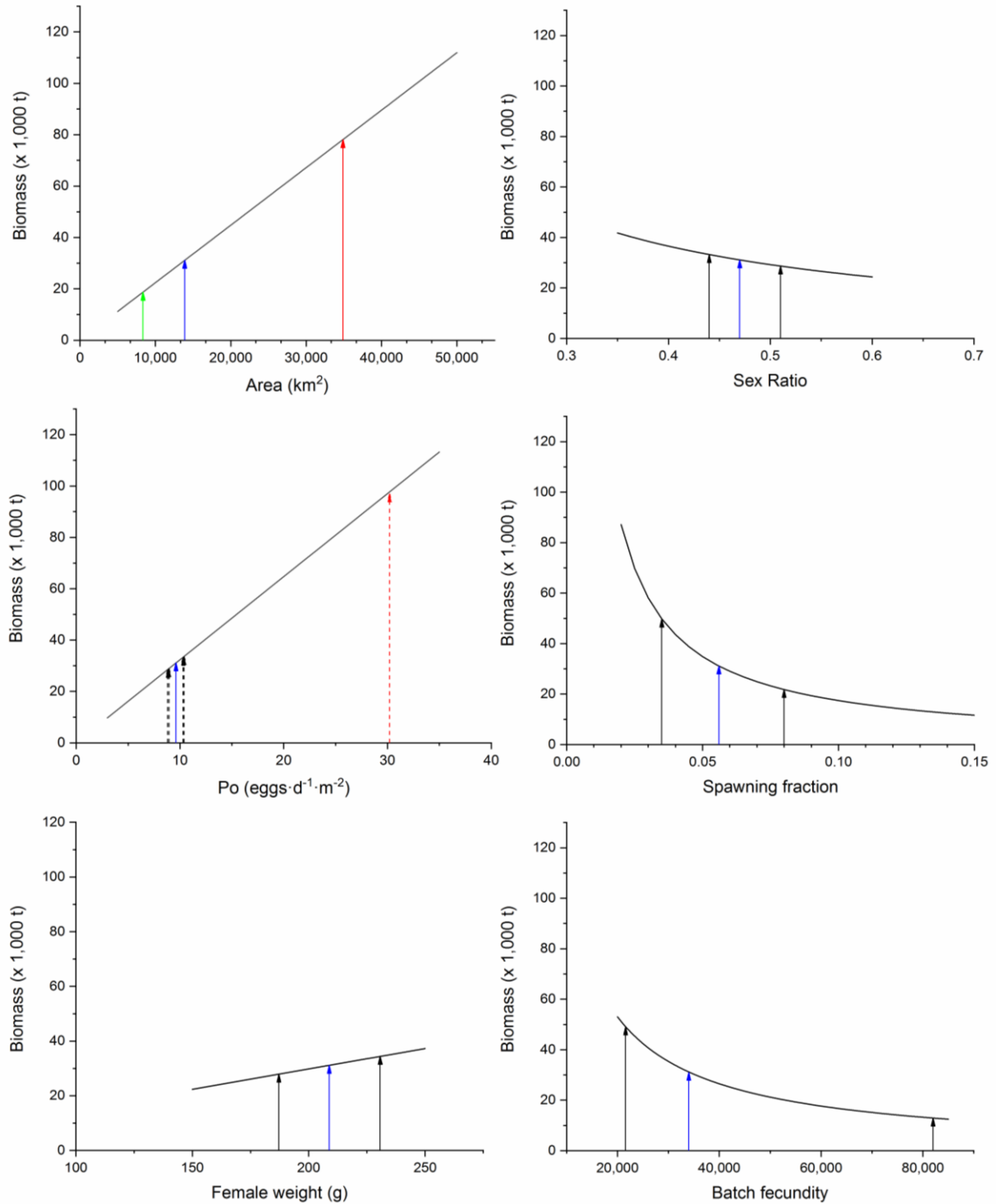


Figure 7. Sensitivity analysis of the effects of each parameter on estimates of spawning biomass of Jack Mackerel. Blue: parameters used in the current DEPM calculations to estimate spawning biomass; Green: spawning area of main survey; Red solid: estimated spawning area of SPF West sub-area from 146°30'E to Kangaroo Island; Black dashed: P_o values from all egg production models, excluding the log-linear model. Red dashed: P_o value from the 2014 Jack Mackerel survey; Black arrows: minimum and maximum values from the 2014 Jack Mackerel survey.

3.2 Sardine

A total of 4,837 live Sardine eggs were collected at 105 of 347 sites in the entire extended survey (Figure 8). This included 4,180 live Sardine eggs collected at 73 of the 306 sites in the main survey and 657 live eggs at 32 of the 41 opportunistic sites. Bottom depths where live eggs were collected ranged from 22–156 m (mean: 68.9 m), and SST was 15.8–20.4°C (mean 17.4°C).

3.2.1 Egg density (P_s)

Most Sardine eggs were collected from Bass Strait waters west to Portland, Victoria and in shelf waters to the south and south-east of Kangaroo Island (Figure 8). The majority ($P_t > 10$ eggs·m⁻²) of eggs were collected at sites where the bottom depth was 22–156 m (mean: 67.4 m). The highest density of eggs was 2,614 eggs·m⁻² south-east of Kangaroo Island. The second highest density was 1,315 eggs·m⁻² south-west of Cape Otway (VIC). Most Sardine eggs were collected at sites with SSTs ranging from 15.8–20.4 °C (mean 17.4°C). The mean SST of the sites with eggs between Cape Otway and King Island was 17.7°C. The mean SST of sites with eggs south and south-west of Kangaroo Island was 16.7°C.

3.2.2 Spawning area (A)

The estimated spawning area for Sardine in the extended survey was 26,366 km², comprising 30.2% of the total area sampled (87,374 km², Table 8). The main survey covered 77,051 km² with a spawning area of 18,309 km² (23.8%, Table 8). The spawning area around Kangaroo Island was 9,441 km² of the extended survey spawning area (36%; A excluding Kangaroo Island: 16,925 km²).

Table 8. Spawning Area (A) and total area surveyed for Australian Sardine in the main survey, and with the addition of the Bass Strait exploratory sites (extended survey).

Region	Survey Area (km ²)	Spawning Area (A)	Area with Eggs (%)
Extended Survey	87,374	26,366	30.2
Main Survey	77,051	18,309	23.8

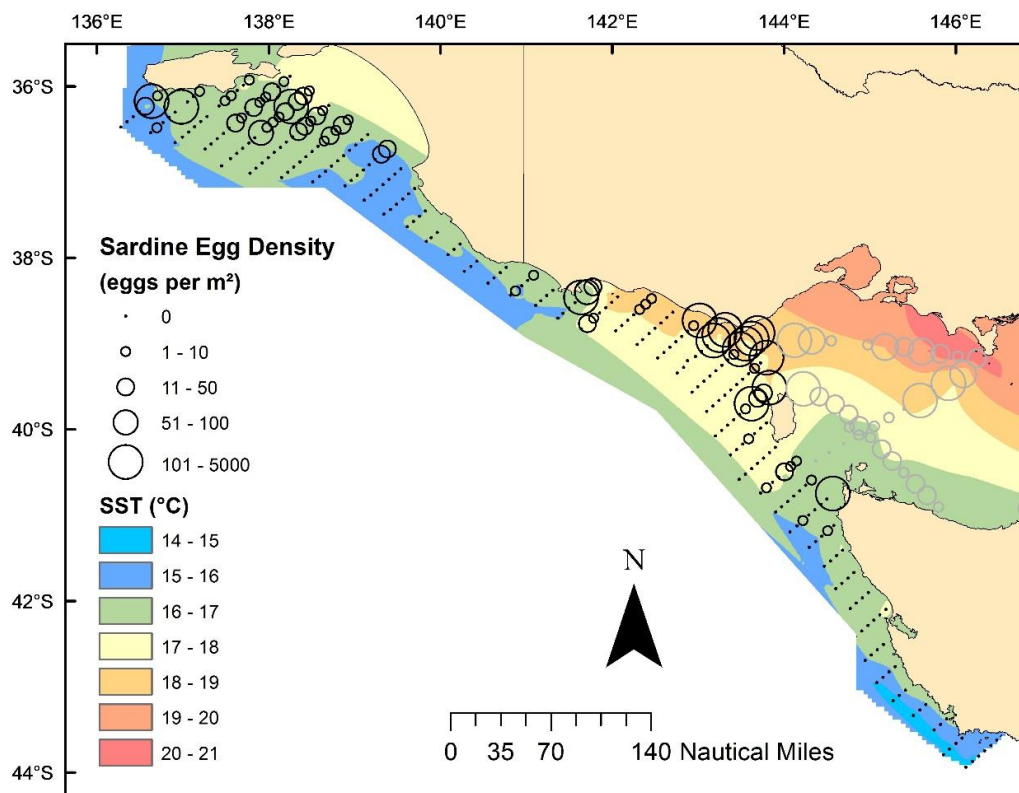


Figure 8: Spatial patterns of Sardine egg distribution and abundance between December 2016 and February 2017. Black circles are egg density (eggs·m⁻²) in main survey area. Grey circles are egg density from opportunistic sampling in Bass Strait. Sea surface temperature (SST) data in Bass Strait are approximations from vessel surface thermometers.

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

The estimate of mean daily egg production (P_0) for the extended survey obtained using the log-linear model was 33.2 eggs·day⁻¹·m⁻² and instantaneous daily egg mortality was 0.33 (z , day⁻¹) (Table 9, Figures 9 and 10). Ward et al. (2018) recommended the use of the log-linear model for estimating egg production in Australian Sardine because this model is more precise and not influenced as strongly as other models by a few samples with very high densities of eggs (e.g. > 1,000 eggs·m⁻² in some of the current survey samples; Figures 9 and 10).

Table 9. Point estimates of mean daily egg production (P_0 , eggs·day⁻¹·m⁻²) and instantaneous daily mortality (z , day⁻¹) for Australian Sardine in the main and extended survey generated by the five egg production models fits.

Egg Production Model	Extended Survey		Main Survey	
	P_0 eggs·day ⁻¹ ·m ⁻²	z day ⁻¹	P_0 eggs·day ⁻¹ ·m ⁻²	z day ⁻¹
Log-Linear	33.2	0.33	19.7	0.13
Non-linear Least Squares	100.0	0.45	46.4	0.21
Quasi GLM	415,942.1	4.90	264.0	1.46
Quasi-Poisson GLM	45.2	0.40	47.0	0.33
Negative Binomial GLM	263.6	1.46	88.4	0.77

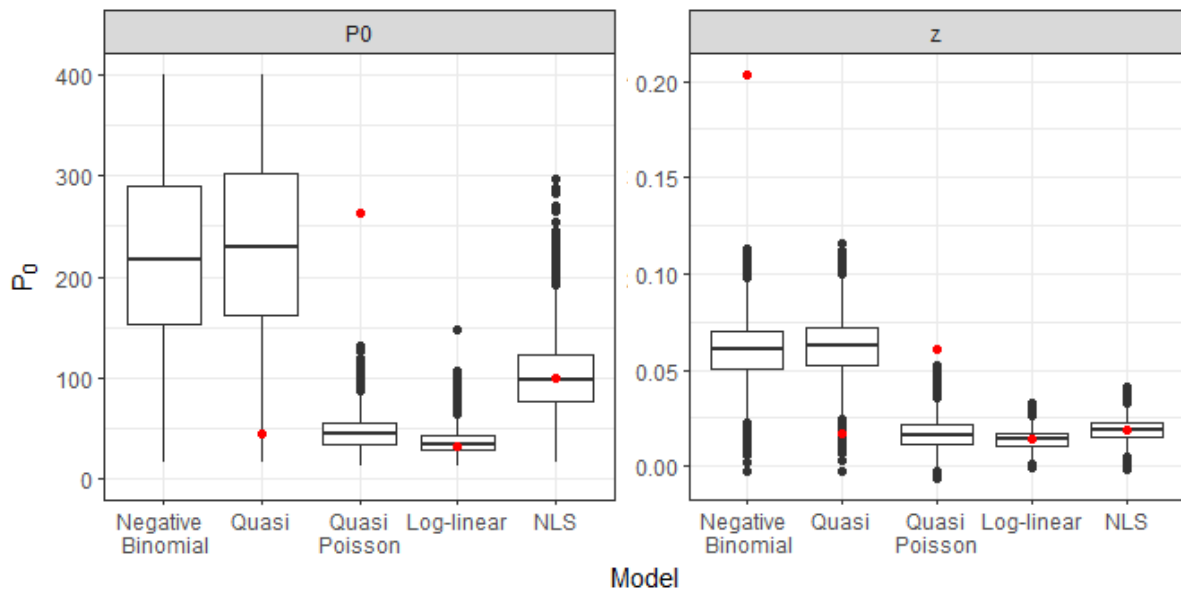


Figure 9. Estimates of mean daily egg production (P_0 , eggs·day⁻¹·m⁻²) and instantaneous daily mortality (z , day⁻¹) for Australian Sardine in the extended survey from the five egg production model fits. NLS: Non-linear Least Squares. Note: Extreme P_0 values (negative binomial and Quasi fits) beyond 400 eggs·day⁻¹·m⁻² are not shown on plot.

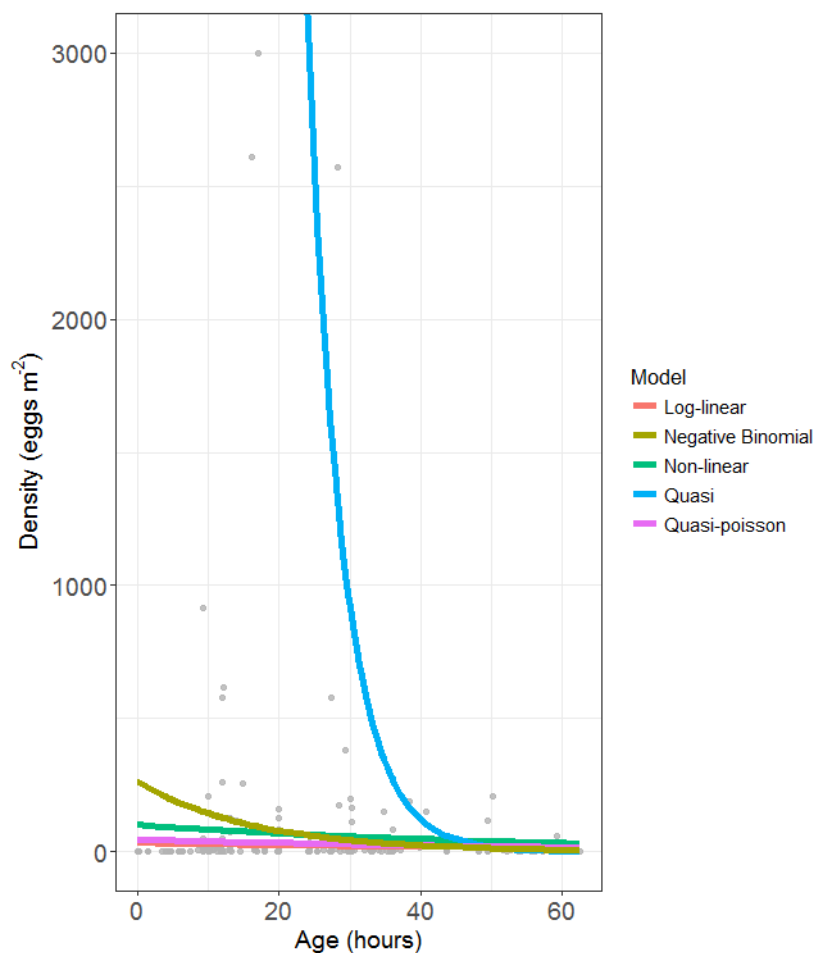


Figure 10. Egg production model fits to cohort egg densities (eggs·m⁻²) plotted by egg age (hours) for Sardine

3.2.4 Female weight (W)

Adult Sardine were not collected during the survey. Data from South Australia between 1998 and 2016 were used to estimate the reproductive parameters of adult Australian Sardine (Table 10). The mean weight of mature females collected during these surveys was 57.0 g. The minimum and maximum weights were 45.2 g (1998) and 78.7 g (2004) (Table 10).

3.2.5 Batch fecundity (F)

The mean batch fecundity for Sardine from South Australia during 1998–2016 was 17,116 eggs·female⁻¹. The minimum and maximum values were 10,904 eggs·female⁻¹ (2003) and 24,790 eggs·female⁻¹ (2004) (Table 10).

Table 10. Mean, minimum and maximum adult parameters calculated for Australian Sardine from South Australian DEPM surveys between 1998 and 2016.

Reproductive Parameter	Mean (min–max)
Female Weight (W , g)	57.0 (45.2–78.7)
Batch Fecundity (F , eggs·female ⁻¹)	17,116 (10,904–24,790)
Sex Ratio (R)	0.54 (0.36–0.68)
Spawning Fraction (S)	0.114 (0.040–0.179)

3.2.6 Sex ratio (R)

The mean sex ratio for Australian Sardine collected during South Australian surveys from 1998 to 2016 was 0.54. The minimum and maximum values were 0.36 (2009) and 0.68 (2013) (Table 10).

3.2.7 Spawning fraction (S)

The mean spawning fraction for Australian Sardine collected during South Australian surveys from 1998 to 2016 was 0.114. The minimum and maximum values were 0.040 (2014) and 0.179 (2001) (Table 10).

3.2.8 Spawning Biomass (SB)

The estimate of spawning biomass for Sardine from the extended survey was 47,283 t. These estimates were calculated using the log-linear model to estimate P_0 (Table 9) and the mean values of adult parameters from South Australia from 1998 to 2016 (Table 10).

3.2.9 Sensitivity Analysis

The sensitivity analysis for Sardine shows the strong influence that the model used to estimate P_0 had on estimates of spawning biomass (Figure 11). Using the log-linear model for Sardine usually provides estimates that are more precise and lower (probably negatively biased) than the other models (Ward *et al.* 2018). In some situations, all other models produce estimates of P_0 that are implausible. The presence of Sardine eggs in 78% of the opportunistic sites in Bass Strait, suggests that the survey did not cover the entire spawning area and that spawning biomass was under-estimated. If spawning occurred in 78% of the area that was not surveyed, the estimate of spawning biomass for Sardine in the West sub-area (146°30'E to Kangaroo Island) would have been ~97,000 t (Figure 11).

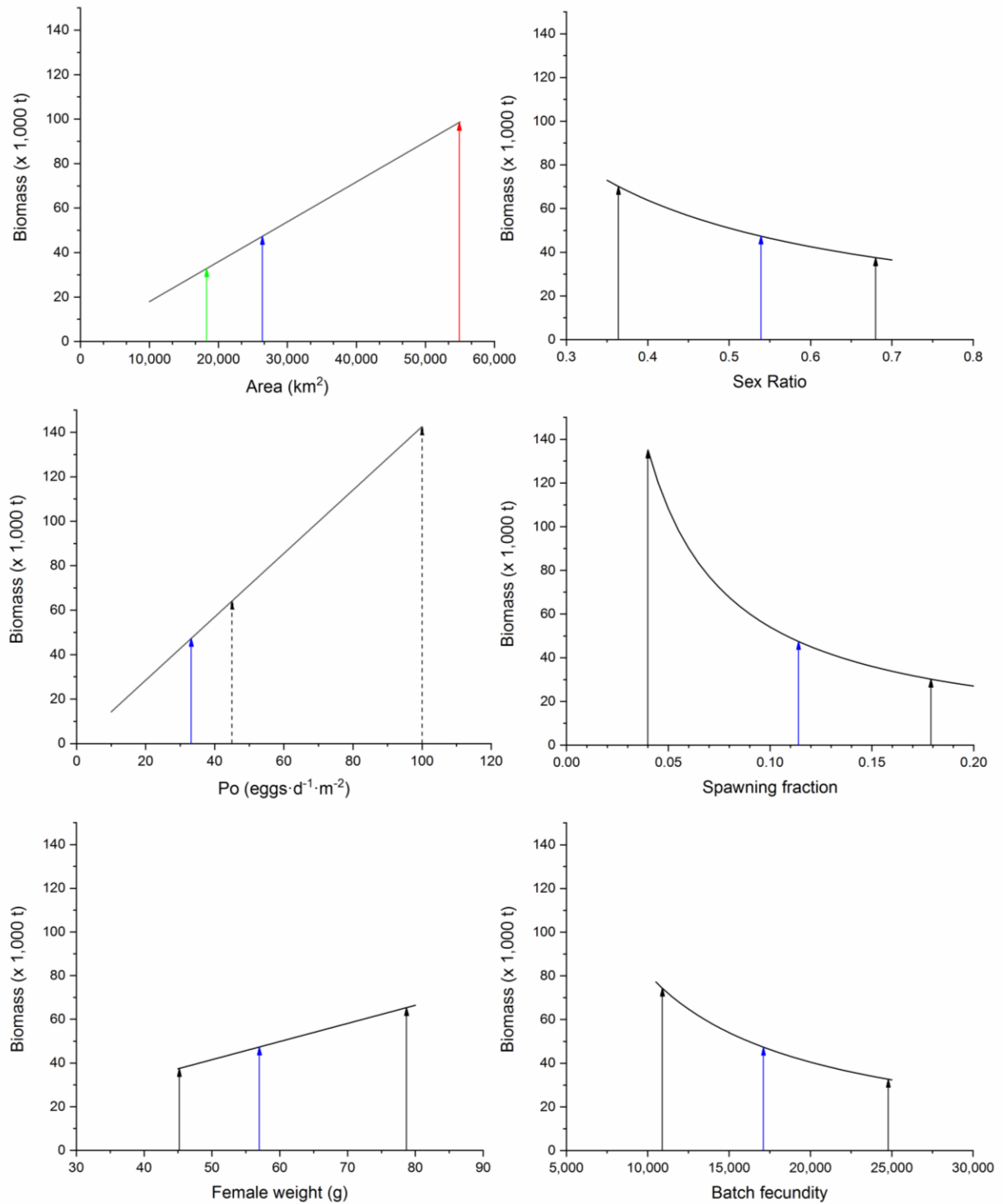


Figure 11. Sensitivity analysis of the effects of each parameter on estimates of spawning biomass of Sardine. Blue: parameters used in the current DEPM calculations to estimate spawning biomass; Green: spawning area of main survey; Red: estimated spawning area of SPF West sub-area from 146°30'E to Kangaroo Island; Dashed: P_0 values from the non-linear least squares and Quasi-Poisson GLM egg production models. Black arrows: minimum and maximum values for South Australian Sardine DEPM surveys between 1998 and 2016.

4 DISCUSSION

4.1 Distribution and stock structure

This study provides important new insights into the distribution and stock structure of Jack Mackerel off southern Australia. The presence of Jack Mackerel eggs in waters north-west of King Island (TAS) and in Bass Strait during summer has significant implications. In combination with the results of a previous survey that showed Jack Mackerel spawns off eastern Tasmania, in eastern Bass Strait and off eastern Victoria during summer (Ward *et al.* 2015b), these findings suggest that previous understanding of the stock structure of Jack Mackerel in Australian waters (e.g. Bulman *et al.* 2008) may need to be re-evaluated. Specifically, our results suggest that Bass Strait may not act as a barrier to mixing of Jack Mackerel from the East and West sub-areas of the SPF (e.g. AFMA 2009). Rather, our results suggest that a Jack Mackerel sub-population may occur v2w off northern Tasmania and Victoria, including Bass Strait. This stock structure is similar to that identified by Izzo *et al.* (2017) for Sardine, a finding which was supported by the presence of Sardine eggs in Bass Strait during the present study. Both Izzo *et al.* (2017) and the results of the present study suggest that a Sardine sub-population occurs from western Victoria, through Bass Strait to eastern Victoria and southern NSW. Our results also suggest that a sub-population of Jack Mackerel occupies a similar area.

The discontinuity in the egg distributions of Jack Mackerel and Sardine observed in the upwelling region of the Bonney Coast suggests that for both species, this area may separate sub-populations in the GAB from those off south-eastern Australia. Izzo *et al.* (2017) provided clear evidence that two populations of Sardine occur off eastern Australia. Sexton *et al.* (in press) demonstrated the existence of two spawning groups separated by a discontinuity (egg barren) where Sardines do not spawn, even when environmental conditions (i.e. SST and depth) are suitable for spawning. In contrast, there appears to be considerable overlap in the areas where Jack Mackerel spawn during summer and winter/spring (Neira 2011), suggesting that there are not spatially distinct spawning grounds for Jack Mackerel off the east coast. It would be useful to evaluate this interpretation more thoroughly by undertaking a detailed analysis of Jack Mackerel data from historical ichthyoplankton surveys, similar to that which Sexton *et al.* (in press) undertook for Sardine.

4.2 Jack Mackerel

The results of both the ichthyoplankton and trawl surveys conducted in this study suggest that the spawning biomass of Jack Mackerel in waters between western Kangaroo Island and

south-western Tasmania during summer is relatively small compared to the spawning biomass off the east coast (Ward *et al.* 2016). Fewer eggs were collected in the current survey compared to 2014 (639 versus 3,530 eggs) from fewer sites (55 versus 117 sites). The mean estimate of P_0 obtained in the current survey ($9.6 \text{ eggs}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) was low in comparison to that obtained in the 2014 survey ($28.9 \text{ eggs}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$). Relatively few adults were collected in the trawl survey reported in this study, whereas adult Jack Mackerel were highly abundant in every daylight trawl conducted off eastern Australia in 2014. These results are also consistent with the sporadic and localised catches of the factory trawler that operated between western Kangaroo Island, South Australia and south-western Tasmania in 2014/15 and 2015/16 (Ward and Grammer 2017).

Opportunistic sampling showed that substantial spawning occurred in western Bass Strait. The estimate of spawning biomass of ~31,000 t from the extended survey area is likely to be an under-estimate of the spawning biomass of Jack Mackerel in the West sub-area. It is likely that spawning occurred in the large area of western Bass Strait that was not surveyed. Previous studies have provided strong evidence that sizeable quantities of Jack Mackerel occur west of the study area in the Great Australia Bight (Shuntov 1969, Stevens *et al.* 1984, Bulman *et al.* 2008). For these reasons, the estimate of spawning biomass of ~31,000 t is considered to be a conservative figure for setting RBC for the West sub-area.

Approximately 20% of the Jack Mackerel spawning area identified in this study occurred off the south-western coast of Kangaroo Island, suggesting that the spawning biomass in this area may have been in the order of ~6,000 t. Recent SPF fishing activity in the West sub-area was concentrated in this location. Limiting the proportion of future catches from the West sub-area that can be taken off the south-western coast of Kangaroo Island may warrant consideration.

4.3 Australian Sardine

The estimate of spawning biomass of Sardine from the current extended survey was ~47,000 t, comprised of ~17,000 t off Kangaroo Island and ~30,000 t east of the Bonney Coast. Similar to Jack Mackerel, the presence of Sardine eggs at the opportunistic sites in Bass Strait suggests that a substantial part of the spawning area was not covered by the survey.

5 CONCLUSIONS

This is the first dedicated application of the DEPM to Jack Mackerel in the West sub-area. It provided important new insights into the stock structure of Jack Mackerel off southern Australia and confirmed a key element of the findings of a recent study of the stock structure of Sardine. Our results suggest that a distinct sub-population of each species occurs in the area between the Bonney Coast and southern NSW, including north-eastern Tasmania, and that Bass Strait is an important spawning area for both species. These findings have implications for the management of the SPF, especially the separation of the fishery into East and West sub-areas.

The estimates of spawning biomass of Jack Mackerel (31,069 t) and Sardine (47,283 t) for the portion of the West Sub-area between western Kangaroo Island and south-western Tasmania, where a factory trawler operated in 2014/15 and 2015/16 are underestimates of the spawning biomass in the West sub-area. However, exploratory ichthyoplankton sampling in Bass Strait suggested that the survey did not include key spawning habitat and under-estimated the spawning biomass of both species. Habitat modelling could be undertaken to predict the occurrence of eggs in these areas.

Future DEPM studies of the south-eastern population of each of these two species should be designed to cover the entire spawning area. Given the large size of the West Sub-area, ongoing spatial management may be needed to prevent concentration of fishing effort in small areas of the fishery. The distribution and abundance of SPF species in parts of the West sub-area located west of Kangaroo Island are poorly understood.

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.
- Barange, M., I. Hampton, and B. A. Roel. 1999. Trends in the abundance and distribution of anchovy and sardine on the South African continental shelf in the 1990s, deduced from acoustic surveys. *South African Journal of Marine Science* **21**:367-391.
- 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.
- Blackburn, M. 1950. Studies on the age, growth, and life history of the sardine *Sardinops neopilchardus* (Steindachner), in Southern and Western Australia. *Australian Journal of Marine and Freshwater Research* **1**:221-258.
- Bulman, C., S. Condie, J. Findlay, B. Ward, and J. Young. 2008. Management zones from small pelagic fish species stock structure in southern Australian waters. Final Report for FRDC Project 2006/076. CSIRO Marine and Atmospheric Research: Hobart, Tasmania. .
- Bulman, C. M., E. A. Fulton, and A. D. M. Smith. 2015. Jack mackerel stock structure in the SPF. CSIRO Marine and Atmospheric Research. Hobart, Tasmania. 19 pp.
- Coetzee, J. 2000. Use of a shoal analysis and patch estimation system (SHAPES) to characterise sardine schools. *Aquatic Living Resources* **13**:1-10.
- 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.
- Fletcher, W., and R. Tregonning. 1992. Distribution and timing of spawning by the Australian pilchard (*Sardinops sagax neopilchardus*) off Albany, Western Australia. *Marine and Freshwater Research* **43**:1437-1449.
- 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.
- Fletcher, W. J., and K. Santoro. 2015. Status Reports of the Fisheries and Aquatic Resources of Western Australia 2014/15: The State of the Fisheries. Department of Fisheries, Western Australia. 353 pp.
- Fletcher, W. J., R. J. Tregonning, and G. J. Sant. 1994. Interseasonal variation in the transport of pilchard eggs and larvae off southern Western Australia. *Marine Ecology Progress Series* **111**:209-224.
- Fulton, E. A. 2013. Simulation analysis of jack mackerel stock size: Ecosystem model based plausibility study. CSIRO, Australia. 24 pp.
- Gaertner, D., and M. Dreyfus-Leon. 2004. Analysis of non-linear relationships between catch per unit effort and abundance in a tuna purse-seine fishery simulated with artificial neural networks. *ICES Journal of Marine Science* **61**:812-820.

- 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. Kuitert. 2008. *Fishes of the Australia's South Coast*. Museum of Victoria. New Holland Publishers (Australia).
- Hijmans, R. J. 2015. geosphere: Spherical Trigonometry. R package version 1.3-13. <http://CRAN.R-project.org/package=geosphere>.
- 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.
- Izzo, C., T. M. Ward, A. R. Ivey, I. M. Suthers, J. Stewart, S. C. Sexton, and B. M. Gillanders. 2017. Integrated approach to determining stock structure: implications for fisheries management of sardine, *Sardinops sagax*, in Australian waters. *Reviews in Fish Biology and Fisheries* **27**:267-284.
- Jordan, A. R. 1994. Age, growth and back-calculated birthdate distributions of larval jack mackerel *Trachurus declivis* (Pisces: Carangidae), from eastern Tasmanian coastal waters. *Journal of Marine and Freshwater Research* **45**:19-33.
- Jordan, A. R., G. Pullen, J. Marshall, and H. Williams. 1995. Temporal and spatial patterns of spawning in jack mackerel, *Trachurus declivis* (Pisces: Carangidae), during 1988-91 in eastern Tasmanian waters. *Marine and Freshwater Research* **46**:831-842.
- Kailola, P. J., M. J. Williams, P. C. Stewart, A. M. Reichelt, and C. Grieve. 1993. *Australian Fisheries Resources*. Bureau of Resource Sciences, Department of Primary Industries and Energy and the Fisheries Research and Development Corporation, Canberra.
- Keane, J. P. 2009. Mesoscale Characterisation of the Pelagic Shelf Ecosystem of South-Eastern Australia: Integrated Approach Using Larval Fish Assemblages and Oceanography. PhD Thesis, University of Tasmania. 269 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.
- Marshall, J. M., G. Pullen, and A. R. Jordan. 1993. Reproductive biology and sexual maturity of female jack mackerel, *Trachurus declivis* (Jenyns), in eastern Tasmanian waters. *Australian Journal of Marine and Freshwater Research* **44**:799-809.
- Maxwell, J. G. H. 1979. Jack mackerel. Fishery Situation Report, no. 2. Division of Fisheries and Oceanography. CSIRO Australia, 18 pp.
- 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*:fsy021-fsy021.
- McLeod, D. J., A. J. Hobday, J. M. Lyle, and D. C. Welsford. 2012. A prey-related shift in the abundance of small pelagic fish in eastern Tasmania? *ICES Journal of Marine Science: Journal du Conseil*.
- 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. 2009. Provisional spawning biomass estimates of yellowtail scad (*Trachurus novaezelandiae*) off south-eastern Australia. Report to New South Wales Department of Primary Industries (NSW DPI). 31 pp.
- Neira, F. J. 2011. Application of daily egg production to estimate biomass of jack mackerel, *Trachurus declivis* - a key fish species in the pelagic ecosystem of south-eastern Australia. Final Report to the Winifred Violet Scott Charitable Trust. Fisheries, Aquaculture and Coasts Centre, Institute for Marine and Antarctic Studies (IMAS), University of Tasmania. 42 pp.
- Neira, F. J., R. A. Perry, C. P. Burrige, 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.
- Oxley, A. P. A., S. R. Catalano, M. L. Wos-Oxley, E. L. Westlake, G. L. Grammer, and M. A. Steer. 2017. Using in situ hybridization to expand the daily egg production method to new fish species. *Molecular Ecology Resources* **17**:1108-1121.
- Parker, K. 1980. A direct method for estimating northern anchovy, *Engraulis mordax*, spawning biomass. *Fisheries Bulletin* **84**:541-544.
- Pauly, D., and R. S. V. Pullin. 1988. Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental biology of fishes* **22**:261-271.
- 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.
- Ruiz, P., A. Sepúlveda, L. Cubillos, C. Oyarzún, and J. Chong. 2008. Reproductive Parameters and Spawning Biomass of Jack Mackerel (*Trachurus murphyi*), in 1999-2006, determined by The Daily Egg Production Method. Chilean Jack Mackerel Workshop. Paper No. 13. 9 pp.
- Schwartzlose, R. A., J. Alheit, A. Bakun, T. R. Baumgartner, R. Cloete, R. J. M. Crawford, W. J. Fletcher, Y. Green-Ruiz, E. Hagen, T. Kawasaki, D. Lluch-Belda, S. E. Lluch-Cota, A. D. MacCall, Y. Matsuura, M. O. Nevárez-Martínez, R. H. Parrish, C. Roy, R. Serra, K. V. Shust, M. N. Ward, and J. Z. Zuzunaga. 1999. Worldwide large-scale fluctuations of sardine and anchovy populations. *South African Journal of Marine Science* **21**:289-347.

- Sexton, S. C., T. M. Ward, and C. Huvneers. 2017. Characterising the spawning patterns of Jack Mackerel (*Trachurus declivis*) off eastern Australia to optimise future survey design. *Fisheries Research* **186**:223-236.
- Sexton, S. C., T. M. Ward, J. Stewart, K. M. Swadling, and C. Huvneers. In press. Spawning patterns provide further evidence for multiple stocks of sardine (*Sardinops sagax*) off eastern Australia. *Fisheries Oceanography*.
- Shuntov, V. P. 1969. Some features of the ecology of pelagic fishes in the Great Australian Bight. *Problems of Ichthyology* **9**:801-809.
- Smith, A. D. M., T. M. Ward, F. Hurtado, N. Klaer, E. Fulton, and A. E. Punt. 2015. Review and update of harvest strategy settings for the Commonwealth Small Pelagic Fishery: Single species and ecosystem considerations. Final Report of FRDC Project No. 2013/028. CSIRO Oceans and Atmosphere Flagship, Hobart. 74 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.
- Stevens, J. D., H. F. Hausfeld, and S. R. Davenport. 1984. Observations on the biology, distribution and abundance of *Trachurus declivis*, *Sardinops neopilchardus* and *Scomber australasicus* in the Great Australian Bight. CSIRO Marine Laboratories, Cronulla.
- Stratoudakis, Y., M. Bernal, K. Ganiias, 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>.
- Walters, C., and J.-J. Maguire. 1996. Lessons for stock assessment from the northern cod collapse. *Reviews in Fish Biology and Fisheries* **6**:125-137.
- Ward, T. M., M. M. Angelico, L. A. Cubillos, C. J. G. van Damme, K. Ganiias, L. Ibaibarriaga, and N. C. H. Lo. 2015a. Benchmarking Australia's small pelagic fisheries against world's best practice. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. FRDC project No. 2013/063. 280 pp.
- 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., O. Burnell, A. Ivey, J. Carroll, J. Keane, J. Lyle, and S. Sexton. 2015b. Summer spawning patterns and preliminary daily egg production method survey of Jack Mackerel and Australian Sardine off the East Coast. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.
- Ward, T. M., O. W. Burnell, A. Ivey, S. C. Sexton, J. Carroll, J. Keane, and J. M. Lyle. 2016. Spawning biomass of jack mackerel (*Trachurus declivis*) off eastern Australia: Critical knowledge for managing a controversial fishery. *Fisheries Research* **179**:10-22.
- 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. 90pp.
- Ward, T. M., and G. L. Grammer. 2017. Commonwealth Small Pelagic Fishery: Fishery Assessment Report 2014-2016. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences),

- Adelaide. SARDI Publication No. F2010/000270-8. SARDI Research Report Series No. 948. 123 pp.
- Ward, T. M., G. L. Grammer, A. R. Ivey, J. R. Carroll, J. P. Keane, J. Stewart, and L. Litherland. 2015c. Egg distribution, reproductive parameters and spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the East Coast during late winter and early spring. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. FRDC Project No. 2014/033. 77 p.
- Ward, T. M., F. Hoedt, L. McLeay, W. F. Dimmlich, G. Jackson, P. J. Rogers, and K. Jones. 2001a. Have recent mass mortalities of the sardine *Sardinops sagax* facilitated an expansion in the distribution and abundance of the anchovy *Engraulis australis* in South Australia? *Marine Ecology-Progress Series* **220**:241-251.
- Ward, T. M., F. Hoedt, L. McLeay, W. F. Dimmlich, M. Kinloch, G. Jackson, R. McGarvey, P. J. Rogers, and K. Jones. 2001b. Effects of the 1995 and 1998 mass mortality events on the spawning biomass of *Sardinops sagax* in South Australian waters. *ICES Journal of Marine Science* **58**:830-841.
- Ward, T. M., and L. J. McLeay. 1998. Use of the Daily Egg Production Method to Estimate the Spawning Biomass of Pilchards (*Sardinops sagax*) in Shelf Waters of Central and Western South Australia in 1998. Report to the South Australian Sardine Working Group. South Australian Research and Development Institute Report.
- Ward, T. M., P. J. Rogers, L. J. McLeay, and R. McGarvey. 2009. Evaluating the use of the daily egg production method for stock assessment of blue mackerel, *Scomber australasicus*. *Journal of Marine and Freshwater Research* **62**:112-128.
- Ward, T. M., J. Smart, and A. Ivey. 2017. Stock assessment of Australian Sardine (*Sardinops sagax*) off South Australia 2017. Report to PIRSA Fisheries and Aquaculture. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2007/000765-6. SARDI Research Report Series No. 971.:107.
- Ward, T. M., and J. Staunton-Smith. 2002. Comparison of the spawning patterns and fisheries biology of the sardine, *Sardinops sagax*, in temperate South Australia and sub-tropical southern Queensland. *Fisheries Research* **56**:37-49.
- Watson, D. F. 1981. Computing the n-dimensional Delaunay tessellation with application to Voronoi polytopes. Pages 167-172 *The Computer Journal*.
- Whittington, I. D., M. Crockford, D. Jordan, and B. Jones. 2008. Herpesvirus that caused epizootic mortality in 1995 and 1998 in pilchard, *Sardinops sagax* (Steindachner), in Australia is now endemic. *Journal of Fish Diseases* **31**:97-105.
- Williams, H., and G. Pullen. 1986. A synopsis of biological data on the jack mackerel *Trachurus declivis* Jenyns. Technical Report No. 10. Department of Sea Fisheries, Tasmania, Marine Laboratories: Taroona. 34 pp.
- Wood, S. N. 2006. *Generalized Additive Models An Introduction* with R. Chapman and Hall/CRC.
- Zwolinski, J. P., D. A. Demer, K. A. Byers, G. R. Cutter, J. S. Renfree, T. S. Sessions, and B. J. Macewicz. 2012. Distributions and abundances of Pacific sardine (*Sardinops sagax*) and other pelagic fishes in the California Current Ecosystem during spring 2006, 2008, and 2010, estimated from acoustic-trawl surveys *Fishery Bulletin* **110**:110-122.

APPENDICES

APPENDIX 1: Genetic identification of Jack Mackerel eggs

J P. Keane

Introduction

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.

Eggs of *Trachurus declivis* (Jack Mackerel) are known to occur in shelf waters of south eastern Australia simultaneously with morphologically similar eggs of other carangids such as *T. novaezelandiae* (yellowtail scad) and *Pseudocaranx dentex* (silver trevally). The eggs of these species possess almost identical morphological characteristics apart from slightly differing but overlapping egg diameters (Neira *et al.* 2014). Furthermore, it is not known if eggs of other species present in the area possess similar morphological characters to *T. declivis* as the eggs of just a few fish species have been described in Australia. As a consequence morphological identification of Carangid eggs to species level remains challenging.

In this study we employ a molecular approach to identify and validate ethanol preserved eggs of *T. declivis*, as well as of eggs that possess similar morphological characteristics, to validate morphologically identified formalin preserved *T. declivis* eggs.

Methods

Samples

Ichthyoplankton samples were collected by vertical bongo hauls over two surveys in southern Australia: Survey 1, western region, (Kangaroo Island, SA, to Portland, Vic) and Survey 2 eastern region (Portland, Vic to SE Tasmania). A total of 305 sites were sampled and preserved in formalin. Replicate hauls were completed at 70 sites and captured plankton was immediately drained of excess seawater and preserved in 96% ethanol. Replicate hauls were conducted at even sites on every second transect. Ichthyoplankton samples were collected using a bongo sampler equipped with 500 μ m mesh, and 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 ca. 1.0 ms⁻¹.

Samples were sorted at the South Australian Research and Development Institute (SARDI) with eggs possessing similar morphological characters as *T. declivis* sent to the Institute for Marine and Antarctic Studies (IMAS) for validation. This included 2025 eggs from 134 sites preserved in formalin and 213 eggs from 11 sites preserved in ethanol (Table 1).

Table 1. Samples provided for *T. declivis* identification

	Formalin		Ethanol	
	Sites	Eggs	Sites	Eggs
Western region	76	993	5	57
Eastern region	58	1032	6	156
Total	134	2025	11	213

Morphometric identification

Eggs were identified using a combination of morphological characters described for *Trachurus* eggs by Ahlstrom and Ball (1954), Crossland (1981) and Cunha *et al.* (2008). The main diagnostic features are: a) spherical with a diameter ranging from approximately 0.70 to 1.03 mm, b) smooth chorion, c) narrow perivitelline space d) prominent segmented yolk sac (irregular and indistinct in early stages), e) single pigmented oil globule oriented posteriorly on yolk sac in later stages of development, f) stout bodied embryo with prominent melanophores along the dorsal surface. Formalin preserved eggs off south-eastern Australia were found to have a mean diameter of 0.954 mm (range 0.853–1.046 mm; Keane and Lyle 2015).

Preserved eggs were rehydrated in distilled water to better reflect diameters of fresh eggs, and measured digitally to 0.02 mm under a stereomicroscope.

Molecular identification

A molecular approach of Mitochondrial DNA (mtDNA) extraction, amplification, and sequencing for *Trachurus* spp. developed by Perry (2011) and refined by Neira *et al.* (2014) was employed to identify eggs of *T. declivis*. DNA extractions from eggs identified based on morphological characters were carried out using the QIAamp DNA Micro Kit (QIAGEN, USA) following the manufacturer's protocol for tissue extraction. Amplification by polymerase chain reactions (PCRs) were performed using MyTaq HSTM DNA Polymerase (Bioline) with PCR product purification and bi-directional sequencing performed by Macrogen Inc. (Seoul, Republic of Korea) (see Neira *et al.* 2014 for full methods). Sequences were aligned to reference data in the Fish Barcode of Life Database (BOLD) using BioEdit biological sequence alignment editor.

The number of eggs subjected to mtDNA testing varied between sites according to raw abundances of eggs morphologically identified as *Trachurus*. All such eggs were tested from sites with 10 or fewer eggs, while for sites with >10 eggs a minimum of 10 eggs were randomly selected for testing, with a minimum of 5 from each stage present. In addition, some eggs possessing similar characteristics to *Trachurus* (e.g. diameter, pigmentation) were selected if present for mtDNA testing.

Results of molecular identifications were used to aid identifications of formalin preserved eggs.

Results

A total of 72 eggs were selected for mtDNA analysis; 40 identified morphologically as *T. declivis*, 18 indeterminable, and 14 similar but morphologically different to *T. declivis* (Tables 2 and 3). Indeterminable eggs consisted of early stage eggs whose morphological characteristics were masked by ethanol preservation, making morphological identification problematic.

Of the 72 eggs subjected to molecular analyses, 61 yielded quality mtDNA. Seven of the 11 eggs that failed to yield quality mtDNA were from one site (JA2), which was reported to have preserved poorly (A. Ivey, SARDI, pers. comm.; Table 2). All eggs morphologically identified as *T. declivis* across five sites were successfully confirmed via mtDNA when quality mtDNA was present. Four eggs from an additional two sites, which were indeterminable by morphological identification, were also genetically identified as *T. declivis* (Table 2).

The mtDNA analysis also facilitated the identification of some non *T. declivis* eggs within the sample, including Barracouta (*Thyrsites atun*) and redbait (*Emmelichthys nitidus*) (Tables 2 and 3).

Using morphological characters and molecular validation results, 587 formalin preserved *T. declivis* eggs from the primary survey were identified from the 2025 eggs preliminarily sorted by SARDI (Table 4.)

Table 2. Eggs from 5 sites subjected to mtDNA genetic analysis from Survey 1, western region, (Kangaroo Island, SA, to Portland, Vic). Indeterminable refers to eggs whose morphological characteristics were masked by ethanol preservation, making morphological identification problematic. Eggs where quality mtDNA was unable to be extracted are listed as 'Fail'.

Site	Morphological ID	Diameter		Comments	Genetic ID
		Stage (mm)	(mm)		
JA2	<i>T. declivis</i>	5	0.98		Fail
JA2	<i>T. declivis</i>	5	0.96		Fail
JA2	<i>T. declivis</i>	5	0.98		Fail
JA2	<i>T. declivis</i>	5	1		Fail
JA2	<i>T. declivis</i>	5	0.98		Fail
JA2	<i>T. declivis</i>	5	1.04		Fail
JA2	<i>T. declivis</i>	5	1.02		<i>T. declivis</i>
JA2	<i>T. declivis</i>	5	1.06		Fail
JA2	<i>T. declivis</i>	5	1.02		<i>T. declivis</i>
JA2	<i>T. declivis</i>	5	1.04		<i>T. declivis</i>
JC6	Unknown - Sp. A	6	0.96	Two distinct pig lines t	<i>Thyrsites atun</i>
JC6	Unknown	6	1	Unpigmented embryo, pigmented oil globule.	<i>Centroberyx sp.</i>
JC6	<i>T. declivis</i>	6	0.94		<i>T. declivis</i>
JC6	<i>T. declivis</i>	2	1		<i>T. declivis</i>
JC6	<i>T. declivis</i>	2	1		<i>T. declivis</i>
JG8	Indeterminable	2	0.9	No distinguishing characters visible	No match
JG8	Indeterminable	2	0.9	No distinguishing characters visible	<i>T. declivis</i>
JG8	Indeterminable	3	0.9	No distinguishing characters visible	Fail
JG8	Indeterminable	3	0.9	No distinguishing characters visible	Fail
JG8	Indeterminable	3	0.92	No distinguishing characters visible	<i>Thyrsites atun</i>
JG8	Indeterminable	3	0.94	No distinguishing characters visible	Fail
JG8	Indeterminable	3	0.94	No distinguishing characters visible	<i>Centroberyx sp.</i>
JG8	Indeterminable	3	1	No distinguishing characters visible	<i>Thyrsites atun</i>
JS2	Indeterminable	3	1.06	No distinguishing characters visible	<i>T. declivis</i>
JS2	Indeterminable	3	1.06	No distinguishing characters visible	<i>T. declivis</i>
JS2	Indeterminable	3	1.06	No distinguishing characters visible	<i>T. declivis</i>
JS2	Unknown	7	1.16	Too large for <i>T. declivis</i>	No match
JS6	Unknown - Sp. A	7	0.96	2 pig lines to snout, 2/3, pig yolk, like 21-24	<i>Thyrsites atun</i>
JS6	Unknown - Sp. A	7	0.96	2 pig lines to snout, 2/3, pig yolk, like 21-24	<i>Thyrsites atun</i>
JS6	Indeterminable	3	1	No distinguishing characters visible	<i>Emmelichthys nitidus</i>

Table 3. Eggs from 5 sites subjected to mtDNA genetic analysis from Survey 2, eastern region (Portland, Vic to SE Tasmania). Indeterminable refers to eggs whose morphological characteristics were masked by ethanol preservation, making morphological identification problematic. Eggs where quality mtDNA was unable to be extracted are listed as 'Fail'.

Site	Morphological ID	Stage	Diameter (mm)	Comments	Genetic ID
KD02	<i>T. declivis</i>	7	0.98		<i>T. declivis</i>
KD02	<i>T. declivis</i>	7	0.96		<i>T. declivis</i>
KD02	<i>T. declivis</i>	7	1		<i>T. declivis</i>
KD02	<i>T. declivis</i>	7	0.98		<i>T. declivis</i>
KD02	<i>T. declivis</i>	7	0.98		<i>T. declivis</i>
KD02	Unknown - Sp. A	8	0.98	Two distinct pig lines to snout	<i>Thyrsites atun</i>
KD02	Unknown - Sp. A	8	1.02	Two distinct pig lines to snout	<i>Thyrsites atun</i>
KD02	Unknown - Sp. A	8	1	Two distinct pig lines to snout	<i>Thyrsites atun</i>
KD02	Unknown - Sp. A	8	1	Two distinct pig lines to snout	<i>Thyrsites atun</i>
KD02	<i>T. declivis</i>	5	0.96		<i>T. declivis</i>
KD02	<i>T. declivis</i>	5	1		<i>T. declivis</i>
KD02	<i>T. declivis</i>	5	1		<i>T. declivis</i>
KD02	<i>T. declivis</i>	5	0.98		<i>T. declivis</i>
KD02	<i>T. declivis</i>	5	0.98		<i>T. declivis</i>
KD02	<i>T. declivis</i>	5	1		<i>T. declivis</i>
KF2	<i>T. declivis</i>	8	1		<i>T. declivis</i>
KF2	<i>T. declivis</i>	8	1		<i>T. declivis</i>
KF2	<i>T. declivis</i>	8	1.02		<i>T. declivis</i>
KF2	<i>T. declivis</i>	8	1.04		<i>T. declivis</i>
KF2	<i>T. declivis</i>	8	1		<i>T. declivis</i>
KF2	<i>T. declivis</i>	4	0.98		<i>T. declivis</i>
KF2	<i>T. declivis</i>	4	1.02		<i>T. declivis</i>
KF2	<i>T. declivis</i>	4	1		<i>T. declivis</i>
KF2	<i>T. declivis</i>	4	1		<i>T. declivis</i>
KF2	<i>T. declivis</i>	4	0.98		<i>T. declivis</i>
KF2	<i>Lepidotrigla</i>	6	1.26	<i>Lepidotrigla</i> characteristics. Pigmented chorion opposite embryo	<i>Lepidotrigla</i> sp.
KF2	<i>T. declivis</i>	7	0.94		<i>T. declivis</i>
KF6	Unknown	8	0.88	Too small for <i>T. declivis</i>	<i>Neoplatycephalus</i> sp.
KF6	Unknown	8	0.8	Too small for <i>T. declivis</i>	No match
KF6	Unknown	7	0.82	Too small for <i>T. declivis</i>	No match
KH2	<i>T. declivis</i>	8	0.96		<i>T. declivis</i>
KH2	<i>T. declivis</i>	8	0.98		<i>T. declivis</i>
KH2	<i>T. declivis</i>	8	0.96		Fail
KH2	<i>T. declivis</i>	8	0.96		<i>T. declivis</i>
KH2	<i>T. declivis</i>	8	0.98		<i>T. declivis</i>
KH2	Indeterminable	2	0.98	No distinguishing characters visible	<i>T. declivis</i>
KH2	Indeterminable	3	0.94	No distinguishing characters visible	<i>Centroberyx</i> sp.
KJ6	Unknown	6	1.04	Pigment just forming. No sign of segmentation.	<i>Thyrsites atun</i>
KP4	Indeterminable	3	1	No distinguishing characters visible	<i>Emmelichthys nitidus</i>
KP4	Indeterminable	3	1	No distinguishing characters visible	Fail
KP4	Indeterminable	3	0.9	No distinguishing characters visible	<i>Thyrsites atun</i>
KP4	Indeterminable	3	0.9	No distinguishing characters visible	<i>Thyrsites atun</i>

Table 4. Counts of morphologically identified *T. declivis* eggs at each developmental stage. Only positive sites listed.

Site	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Total
JA1					2		17				19
JA2				1	2		3				6
JB3					11	2	2	4	16		35
JC9	5										5
JD11		1					2			1	4
JD12		2			1			2			5
JD8		8									8
JD9		1					6		1		8
JE10	1										1
JE11				1	3		10				14
JE12				1							1
JE7	3			1							4
JF7										2	2
JG5								1			1
JG8	1										1
JS2					4						4
JS7	10				1						11
KA8	1	1									2
KC7	4										4
KD2	2				63			1			66
KD3	4					2					6
KE1	2					39					41
KE2						1					1
KF1					1			2	1		4
KF2				4	22		2	10			38
KF3				4	1						5
KF4					1						1
KF6									2		2
KG1							1	4	5		10
KG2					4	4	5	5	1		19
KG3					2		2	1	25	5	35
KG4						1				1	2
KG10		3	3	3	1						10
KH1	1	1		2			17	68			89
KH2		1				1	13	6			21
KH3								1	1		2
KH4				33	2						35
KH5				3			20		3	3	29
KJ7	1										1
KK3			1								1
KP3					2		1				3
KR1		3		1		27					31
Total	25	19	16	53	123	78	101	103	57	12	587

Discussion

Molecular analyses successfully validated eggs identified as *T. declivis* using species-specific morphological characters. The analysis further confirmed the presence of *T. declivis* eggs where morphological identification was problematic in ethanol preserved samples.

The molecular analyses confirmed the presence of *T. declivis* eggs within the vicinity of Kangaroo Island and Portland in Survey 1, as well as in the vicinity of Portland, King Island and north-western Tasmania in Survey 2. Highest raw abundances were collected near Kangaroo Island and King Island.

Egg diameters of *T. declivis* eggs within this study off southern Australia (mean 0.99 mm) were similar to those reported off eastern Australia (0.95 mm; Ward *et al.* 2015).

The molecular analyses also facilitated the identification of some eggs possessing similar morphological characters to *T. declivis*, including barracouta, *T. atun*. Although eggs of this species were not quantified in this study, their presence was observed over a broad range and in substantial numbers, indicating the region may be a key spawning area for this species.

Molecular analyses should continue to be employed to validate fish eggs when used in stock assessments, such as the Dailey Egg Production Method (DEPM), given the complexity of fish egg identification and poor taxonomic knowledge of fish eggs in Australian waters.

References

- Ahlstrom, E.H., Moser, H.G. (1980) Characters Useful in Identification of Pelagic Marine Fish Eggs. CA COOP. Oceanic Fisheries Investment Reports 21:131pp.
- Crossland, J. (1981) Fish eggs and larvae of the Hauraki Gulf, New Zealand. Fisheries Research Bulletin of the New Zealand Ministry of Agriculture and Fisheries 23:1-61
- Cunha, M.E., Vendrell, C., Goncalves, P. (2008) Experimental study of the dependence of embryonic development of *Trachurus trachurus* eggs on temperature. ICES Journal of Marine Science 65:17-24
- Goodsir, F., Armstrong, M.J., Witthames, P.R., Maxwell, D.L., and Fox, C.J. (2008) The use of species-specific TaqMan probes for identifying early stage gadoid eggs following formaldehyde fixation. ICES Journal of Marine Science, 65: 1573–1577.
- Karaiskou, N., Triantafyllidis, A., Alvarez, P., Lopes, P., Garcia-Vazquez, E., Triantaphyllidis, C. (2007) Horse mackerel egg identification using DNA methodology. Marine Ecology-an Evolutionary Perspective 28:429-434
- Neira, F.J., Perry, R.A., BurrIDGE, C.P., Lyle, J.M. and Keane, J.P. (2014) Molecular discrimination of shelf-spawned eggs of two co-occurring *Trachurus* spp. (Carangidae) in south-eastern Australia: a key step to future egg-based biomass estimates. ICES Journal of Marine Science; doi: 10.1093/icesjms/fsu151

- Perry, R.A. (2011) Spawning habitat of two commercial mackerel species (*Trachurus* spp.) in south-eastern Australia based on distribution of eggs identified using molecular techniques. Honours Thesis, University of Tasmania. 111pp.
- Oxley, A. P. A., S. R. Catalano, M. L. Wos-Oxley, E. L. Westlake, G. L. Grammer, and M. A. Steer. 2017. Using in situ hybridization to expand the daily egg production method to new fish species. *Molecular Ecology Resources* 17:1108-1121.
- Ward, T.M., Burnell, O., Ivey, A. R., & Carroll, J. and Keane, J. P. (2015). Summer spawning patterns and preliminary daily egg production method survey of jack mackerel and Australian sardine off the east coast. South Australian Research and Development Institute, Adelaide. 65p.

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 End Latitude	Longitude 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