



**FRDC**  
FISHERIES RESEARCH &  
DEVELOPMENT CORPORATION

**PREMIUM**  
FOOD AND WINE FROM OUR  
**CLEAN**  
ENVIRONMENT



**SOUTH**  
AUSTRALIA



Government  
of South Australia  
Primary Industries  
and Regions SA



**SARDI**  
SOUTH AUSTRALIAN  
RESEARCH AND  
DEVELOPMENT  
INSTITUTE

# **Egg distribution, reproductive parameters and spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the east coast during late winter and early spring**

**Tim M. Ward, Gretchen Grammer, Alex Ivey, Jonathan Carroll, John Keane,  
John Stewart and Lenore Litherland**

**December 2015**

**FRDC Project No 2014/033**

© 2015 Fisheries Research and Development Corporation and South Australian Research and Development Institute  
All rights reserved.

ISBN 978-921563-85-0

Egg distribution, reproductive parameters and spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the east coast during late winter and early spring.

2014/033

2015

### Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Fisheries Research and Development Corporation and the South Australian Research and Development Institute. 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.

This publication (and any information sourced from it) should be attributed to Ward, T .M., Grammer, G. L., Ivey, A., Carroll, J., Keane, J., Stewart, J. and Litherland, L. South Australian Research and Development Institute (Aquatic Sciences) 2015, *Egg distribution, reproductive parameters and spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the east coast during late winter and early spring*, December.

### Creative Commons licence

All material in this publication is licensed under a Creative Commons Attribution 3.0 Australia Licence, save for content supplied by third parties, logos and the Commonwealth Coat of Arms.



Creative Commons Attribution 3.0 Australia Licence is a standard form licence agreement that allows you to copy, distribute, transmit and adapt this publication provided you attribute the work. A summary of the licence terms is available from [creativecommons.org/licenses/by/3.0/au/deed.en](http://creativecommons.org/licenses/by/3.0/au/deed.en). The full licence terms are available from [creativecommons.org/licenses/by/3.0/au/legalcode](http://creativecommons.org/licenses/by/3.0/au/legalcode).

Inquiries regarding the licence and any use of this document should be sent to: [frdc@frdc.com.au](mailto:frdc@frdc.com.au)

### 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 does not accept any liability for the contents of this report or for any consequences arising from its use or any reliance placed upon it. Material presented in these Administrative Reports may later be published in formal peer-reviewed scientific literature.

The information, opinions and advice contained in this document may not relate, or be relevant, to a readers particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the FRDC.

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

#### Researcher Contact Details

Name: A/Prof Tim M. Ward  
Address: 2 Hamra Avenue, West Beach  
PO Box 120 Henley Beach 5022  
Phone: 08 8207 5433  
Fax: 08 8207 5481  
Email: Tim.Ward@sa.gov.au

#### FRDC Contact Details

Address: 25 Geils Court  
Deakin ACT 2600  
Phone: 02 6285 0400  
Fax: 02 6285 0499  
Email: [frdc@frdc.com.au](mailto:frdc@frdc.com.au)  
Web: [www.frdc.com.au](http://www.frdc.com.au)

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

# Contents

<b>Contents</b> .....	<b>3</b>
<b>Acknowledgments</b> .....	<b>9</b>
<b>Abbreviations</b> .....	<b>10</b>
<b>Executive Summary</b> .....	<b>11</b>
<b>1. Introduction</b> .....	<b>14</b>
1.1 Small Pelagic Fishes .....	14
1.2 Daily Egg Production Method .....	15
1.3 Blue Mackerel .....	16
1.4 Australian Sardine .....	17
1.5 Tailor .....	18
1.6 Need .....	19
<b>2. Objectives</b> .....	<b>20</b>
<b>3. Methods</b> .....	<b>20</b>
3.1 Study Area and Environmental Variables.....	20
3.2 Daily Egg Production and Spawning Area .....	20
3.3 Adult Reproductive Parameters.....	31
3.4 Spawning Biomass and Bootstrapping Procedures .....	36
3.5 Sensitivity Analysis .....	37
<b>4. Results</b> .....	<b>39</b>
4.1 Environmental Variables.....	39
4.2 Blue Mackerel .....	40
4.3 Australian Sardine .....	46
4.4 Tailor .....	56
<b>5. Discussion</b> .....	<b>60</b>
5.1. Blue Mackerel .....	60
5.2 Australian Sardine .....	62
5.3 Tailor .....	64
<b>6. Conclusions</b> .....	<b>65</b>
<b>7. Implications and recommendations</b> .....	<b>66</b>
<b>8. Further development</b> .....	<b>66</b>
<b>9. Extension and Adoption</b> .....	<b>66</b>
Planned Outcomes and Benefits .....	66

<b>10. Project materials developed</b> .....	<b>67</b>
Outputs and outcomes .....	67
<b>11. References</b> .....	<b>68</b>
<b>12. Appendices</b> .....	<b>73</b>
Appendix 1. Genetic identification of Blue Mackerel ( <i>Scomber australasicus</i> ) and Tailor ( <i>Pomatomus saltatrix</i> ) eggs .....	73
Appendix 2a: Egg density (eggs·m <sup>-2</sup> ) versus egg age (hours) for Blue Mackerel. Lines represent methods used to estimate mean daily egg production ( $P_0$ ) of Blue Mackerel on the east coast of Australia.. .....	84
Appendix 2b: Egg density (eggs·m <sup>-2</sup> ) versus egg age (hours) for Australian Sardine. Lines represent methods used to estimate mean daily egg production ( $P_0$ ) of Australian Sardine on the east coast of Australia.. .....	85
Appendix 3: Project Staff .....	86

## Tables

Table 1: Date, time and locations of trawls along the east coast of Australia for adult Blue Mackerel and Australian Sardine during August and September 2014. Shot locations are shown in Fig. 2. ....	23
Table 2. Mean, minimum and maximum adult Blue Mackerel parameters determined in DEPM surveys in South Australia between 2001 and 2006 (Ward and Rogers 2007).....	36
Table 3. Adult parameters of <i>Scomber</i> spp. sourced from available literature used to inform sensitivity analysis for female weight ( $W$ ), sex ratio ( $R$ ), spawning fraction ( $S$ ), and batch fecundity ( $F$ ). Values presented are study means. NSW: New South Wales; SA: South Australia; USA: United States of America	38
Table 4. Adult parameters of Australian Sardine determined in DEPM surveys in South Australia between 1998 and 2014 used to inform sensitivity analysis for egg production ( $P_0$ , eggs·day <sup>-1</sup> ·m <sup>-2</sup> ), female weight ( $W$ , g), sex ratio ( $R$ ), spawning fraction ( $S$ ), and batch fecundity ( $F$ , eggs·female <sup>-1</sup> ) (Ward et al. 2014b). ....	38
Table 5. Total survey area, spawning area ( $A$ ), percent area containing eggs, and spawning biomass of Blue Mackerel. ....	42
Table 6. Mean daily egg production ( $P_0$ ) of Blue Mackerel estimated using assumed egg mortality rates ( $z$ ) ranging from 0 (mean) to 0.6 day <sup>-1</sup> . The value used for biomass estimation is highlighted in bold. Ranges are 95% confidence intervals.....	43
Table 7. Parameters used in the calculations of spawning biomass of Blue Mackerel in 2014. #Range is 95% CI for $P_0$ at $Z = 0.3$ eggs·m <sup>-2</sup> ·day <sup>-1</sup> . *Source: Ward and Rogers (2007).....	44
Table 8. Total survey area, spawning area ( $A$ ), percent area containing eggs, and spawning biomass of Australian Sardine. ....	50
Table 9. Mean daily egg production ( $P_0$ ) of Australian Sardine estimated using four alternate models. The value used for biomass estimation is highlighted in bold. Ranges are 95% confidence intervals. ....	51
Table 10. Total number, mean weights (g) by sex, and sex ratio ( $R$ ; proportion of females by weight) of adult Australian Sardine collected off the east coast in 2014. Values in bold are sums (*) or weighted means (#). Refer to Fig. 2 for location of Site 1f. NSW: New South Wales.....	51
Table 11. Total number of post ovulatory follicles (POF) and estimates of spawning fraction ( $S$ ) for samples of female Australian Sardine collected along the East Australian Coast in 2014. Refer to Fig. 2 for location of Site 1f. Values in the bottom row are sums (*) or a weighted mean (#). NSW: New South Wales .....	53
Table 12. Parameters used in the calculations of spawning biomass of Australian Sardine in 2014. ....	53
Table 13. Total survey area, spawning area ( $A$ ), percent area containing eggs, and spawning biomass of Tailor. ....	56
Table 14. Total number, mean weights (g) by sex, and sex ratio ( $R$ ; proportion of females by weight) of adult Tailor measured on Fraser Island, Queensland during two recreational fishing surveys in August -September 2014. Values in bold are sums (*) or weighted means (#). QLD: Queensland .....	57
Table 15. Total number of post ovulatory follicles (POF) and estimates of spawning fraction ( $S$ ) for samples of female Tailor collected on Fraser Island, Queensland during two recreational fishing surveys in August -	

September 2014. Values in the bottom row are sums (\*) or a weighted mean (#). \*Includes hydrated females.  
QLD: Queensland ..... 58  
Table 16. Parameters estimated for use in future spawning biomass calculations of Tailor. .... 59

## Figures

- Fig. 1. Locations of ichthyoplankton sites sampled from the *FV Dell Richie II* along the Australian east coast during August-September 2014. Open circles indicate replicate hauls were samples were fixed in ethanol. The northern-most transect is designated as number 1 and the southern-most is number 45. Station numbers increased westward, with the eastern-most station being Station 1. .... 21
- Fig. 2 Trawl locations for Blue Mackerel and Australian Sardine conducted from the *FV Hazel-K* in August-September 2014 along the Australian east coast..... 22
- Fig. 3. Blue Mackerel egg stages used in this study following the characteristics of embryonic development described for Blue Mackerel (Ward and Rogers 2007, Neira and Keane 2008) and Chub Mackerel (Kramer 1960). a) Stage 2; b) Stage 3; c) Stage 4; d) Stage 5; e) Stage 6; f) Stage 7; g) Stage 8. See document text for specific descriptions. AN: anus; BC: blastodermal cap; BP: blastopore; CF: caudal fold; ES: embryonic shield; OG: oil globule; OP: optic cup; UN: unpigmented nape; SN: snout TL: tail tip. .... 26
- Fig. 4. Voronoi natural neighbour polygons used to estimate spawning area..... 29
- Fig. 5. Photomicrographs of ovarian histology from spawning capable Tailor collected on Fraser Island, Queensland during August-September 2014. A) spawning capable phase showing asynchronous oocyte development; B) actively spawning sub-phase showing germinal vesicle break down and hydration; C) spawning capable phase with day-1 post-ovulatory follicles; D) spawning capable phase with day-2 post-ovulatory follicles; E) spawning capable phase with alpha atresia present; F) spawning capable phase with beta atresia present. Mid- to late-stages of atresia can sometimes be mistaken for post-ovulatory follicles. Oocyte development and maturation stages: PG = primary growth; CA = cortical alveolar; Vtg1 = primary vitellogenic; Vtg2 = secondary vitellogenic; Vtg3 = tertiary vitellogenic; GVM = germinal vesicle migration; GVBD = germinal vesicle breakdown; HYD = hydration; POF 1 = day-1 post-ovulatory follicles; POF 2 = day-2 post-ovulatory follicles;  $\alpha$ -atresia = early-stage atresia;  $\beta$ -atresia = mid-stage atresia. .... 35
- Fig. 6. Blue Mackerel egg densities (eggs-m<sup>-2</sup>) and associated sea surface temperatures (SST, °C) along the east coast of Australia during August-September 2014. The northern-most transect is designated as number 1 and the southern-most is number 45..... 39
- Fig. 7. Kernel Density Growth Profile of each Blue Mackerel egg stage plotted by decimal time (hours) after spawning. Decimal time is based on time of sample collection. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample. Solid coloured lines represent the Kernel Density Smoothing and temporal extent of each egg stage. The solid black line is the modal time of developmental progression through the egg stages from post-spawn to pre-hatch. Grey points indicate eggs that were shifted  $\pm 24$  hours to assign egg stages to the correct timeframe (see Section 3.2.3). Red dashed lines specify midnight on the spawning night and 24 hr increments thereafter. .... 41
- Fig. 8. A cohort-by-cohort profile of Blue Mackerel eggs over time. The joined peaks of the Kernel Density Smoothing for each Blue Mackerel egg stage (grey lines) are shown at 24 hours intervals from the estimated peak spawning time. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample. .... 42

Fig. 9. Sensitivity analyses of the effects of individual parameters on estimates of spawning biomass of Blue Mackerel. Blue arrows are values estimated in the current survey, green arrows are values representing 95% CI for that parameter, and red and black arrows are mean, minimum and maximum values sourced from the literature as described in Table 2. ....	45
Fig. 10. Australian Sardine egg densities (eggs·m <sup>-2</sup> ) and associated sea surface temperatures (SST, °C) along the east coast of Australia during August-September 2014. Note: The northern-most transect is designated as number 1 and the southern-most is number 45. ....	46
Fig. 11. Kernel Density Growth Profile of each Australian Sardine egg stage plotted by decimal time (hours) after spawning. Decimal time is based on time of sample collection. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample. Solid coloured lines represent the Kernel Density Smoothing and temporal extent of each egg stage. The solid black line is the modal time of developmental progression through the egg stages from post-spawn to pre-hatch. Grey points indicate eggs that were shifted ±24 hours to assign egg stages to the correct timeframe (see Section 3.2.3). Red dashed lines specify midnight on the spawning night and 24 hr increments thereafter. ....	48
Fig. 12. A cohort-by-cohort profile of Australian Sardine eggs as they age. The joined peaks of the Kernel Density Smoothing for each Australian Sardine egg stage (grey lines) are shown at 24 hour separations from the estimated peak spawning time. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample (binned). ....	49
Fig. 13. Modal age of each egg stage (hours; estimated with the Kernel Density Growth Profile; solid line) plotted against temperature-development curves for Australian Sardine (White and Fletcher 1998; dashed lines). The mean <i>in situ</i> temperature encountered during the current survey was 20°C (range: 17 to 22°C). ....	50
Fig. 14. Relationship between gonad-free female weight and batch fecundity of Australian Sardine from 1998 to 2014 in South Australia (n = 1052) used to calculate batch fecundity of mature east coast Australian Sardine. Red shaded area = 95% CI. ....	52
Fig. 15. Sensitivity analysis of the effects of individual parameters on estimates of spawning biomass of Australian Sardine. Red arrows are values estimated in the current survey, and black arrows are the minimum and maximum values estimated for South Australian DEPM surveys between 1998 and 2014 as described in Table 4. ....	55
Fig. 16. Relationship between gonad-free female weight and batch fecundity of Tailor collected during the 2014 survey on Fraser Island, Queensland (n = 4). ....	58



# Acknowledgments

The Fisheries Research and Development Corporation (FRDC) provided funding (Project No: 2014/033). Mr John Wilson's assistance with organising the surveys is gratefully acknowledged. We thank the masters, crews, owners and staff of the FV *Dell Richie II* and FV *Hazel-K* for their professional and cheerful assistance. The efforts of Mr Stuart Richie and Mr Russell Kerr in ensuring the success of the surveys are recognized with gratitude. Mr Lorenzo Andreacchio, Mr Mathew Lloyd, Graham Hooper, and Joshua Fredberg (SARDI) collected ichthyoplankton samples from the FV *Dell Richie II*. Dr Owen Burnell and Mr Leonardo Mantilla assisted with collection of adult samples from the FV *Hazel-K*. Mr Nicholas Sarapuk, Ms Anne-Marie Hegary, Dr Ash Fowler and Ms Caitlin Young assisted with collection of adult samples in NSW. Mr Lorenzo Andreacchio, Mr Josh Fredberg, Mr Damian Matthews (SARDI) and Mr Nathavong Navong helped to sort the ichthyoplankton samples and identify eggs and larvae. Dr Chris Leigh (University of Adelaide) performed the histological analyses. Dr Richard McGarvey (SARDI) provided statistical advice on model selection. Dr Michael Steer, Dr Richard McGarvey and Dr Stephen Mayfield (SARDI) and two external reviewers provided comments on a draft of the report. The final report was approved for release by Dr Stephen Mayfield (SARDI Aquatic Sciences).

# Abbreviations

AFMA	Australian Fisheries Management Authority
BLAST	Basic Local Alignment Search Tool
BOLD	Fish Barcode of Life Database
CTD	Conductivity Temperature Depth
DEPM	Daily Egg Production Method
FRDC	Fisheries Research and Development Corporation
GLM	Generalised Linear Models
GSI	Gonadosomatic Index
IMAS	Institute of Marine and Antarctic Studies
ITQ	Individual Transferable Quotas
KDS	Kernel Density Smoothing
mtDNA	Mitochondrial DNA
NSW	New South Wales
NSW DPI	New South Wales Department of Primary Industries
PCR	Polymerase Chain Reaction
POF	Post-Ovulatory Follicles
DAF	Queensland Department of Agriculture and Fisheries
RAG	Resource Assessment Group
RBC	Recommended Biological Catches
SASF	South Australian Sardine Fishery
SARDI	South Australian Research and Development Institute
SPF	Small Pelagic Fishery
SPF RAG	Small Pelagic Fishery Resource Assessment Group
SST	Sea Surface Temperature
TAC	Total Allowable Catch
Tas DPIPWE	Tasmanian Department of Primary Industries, Parks, Water and Environment
VNN	Voronoi Natural Neighbour

# Executive Summary

## Overview

This study was undertaken collaboratively by the South Australian Research and Development Institute (SARDI), University of Tasmania, Fisheries Queensland and New South Wales Department of Primary Industries (NSW DPI). The Daily Egg Production Method (DEPM) was applied to Blue Mackerel (*Scomber australasicus*) and Australian Sardine (*Sardinops sagax*) off the east coast of Australia to inform future management of these species. This was the first study to estimate adult reproductive parameters of Tailor (*Pomatomus saltatrix*) required for future application of the DEPM.

The spawning biomass of Blue Mackerel off eastern Australia during August-September 2014 was estimated to be ~83,300 t (95% CI = 35,100 - 165,000 t). Most estimates of spawning biomass obtained in the sensitivity analyses were mainly 50,000 t and 100,000 t. The estimate of spawning biomass should be treated with caution as adult samples were not collected during the study. Sampling intensity for estimates of egg production in the region was higher than in exploratory surveys conducted in 2003 and 2004. Current estimates of egg production and spawning area are likely to be more robust than those previously reported.

The spawning biomass of Australian Sardine off eastern Australia during August-September 2014 was estimated to be ~49,600 t (95% CI = 24,200 - 213,300 t). Most estimates of spawning biomass obtained in sensitivity analyses were between 30,000 t and 110,000 t. Credible values for only one parameter (spawning fraction) provided estimates of spawning biomass that were outside that range; this parameter was estimated with a high degree of confidence in the present study. The proportion of the adult biomass of Australian Sardine off eastern Australia that occurred outside the survey area during the survey period is unknown.

## Background

Small pelagic fishes such as Blue Mackerel and Australian Sardine are known as low trophic level species or forage fish. These species are critical components of pelagic ecosystems and prey items for a range of predatory fishes, seabirds and marine mammals. There is growing international consensus about how fisheries for these species should be managed to ensure their ecological sustainability. Estimates of abundance/biomass obtained from fishery-independent surveys underpin the assessment and management frameworks of most modern fisheries for small pelagic species. For example, estimates of spawning biomass obtained using the DEPM are the key biological performance indicators in the South Australian Sardine Fishery (SASF) and the Commonwealth Small Pelagic Fishery (SPF).

Off the east coast of Australia, Blue Mackerel and Australian Sardine are targeted in several commercial fisheries and are quota species of the SPF. Blue mackerel are also targeted by recreational fishers. Preliminary evaluations suggest that the DEPM is a suitable method for stock assessment of both species.

The current project also evaluated the application of the DEPM to Tailor, an iconic recreational fishing species off the coasts of Queensland and New South Wales (NSW).

## Objectives of the study

1. Determine distribution and abundance of eggs and larvae of Blue Mackerel, Australian Sardine and Tailor off the east coast during winter/spring;
2. Establish methods for estimating adult reproductive parameters of Blue Mackerel, Australian Sardine and Tailor off the east coast during winter/spring;
3. Produce preliminary estimates of the spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the east coast during winter/spring.

## Methods

Surveys to estimate DEPM parameters were conducted concurrently from two vessels during August-September 2014. Ichthyoplankton samples were collected from the *FV Dell Richie II* at 262 stations along 45 transects perpendicular to the coastline between Sandy Cape, Queensland and Bateman's Bay, NSW. Fish trawls for adult Australian Sardine and Blue Mackerel were undertaken from the *FV Hazel-K* at 22 locations between Byron Bay and Stockton Beach, NSW. Additional samples of adult Australian Sardine were collected from commercial purse seine catches off Iluka, NSW. Adult Tailor samples were collected during surveys of recreational fishers off Fraser Island, Queensland.

Standard laboratory procedures were used to identify and stage eggs of Blue Mackerel, Australian Sardine and Tailor. Egg identifications of Blue Mackerel and Tailor were confirmed using molecular techniques. Kernel Density Growth Profiling was used to estimate ages of eggs and infer the time of peak spawning. Four model fits were used to estimate egg production ( $P_0$ ) of Blue Mackerel and Australian Sardine. Tailor eggs were not found in sufficient numbers in the samples to calculate egg production ( $P_0$ ). Spawning area was estimated using the Voronoi Nearest Neighbour method. Standard statistical approaches were used to estimate adult parameters. Adult samples of Blue Mackerel were not collected during the trawl survey conducted in this study; adult parameters estimated for South Australia between 2001 and 2006 were used to estimate spawning biomass. Sensitivity analyses were undertaken to determine the influence of uncertainty in individual parameters on estimates of spawning biomass.

## Results, implications and recommendations

Live Blue Mackerel eggs ( $n = 2,330$ ) were collected from 70 of the 262 (26.7%) stations between Sandy Cape, Queensland to just south of Newcastle, NSW. The highest densities of Blue Mackerel eggs were recorded in waters north of Coffs Harbour and off Port Stephens where sea surface temperatures (SSTs) ranged between 18 and 20°C. The estimated spawning area ( $A$ ) was 17,911 km<sup>2</sup>, comprising 27.3% of the total area sampled (65,528 km<sup>2</sup>). Mean daily egg production ( $P_0$ ) was 34.6 eggs.day<sup>-1</sup>.m<sup>-2</sup> (95% CI = 14.6 - 69.1). The estimate of spawning biomass of 83,300 t was based on estimates of adult parameters from South Australia and should be treated with caution. Sensitivity analyses showed that realistic variations of each parameter produced estimates of spawning biomass for Blue Mackerel that were mainly between about 50,000 t and 100,000 t. The exceptions were the lower estimates of spawning fraction (0.05,  $S$ ) and batch fecundity (22,085 eggs.female<sup>-1</sup>;  $F$ ) and the higher estimate of daily egg production (69.1 eggs.day<sup>-1</sup>.m<sup>-2</sup>;  $P_0$ ), which produced estimates of spawning biomass between 150,000 and 250,000 t.

A total of 3,461 live Australian Sardine eggs were collected from 89 of the 262 (34.0%) stations sampled. Most Australian Sardine eggs were collected from waters between Sandy Cape and south of Newcastle where SSTs ranged between 17 and 22°C. The highest densities of eggs were collected from sites with SSTs ranging between 18 and 21°C. The estimated spawning area ( $A$ ) was 22,400 km<sup>2</sup>, comprising 34.2% of the total area sampled (65,528 km<sup>2</sup>). Mean daily egg production ( $P_0$ ) was 52.6 eggs.day<sup>-1</sup>.m<sup>-2</sup> (95% CI = 39.1 - 78.1). Mean sex ratio ( $R$ ) was 0.54 (95% CI = 0.40 - 0.70). Mean female weight ( $W$ ) was 38.8 g (95% CI = 36.8 - 42.1 g). Mean batch fecundity ( $F$ ) was 11,942 eggs (95% CI = 11,148 - 13,036). Mean spawning fraction ( $S$ ) was 0.14 (95% CI = 0.04 - 0.21). All DEPM parameters were estimated from a large number of samples and are considered robust. The estimate of the spawning biomass of Australian Sardine, i.e. 49,600 t (95% CI = 24,200 - 213,300 t), is considered suitable for setting recommended biological catches as outlined under the harvest strategy for the SPF. Most of the estimates of spawning biomass obtained in the sensitivity analyses were between approximately 30,000 and 110,000 t. Credible values for only one parameter (spawning fraction, 0.04) provided estimates outside that range (i.e. ~ 175,000 t). The estimate in the present study is larger than a preliminary estimate of 28,809 t for Australian Sardine off eastern Australia (Ward et al. 2007). The proportion of the total adult biomass of Australian Sardine off eastern Australia that occurs outside the survey area during the survey period is unknown.

Adult Tailor ( $n = 278$  males and 600 females) were measured on Fraser Island, Queensland during two recreational fishing surveys in August -September 2014. Average fork length was 359 mm (range: 240 - 560 mm FL). Mean sex ratio ( $R$ ) was 0.70 (95% CI = 0.67 - 0.73). Mean female weight ( $W$ ) was 613 g (95% CI = 534 - 734 g). Ovaries were collected from an additional 206 females for histological analyses (average length: 375 mm FL; range: 310 - 520 mm FL). Mean batch fecundity ( $F$ ) was 102,361 eggs (95% CI = - 189,404 - 290,749). Mean spawning fraction ( $S$ ) was 0.14 (95% CI = 0.11 - 0.32). No Tailor eggs were collected in the main ichthyoplankton survey (262 formalin-preserved samples), but one egg was collected off Fraser Island in samples collected for genetic analysis. Future sampling for tailor eggs should focus on inshore areas. Egg production, spawning area and spawning biomass for Tailor could not be estimated due to the lack of eggs collected. Batch fecundity was estimated from four hydrated individuals; future surveys should attempt to collect more samples over a broader temporal range and consider alternative methods for estimating batch fecundity.

This study made some crucial technical developments (e.g. established a robust method for ageing fish eggs from field surveys) and filled several key knowledge gaps (e.g. estimates of adult reproductive parameters for Australian Sardine and Tailor off the east coast). However, further study is required to fill remaining gaps (e.g. adult parameters for Blue Mackerel off the east coast and egg production/spawning area and batch fecundity for Tailor) and those identified during the course of the project (e.g. spawning habitat and egg stages of Tailor).

**Keywords:** Blue Mackerel, *Scomber australasicus*, Australian Sardine, *Sardinops sagax*, Tailor, *Pomatomus saltatrix*, Daily Egg Production Method, Kernel Density Growth Profile, Spawning Biomass, Small Pelagic Fishery, eastern Australia, Queensland, New South Wales

# 1. Introduction

## 1.1 Small Pelagic Fishes

Small pelagic fishes are also known as low trophic level species or forage fish (Pikitch et al. 2012). These species are critical components of pelagic ecosystems and prey items for a range of predatory fishes, seabirds and marine mammals (e.g. Goldsworthy et al. 2013). Two important small pelagic species in Australian waters are Australian Sardine (*Sardinops sagax*) and Blue Mackerel (*Scomber australasicus*); both are taken in several commercial fisheries off the east coast and are quota species in the Commonwealth Small Pelagic Fishery (SPF). Blue mackerel are also targeted by recreational fishers, mainly for bait. Tailor (*Pomatomus saltatrix*) is a medium-sized pelagic fish that is heavily targeted by recreational fishers off the coasts of Queensland and New South Wales (NSW).

There is growing international consensus about how fisheries for small pelagic species need to be managed to ensure their ecological sustainability (e.g. Smith et al. 2011, Pikitch et al. 2012). Estimates of abundance obtained from fishery-independent surveys underpin the assessment and management frameworks of most modern fisheries for small pelagic species. For example, estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are the key biological performance indicators in both the South Australian Sardine Fishery (SASF) and SPF (Ward et al. 2012, 2015b)

The SPF was established in 1992 and is managed by the Australian Fisheries Management Authority (AFMA). The SPF includes Commonwealth waters (3 to 200 nm) around southern Australia and extends south from the Queensland/ NSW border (28 to 24°S) to near Lancelin, Western Australia (31°S). The fishery is divided into two sub-areas (East and West) by a line through Bass Strait and south of Tasmania (146° 30'S).

A harvest strategy for the SPF was established in 2009 (AFMA 2009) and updated in 2015 (AFMA 2015). The harvest strategy is used to establish recommended biological catches (RBCs) for each quota (target) species. Stocks are allocated to a tier based upon the level of knowledge about stock size, with Tier 1 having the highest level of information available and Tier 3 the lowest (Moore and Skirtun 2012). Total Allowable Catches (TACs) reflect the tier level; Tier 1 stocks have the largest TACs and Tier 3 the smallest (AFMA 2015). The tiered system was introduced to ensure that significant exploitation only occurs in stocks where there is a high level of confidence that this level of exploitation can be sustained (Moore and Skirtun 2012). The SPF harvest strategy specifies that estimates of spawning biomass are obtained using the DEPM (Moore and Skirtun 2012).

RBCs derived from the harvest strategy apply to fish stocks throughout their range and are conservative (less than 20% of the estimated spawning biomass) to account for the ecological importance of SPF species. TACs for each quota species are determined by subtracting other sources of fishing mortality (i.e. catches taken in other Commonwealth and State fisheries) from the corresponding RBCs.

## 1.2 Daily Egg Production Method

The DEPM was developed for stock assessment of the northern anchovy, *Engraulis mordax* (Parker 1980, Lasker 1985). It has been applied to at least 18 species of small pelagic fishes worldwide (Stratoudakis et al. 2006, Dimmlich et al. 2009, Neira et al. 2009, Ward et al. 2009). The DEPM is widely used because it is often the most practical option available for stock assessment of small pelagic species that spawn multiple batches of pelagic eggs over an extended spawning season.

The DEPM relies on the premise that the biomass of spawning adults can be estimated by dividing the mean number of pelagic eggs produced per day throughout the spawning area (i.e. total daily egg production) by the mean number of eggs produced per day per unit biomass of adult fish (i.e. mean daily fecundity; Lasker 1985). Total daily egg production is the product of mean daily egg production ( $P_0$ ) and total spawning area ( $A$ ). Mean daily fecundity is calculated by dividing mean female weight ( $W$ ) by the product of mean sex ratio (by weight,  $R$ ), mean batch fecundity (number of oocytes in a batch,  $F$ ), and mean spawning fraction (proportion of mature females spawning each day/night,  $S$ ).

Spawning biomass ( $SB$ ) is calculated according to the equation:

$$SB = \frac{P_0 A W}{R F S} \quad \text{Equation 1}$$

The DEPM is applied to fishes that spawn multiple batches of pelagic eggs over an extended spawning season (e.g. Parker 1980). Data used to estimate DEPM parameters are typically obtained during fishery-independent surveys involving vertical plankton tows at sites located at regular intervals along parallel cross-shelf transects. Adult samples should be collected at the same time as the egg survey: either opportunistically during egg sampling, from a dedicated survey using another vessel or from the commercial fleet (Stratoudakis et al. 2006). 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). Sensitivity analyses can be used to assess the effects of variations in individual parameters on estimates of spawning biomass.

The DEPM is used widely, but a range of challenges have been encountered and estimates of spawning biomass are generally considered to be accurate (unbiased) but relatively imprecise (e.g. Alheit 1993, Hunter and Lo 1997, Stratoudakis et al. 2006). There are considerable uncertainties associated with the estimation of  $P_0$  and  $S$  in particular (Fletcher et al. 1996, McGarvey and Kinloch 2001, Ward et al. 2001a, Ward et al. 2001b, Gaughan et al. 2004). For example,  $P_0$  has been determined using a variety of statistical approaches. Ward et al. (2011a) showed that these approaches provide different estimates of  $P_0$  and suggested that the log-linear model of Picquelle and Stauffer (1985) should be used for Australian Sardine because it fits strongly over-dispersed egg density data better and provides more consistent and precautionary estimates of  $P_0$  than the exponential mortality model and most generalised linear models. McGarvey and Kinloch (2001) and Bernal et al. (2011) suggested using an “all years” estimate of mortality to estimate egg production, which reduces the number of degrees of freedom in each yearly regression but loses information about inter-annual variations in mortality.

Spawning fraction (*S*) is often the most difficult DEPM parameter to estimate for small pelagic fish. Obtaining representative samples of adults can be difficult because during the spawning period, spawning females are over-represented in ephemeral spawning aggregations and under-represented in the remainder of the population (Stratoudakis et al. 2006). Much of the uncertainty surrounding estimates of *S* is associated with determining whether imminent or recent spawners or both should be used in calculations. However, the size and reproductive characteristics of many small pelagic species can also vary spatially and temporally, and it is critical that the design of the adult sampling program adequately addresses these issues.

In Australia, the DEPM was first used to estimate the spawning biomass of the Australian Sardine, *Sardinops sagax*, in the early-mid 1990s (Fletcher et al. 1996, Ward et al. 1998, Ward and McLeay 1998).

Subsequently, DEPM assessments have been applied to numerous species within the SPF, including Australian Sardine in the East sub-area (Ward et al. 2007, Ward et al. 2015a), Blue Mackerel in the East and West sub-areas (Ward and Rogers 2007, Ward et al. 2009), Jack Mackerel in the East sub-area (Ward et al. 2015a), Redbait in the East sub-area (Neira et al. 2009) and Yellowtail Scad, *Trachurus novaezelandiae*, in the East sub-area (Neira 2009).

### 1.3 Blue Mackerel

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

Commercial fishing for Blue Mackerel began off the east coast in the late 1980s (Stewart and Ferrell 2001), and total annual catches since 1997/98 have ranged between approximately 300 and 1000 tonnes (e.g. Ward et al. 2015b). The east coast stock of Blue mackerel is classified as 'not overfished' and 'not subject to overfishing' (Georgeson et al. 2014). Blue Mackerel may attain lengths up to 650 mm (Hutchins and Swainston 1986) and ages of 8+ years in Australia (Stevens et al. 1984), but results from Ward and Rogers (2007) suggest smaller, younger fish (200 to 300 mm TL; 1 to 2 years) are more common along the Queensland/NSW coast. In NSW, Stewart and Ferrell (2001) reported 70% of the commercial Blue Mackerel purse-seine catch was comprised of 1 year old fish.

Spawning of Blue Mackerel occurs between November and April off southern Australia and between July and October off eastern Australia (Neira and Keane 2008, Rogers et al. 2009). In southern Australia, Ward and Rogers (2007) estimated spawning frequency to be 2 to 11 days (mean: 7 days), and length at 50% maturity for males and females was 236.5 and 286.8 mm FL, respectively. Shelf waters of southern Queensland and northern NSW are the main spawning area for the eastern Blue Mackerel stock (Rogers et al. 2009). Eggs and larvae along the east coast are found in high abundances in shelf waters with mean temperatures of 18 - 21°C and salinities between 35.35 - 35.60 (Neira and Keane 2008). The flow of the East Australian Current (EAC) south along the east Australian coast is thought to link directly with the winter-spring spawning dynamics of Blue Mackerel by providing optimal temperatures for spawning and egg



development, creating spawning 'hotspots' where the upwelling of nutrient rich water occurs, and dispersing eggs and larvae by advection both south and eastwards (Neira and Keane 2008).

Blue Mackerel are pelagic serial spawners whose buoyant, pelagic eggs make them suitable for application of the DEPM (e.g. Lasker 1985, Rogers et al. 2009, Ward et al. 2009). Exploratory surveys in October 2003 and July 2004 along the east coast provided conservative, preliminary estimates of spawning biomass at 29,578 t, and demonstrated that the DEPM is a suitable stock assessment method for Blue Mackerel (Ward and Rogers 2007, Ward et al. 2009). Spawning biomass for the eastern stock was potentially underestimated in these surveys due to limitations in the timing, sampling intensity, and spatial coverage of the surveys.

Adult reproductive characteristics used in DEPM estimates to calculate spawning biomass for the east coast in 2003/04 were a combination of parameters sourced from both the eastern and southern Australian populations of Blue Mackerel (Ward and Rogers 2007). The southern population was surveyed in February-March 2005 and the resulting spawning fraction was used in east coast estimates. Egg production and spawning area are important parameters for estimating spawning biomass, sensitivity analyses suggested that uncertainty in estimates of spawning biomass for the east coast in July 2004 reflected uncertainty in estimates of spawning fraction (Ward and Rogers 2007). This finding demonstrated the importance of developing a method for collecting representative adult data to underpin future application of the DEPM to Blue Mackerel off the east coast.

## 1.4 Australian Sardine

The Australian Sardine (Jenyns 1842, *Clupeidae*) is found in temperate marine waters from southern Queensland to Western Australia, including northern Tasmania (Gomon et al. 2008). It is a small, stream-lined, forage species (Gomon et al. 2008) that supports some of the world's largest fisheries (Schwartzlose et al. 1999). Along the east coast of Australia, Australian Sardine is targeted by fishers operating in the SPF, NSW Ocean Hauling Fishery and Victorian Ocean Purse Seine Fishery (Ward et al. 2014a). Annual catches off the east coast have exceeded 2,000 t since 2004/05, peaked at 4,768 t in 2008/09 before declining to 1,115 t in 2012/13 (Ward et al. 2015b). Recent declines in catches have been accompanied by a decline in the number of vessels reporting catches in the region, reaching a historical low of seven vessels in 2012/13. In 2013/14, the catch of 1,385 t was taken by eight vessels (Ward et al. 2015b).

The gonadosomatic index (GSI) of Australian Sardine peaks from late spring to early summer off Victoria (Hoedt and Dimmlich 1995; Neira *et al.* 1999). Off southern NSW, the peak GSI occurs between winter and summer, i.e. July to December (Stewart *et al.* 2010), while in southern Queensland the peak in GSI occurs in winter to early spring (Ward and Staunton-Smith 2002). This information is consistent with the hypothesis that some fish migrate northward during winter to spawn in waters of southern Queensland when water temperatures are below 23°C (Ward and Staunton-Smith 2002).

The DEPM has been widely applied to clupeoids both within Australia and overseas. Preliminary evaluations suggest that the DEPM is a suitable method for stock assessment of Australian Sardine off the east coast (Staunton-Smith and Ward 2000, Ward et al. 2007). The best estimate of spawning biomass of the East Australian Sardine stock during July 2004 was ~29,000 t, with likely estimates ranging from ~25,000 to

35,000 t (Ward et al. 2007). Recent assessments suggest that annual catches over the last decade have reached levels where a dedicated DEPM survey is needed to assess the status of the east coast stock (e.g. Ward et al. 2014c). The east coast stock of Australian Sardine is currently classified as sustainable (Flood et al. 2014, Ward et al. 2015b).

## 1.5 Tailor

Tailor (*Pomatomus saltatrix*, Linnaeus 1766) is found throughout the world in most tropical and temperate oceans, except the eastern and northern Pacific Ocean. It occurs in continental shelf waters, including bays and estuaries, and is a highly migratory, piscivorous predator. In Australia, Tailor are distributed from southern Queensland to the Bass Strait in Victoria and along the western coast from Exmouth to Esperance, Western Australia (Lenanton et al. 1996, Miskiewicz et al. 1996, Gomon et al. 2008). Fish from the East and west coasts are classified as genetically separate stocks (Nurthen et al. 1992).

Tailor are fished commercially and recreationally on the east and west coasts of Australia, with recreational catches historically being much greater than commercial catches (Leigh and O'Neill 2004, Taylor et al. 2012, Smith et al. 2013). Recreational and commercial catches display a decreasing trend in Queensland-NSW since 2000 (Litherland et al. 2014, Queensland Department of Agriculture, Fisheries and Forestry 2014). The trend can be linked to management arrangements introduced since 2002 that have been effective in lowering fishing pressure (Litherland et al. 2014, Queensland Department of Agriculture, Fisheries and Forestry 2014). The management measures included reduced possession limits, TACs, seasonal closures and increased minimum legal size (Queensland Department of Agriculture 2014). Recreational harvest has reduced from 410 t (2000) to 140 t (2010) in Queensland (Queensland Department of Agriculture 2013). During the 1970s and 1990s, there were periods of high recruitment to the fishery, but since 2001, recruitment has been considered to be below average (Leigh and O'Neill 2004, Litherland et al. 2014). The Queensland-NSW Tailor stock was assessed in 2008 and had a biomass greater than 50% of virgin levels with a total harvest below the estimated maximum sustainable yield (MSY; 1326 t). The general decline in harvest numbers and fishing pressure, particularly in the Queensland component, suggests total harvest has remained below MSY and the stock is unlikely to be or become recruitment overfished (Litherland et al. 2014).

Migratory patterns of Tailor along coastlines are associated with spawning and feeding. Off the east coast during winter and spring, schools of mature Tailor migrate northward to spawn in the subtropical waters of southern Queensland (Ward et al. 2003). The fish are thought to have a protracted spawning season where spawning continues as they return to southern temperate waters during summer- autumn (Ward et al. 2003, Leigh and O'Neill 2004). Conversely, the west coast stock is comprised of both migratory and resident populations with the main spawning event occurring in northern climes during winter-spring and a smaller summer event in temperate waters (Lenanton et al. 1996, Smith et al. 2013). These patterns tend to be driven by a narrow range of SSTs: east coast 21 to 23°C (Ward et al. 2003) and west coast 18 to 24°C (Smith et al. 2013). Tailor have pelagic larval and juvenile phases before the young recruit to estuarine or inshore habitats (Juanes et al. 1996, Lenanton et al. 1996). On the east coast, larvae spawned in northern areas are advected south by the East Australian Current (Miskiewicz et al. 1996, Ward et al. 2003). In

contrast, larvae on the west coast are transported south by wind driven, inner-shelf currents instead of the poleward flowing Leeuwin Current (Lenanton et al. 1996, Smith et al. 2013). Tailor switch from planktivory to piscivory at an early age (<1 yr and 40 mm standard length (SL); Marks and Conover 1993) and prey on a variety of fish, such as Australian Sardine, Blue Mackerel and Garfish (*Hyporhamphus melanochir*) (Beckley and van der Lingen 1999, Anonymous 2014).

Tailor are serial spawners with asynchronous oocyte development and batch fecundities of 114,513 to 920,746 eggs (mean: 402,247 eggs; USA; Robillard et al. 2008). In Western Australia, the sex ratio in fishery catches is 1:1.5 (biased towards females); 50% maturity occurs at 320 mm total length (TL) and <2 years old (Smith et al. 2013). Juveniles (<150 mm TL) display high growth rates of 0.5 to 0.8 mm.day<sup>-1</sup> with faster growth rates related to higher water temperatures (Smith et al. 2013). Tailor may reach a maximum age of 10 years and over 1 m in length; however, smaller younger fish are more common (i.e. <50 mm TL; 2 to 5 years old; Litherland et al. 2014). Natural mortality has been estimated to be 0.34 - 0.42 y<sup>-1</sup> on the west coast (Smith et al. 2013) and 0.8 - 1.3 y<sup>-1</sup> on the east coast (Leigh and O'Neill 2004).

The DEPM has never been applied to Tailor either within Australia or overseas. As the *Pomatomidae* family is monotypic (e.g. Gomon et al. 2008), there are no closely related species from which reproductive parameters can be estimated.

## 1.6 Need

This project was developed to: 1) support the ecologically sustainable management of the SPF, 2) address community concerns regarding ecological and social impacts of large scale harvesting of small pelagic fishes, and 3) provide DEPM-related information for the future management of Tailor, an iconic recreational fishing species. It was developed at the request of the SPF Resource Assessment Group (RAG). The SPF Research Strategy and Research Plan for 2013/14 and 2014/15 identified DEPM surveys of Blue Mackerel and Australian Sardine off the east coast as a high priority for the fishery.

Knowledge of the winter/spring spawning patterns of Blue Mackerel and Australian Sardine is needed to underpin future assessment of these stocks and the ecologically sustainable development of pelagic fish resources off the east coast of Australia. Current and robust estimates of the population size of Blue Mackerel and Australian Sardine off the east coast are needed to address community concerns regarding the potential ecological impacts of large scale fishing for small pelagic fishes along the east coast. Information on the egg distribution, reproductive parameters and spawning biomass of Tailor is needed for future management of this iconic recreational fishing species in southern Queensland and NSW.

Under the current harvest strategy, indicative exploitation rates are reduced from 15% to 7.5% (Blue Mackerel) and 20% to 10% (Australian Sardine) when spawning biomass estimates are more than five years old. Thus, these surveys may facilitate increases in Total Allowable Catches (TACs) for Blue Mackerel and Australian Sardine in the SPF by updating DEPM estimates of spawning biomass. These surveys will also enhance the current knowledge and future sustainability of Tailor off the east coast of Australia.

## 2. Objectives

The objectives of the project were to:

1. Determine distribution and abundance of eggs and larvae of Blue Mackerel, Australian Sardine and Tailor off the east coast during winter/spring;
2. Establish methods for estimating adult reproductive parameters of Blue mackerel, Australian Sardine and Tailor off the east coast during winter/spring;
3. Produce preliminary estimates of the spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the east coast during winter/spring.

## 3. Methods

### 3.1 Study Area and Environmental Variables

#### 3.1.1 Study area

Ichthyoplankton surveys for Blue Mackerel, Australian Sardine and Tailor were conducted from the *FV Dell Richie II* at 262 stations along 45 transects running perpendicular to the coastline in August to September 2014 between Sandy Cape, Queensland and Bateman's Bay, NSW (Fig. 1). Transects were located 15 nm apart, with stations on each transect located 5 nm apart. Adult Blue Mackerel and Australian Sardine were sampled at 22 stations using a modified demersal trawl net deployed from the *FV Hazel-K* during August and September 2014 in shelf and slope waters between Byron Bay and Newcastle, NSW (Fig. 2, Table 1). Adult samples were taken from areas known to be suitable for trawling. Adult Tailor were collected during surveys of recreational fishers between Nkgala Rocks and the Maheno wreck on the eastern beaches off Fraser Island, Queensland. Samples of Australian Sardine were also collected from commercial purse seine catches off Iluka by the NSW Department of Primary Industries.

#### 3.1.2 Water temperature

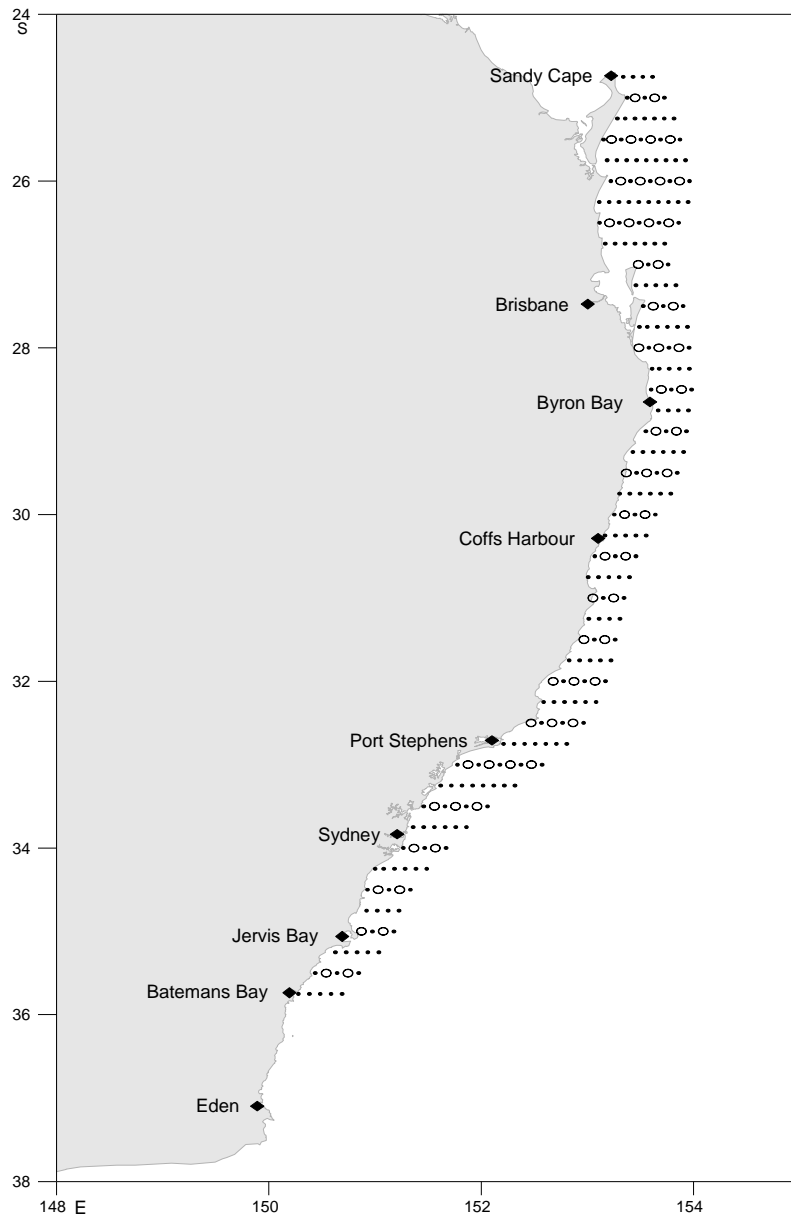
At each ichthyoplankton sampling station (Fig. 1), a *Sea-Bird* Conductivity-Temperature-Depth (CTD) recorder (attached to the bongo net frame, Section 3.2.1) was deployed to collect oceanographic data. Estimates of water temperature at a depth of 3 m were extracted from each profile. Spatial plots of SST were prepared using ArcGIS® (Version 10.1).

### 3.2 Daily Egg Production and Spawning Area

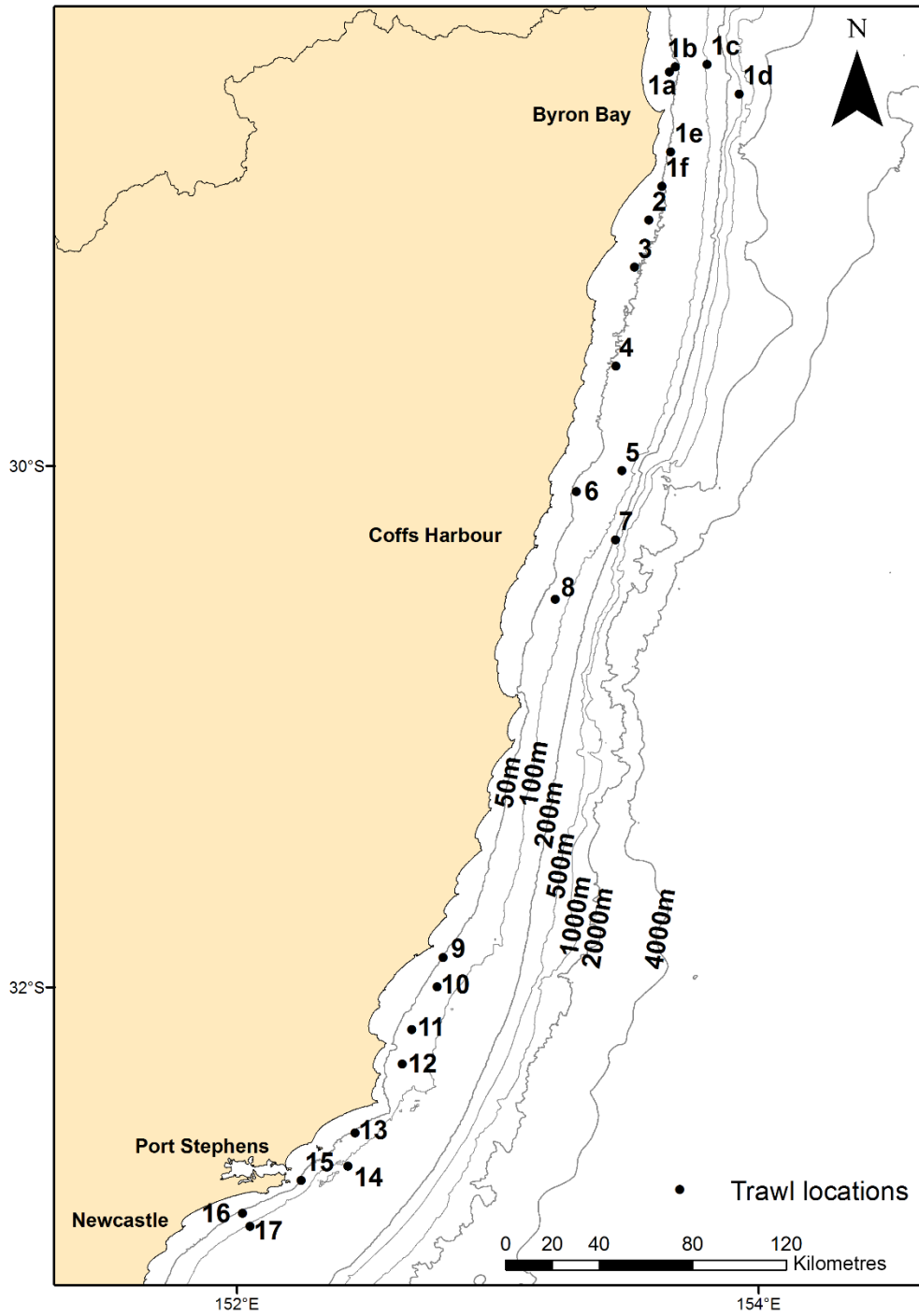
#### 3.2.1 Ichthyoplankton sampling

Ichthyoplankton samples (n = 262) were collected from 19 August to 14 September 2014 in vertical tows of paired bongo plankton nets (Fig. 1). Each net had an internal diameter of 0.6 m, 500 µm mesh and plastic

cod-ends. During each tow, the bongo net was lowered to a depth of 10 m above the seabed to a maximum depth of 200 m and retrieved vertically at a speed of  $\sim 1 \text{ m}\cdot\text{s}^{-1}$ . General Oceanics™ 2030 flow-metres and factory calibration coefficients were used to estimate the distance travelled by the net during each tow. Where there was a discrepancy of more than 5% between flow-metres, the relationship between wire length released and flow-metre units was used to determine which metre was more accurate, and that value was used for both nets. Upon retrieval of the nets, samples from each of the two cod-ends were washed into a sample container. Ichthyoplankton samples were fixed using 5% buffered formaldehyde and seawater. Replicate plankton tows were undertaken for 20% of the sites throughout the study region and preserved using 96% ethanol for genetic validation of Blue Mackerel and Tailor eggs (see Appendix 1).



**Fig. 1.** Locations of ichthyoplankton sites sampled from the *FV Dell Richie II* along the Australian east coast during August-September 2014. Open circles indicate replicate hauls where samples were also fixed in ethanol. The northern-most transect is designated as number 1 and the southern-most is number 45. Station numbers increased westward, with the eastern-most station being Station 1.



**Fig. 2.** Trawl locations for Blue Mackerel and Australian Sardine conducted from the *FV Hazel-K* in August-September 2014 along the Australian east coast.

**Table 1:** Date, time and locations of trawls along the east coast of Australia for adult Blue Mackerel and Australian Sardine during August and September 2014. Shot locations are shown in Fig. 2.

Shot	Date	Start Time End Time	Start latitude longitude End latitude longitude	Depth (m)
1-A	21 August	07:20 09:30	28°33.34'S 153°39.26'E 28°25.37'S 153°39.70'E	44-51
1-B	21 August	10:10 12:25	28°24.71'S 153°40.28'E 28°31.56'S 153°41.38'E	53-55
1-C	22 August	07:10 08:50	28°30.85'S 153°47.96'E 28°24.34'S 153°48.33'E	106-110
1-D	22 August	10:20 12:05	28°31.43'S 153°60.66'E 28°37.39'S 153°50.42'E	146-152
1-E	22 August	14:10 16:10	28°44.46'S 153°39.99'E 28°51.01'S 153°39.56'E	53-53
1-F	22 August	16:55 19:00	28°51.87'S 153°39.11'E 28°59.48'S 153°36.40'E	53-42
2	23 August	06:35 08:50	29°00.09'S 153°36.33'E 29°06.81'S 153°33.18'E	46-48
3	23 August	09:50 12:00	29°10.84'S 153°32.57'E 29°17.79'S 153°30.39'E	51
4	23 August	14:25 17:20	29°32.09'S 153°27.69'E 29°42.07'S 153°26.62'E	49-60
5	24 August	07:05 08:25	29°58.99'S 153°29.42'E 30°03.33'S 153°27.72'E	86-93
6	8 September	08:10 09:55	30°07.98'S 153°17.22'E 30°03.90'S 153°18.90'E	49-53
7	8 September	11:55 14:00	30°11.43'S 153°28.41'E 30°22.69'S 153°25.82'E	132-134
8	8 September	16:15 18:05	30°27.57'S 153°13.72'E 30°33.91'S 153°12.73'E	60-69
9	9 September	05:30 07:10	31°51.24'S 152°49.73'E 31°55.34'S 152°45.17'E	64-53
10	9 September	07:40 09:30	31°56.71'S 152°47.14'E 32°03.23'S 152°45.04'E	73-91
11	9 September	10:30 12:00	32°07.52'S 152°42.12'E 32°12.18'S 152°38.35'E	68-69
12	11 September	06:20 08:50	32°14.94'S 152°39.24'E 32°20.56'S 152°36.85'E	80-82
13	11 September	10:10 12:10	32°31.50'S 152°29.77'E 32°35.71'S 152°24.70'E	64-79
14	11 September	13:00 15:00	32°38.72'S 152°27.68'E 32°43.84'S 152°23.54'E	104-106
15	11 September	16:15 17:35	32°42.85'S 152°16.35'E 32°46.33'S 152°13.28'E	73-79
16	12 September	07:25 09:30	32°50.99'S 152°04.68'E 32°53.38'S 151°57.93'E	51-69
17	12 September	11:15 12:50	32°56.63'S 152°00.68'E 32°53.71'S 152°05.45'E	104

### 3.2.2 Laboratory analysis

#### *Blue Mackerel*

Eggs of Blue Mackerel, preserved in a formalin solution, were identified, staged and counted using characteristics of embryonic development described for Blue Mackerel (Ward and Rogers 2007, Neira and Keane 2008) and Chub Mackerel (Kramer 1960). General egg developmental characteristics of Horse Mackerel (*Trachurus trachurus*; Cunha et al. 2008) were also used to guide staging. The main diagnostic features of the eggs were: i) spherical (1.00 to 1.35 mm diameter); ii) smooth chorion; iii) small perivitelline space; iv) prominent, unsegmented yolk sac; v) single oil globule (0.22 to 0.38 mm diameter), becomes pigmented in mid-stages and located posterior in yolk in later stage eggs; and vi) prominent paired rows of melanophores along dorsal surface of trunk and tail of embryo, no pigment on nape region (Fig. 3g).

Eleven stages were initially used to classify Blue Mackerel eggs. These were further condensed into eight final stages to categorise temporal egg development in uniform time increments (Fig. 3: Stages 2 to 8, Stage 1 not pictured). This allowed the eggs to be more easily assigned to age day classes when calculating daily egg production ( $P_0$ ). The duration of time spent in each egg stage was estimated from temperature-development keys for Atlantic Mackerel (*Scomber scombrus*; Lockwood et al. 1981, Mendiola et al. 2006) and Chub Mackerel (Hunter and Kimbell 1980), and a Kernel Density Growth Profile (see Section 3.2.3).

The eight stages of Blue Mackerel eggs are as follows:

**Stage 1:** Division of individual cells up to 64 (not pictured). Equates to Stages 1 in Ward and Rogers (2007), and 1A in Lockwood et al. (1981).

**Stage 2:** Cleavage of cells continue until individual cells are not distinguishable and blastodermal cap is formed (Fig. 3a). Equates to Stages 2 in Ward and Rogers (2007) and 1A in Lockwood et al. (1981).

**Stage 3:** Blastopore appears, forms, and begins to close (Fig. 3b). Early stage: first appearance of germ ring and an open blastopore. Mid stage: the embryonic shield appears, the embryonic axis forms, and the blastopore begins to reduce in size. Late stage: blastopore is much reduced and almost closed, eyes begin to differentiate, and oil globule is more posterior. Equates to Stages 3 in Ward and Rogers (2007) and 1B in Lockwood et al. (1981).

**Stage 4:** Blastopore is closed, optic cups are formed, fine melanophores appear on dorsal surface of embryo, embryo extends from around  $\frac{1}{2}$  to just past the yolk, sparse pigment forms on oil globule, and body somites (myomeres) begin to appear along tail (Fig 3c). In late Stage 4 when the tail reaches past the oil globule and begins to separate from yolk, melanophores form two distinct rows reaching to the tail, a few stellate melanophores appear over yolk and around oil globule, and nape region is unpigmented Equates to Stages 4 in Ward and Rogers (2007) and 2 in Lockwood et al. (1981).

**Stage 5:** Head continues to widen, embryo is  $\frac{2}{3}$  around yolk, caudal fold is visible on tail and tip is bent around oil globule (Fig. 3d). Tail hugs oil globule and curves halfway around it, anus is forming but not distinct (straight-line measurement from anus to tail tip is <0.6mm). Equates to Stages 5 in Ward and Rogers (2007) and 3 in Lockwood et al. (1981).



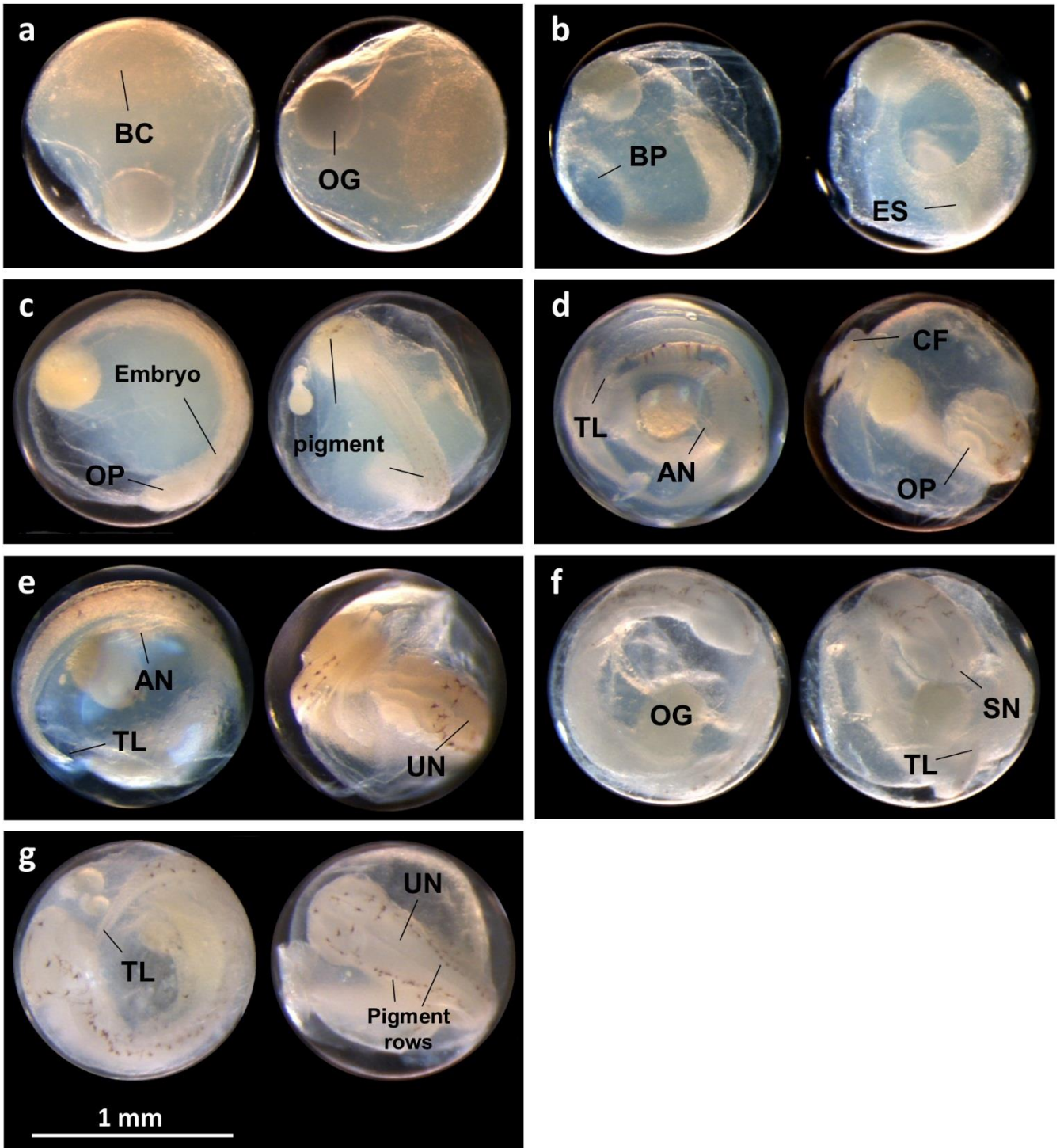
**Stage 6:** Head continues to widen, embryo is  $\frac{3}{4}$  around yolk, caudal fold is visible on tail and tip is bent around oil globule, but tail tip is much longer than in Stage 5 (Fig. 3e). The tail tip is also more detached and well beyond oil globule (straight-line measurement from anus to tail tip is  $>0.6$  mm). Anus and caudal fin fold are more developed. Equates to Stages 5 in Ward and Rogers (2007) and 3 in Lockwood et al. (1981).

**Stage 7:** Embryo extends around entire yolk, tail just reaches the head, eyes and nape region still unpigmented, melanophores develop on yolk surface and on snout, oil globule is  $\frac{3}{4}$  pigmented, and paired rows of melanophores are present on dorsal surface extending on to tail (Fig 3f). Equates to Stages 6 in Ward and Rogers (2007) and 4 in Lockwood et al. (1981).

**Stage 8:** Embryo is fully developed, tail extends past snout and is twisted off embryonic axis, pigment pattern similar to that in Stage 10 (Fig. 3g). Equates to Stages 7 in Ward and Rogers (2007) and 5 in Lockwood et al. (1981).

Sub-samples of the ethanol preserved eggs were identified using the molecular techniques developed by Neira and Keane (2008). Results of the molecular studies were used to evaluate and develop morphological criteria for distinguishing Blue Mackerel eggs from similar eggs of other species, especially gurnards (*Lepidotrigla* spp.) (see Appendix 1).

Formalin-preserved larvae of Blue Mackerel were identified and counted in each ichthyoplankton sample using morphological characteristics from Neira et al. (1998). The presence of Blue Mackerel larvae in a sample supported positive egg identifications.



**Fig. 3.** Blue Mackerel egg stages used in this study following the characteristics of embryonic development described for Blue Mackerel (Ward and Rogers 2007, Neira and Keane 2008) and Chub Mackerel (Kramer 1960). a) Stage 2; b) Stage 3; c) Stage 4; d) Stage 5; e) Stage 6; f) Stage 7; g) Stage 8. See document text for specific descriptions. AN: anus; BC: blastodermal cap; BP: blastopore; CF: caudal fold; ES: embryonic shield; OG: oil globule; OP: optic cup; UN: unpigmented nape; SN: snout TL: tail tip.

### *Australian Sardine*

Australian Sardine eggs were identified and staged in each sample using published descriptions (Neira et al. 1998, White and Fletcher 1998). The duration of time spent in each stage was estimated from temperature-development keys in White and Fletcher (1998) and a Kernel Density Growth Profile (see Section 3.2.3).

### *Tailor*

Eggs preserved in formalin were identified as Tailor based on descriptions from Deuel et al. (1966) and Neira et al. (1998). The main diagnostic features of the eggs were: i) spherical (0.8 to 1.2 mm diameter); ii) smooth chorion; iii) small perivitelline space; iv) segmented yolk sac; v) single oil globule (0.22 to 0.30 mm diameter), becomes pigmented in mid-stages and located posterior in yolk sac of later stage eggs; vi) prominent pigmentation along the dorsal surface of the embryo appears in the mid stages and encircles the eye cups; vii) sparse pigment present on the dorsal surface of the yolk sac, and viii) embryonic tail does not extend around yolk to head before hatching.

Sub-samples of the ethanol preserved eggs were identified using the molecular techniques developed by Neira and Keane (2008). Results of the molecular studies were used to evaluate and develop morphological criteria for distinguishing Tailor eggs from those of other species with similar eggs, particularly Cardinalfish (*Acropomatidae*), Tongue Soles (*Cynoglossus* spp.) and Grunters (*Pelates* spp.) (see Appendix 1).

Formalin-preserved larvae of Tailor were identified and counted in each ichthyoplankton sample using morphological characteristics from Neira et al. (1998), Norcross et al. (1974) and Wilks (1977). The presence of Tailor larvae in a sample was noted in the absence of positive egg identifications.

### **3.2.3 Egg ageing**

A Kernel Density Growth Profile was produced for Blue Mackerel and Australian Sardine to estimate ages of eggs and infer the time of peak spawning. Egg stages were assigned a decimal time based on time of collection in R (R 3.2.0). Corrections were applied to these times to ensure that eggs were assigned to the correct spawning day.

The rules applied to assign Blue Mackerel egg stages to a spawning day were as follows: i) 24 hours were immediately added to Stages 5 to 8; ii) 24 hours subtracted from Stages 2 to 3 where time >22:00; iii) 24 hours subtracted from Stage 1 where time >10:00; iv) 24 hours added to Stage 4 where time <4:00; and v) 24 hours subtracted from Stage 5 where time >40:00.

Similar rules for Australian Sardine egg stages were: i) 24 hours were immediately added to Stages 10 to 12; ii) 24 hours subtracted from Stages 1 to 3 where time >15:00; iii) 24 hours added to Stage 7 to 10 where time <10:00; and iv) 24 hours subtracted from Stage 10 to 11 where time >42:00.

After corrections were applied, Kernel Density Smoothing (KDS) was performed for each egg stage to describe an egg growth-rate profile. The peaks of each KDS were joined and plotted on top of the egg stage data, arranged by date and time, to show the progression of eggs ageing in a cohort-by-cohort profile. Spawning time was estimated to be the peak of the KDS for Stage 1 eggs. Eggs in the first and last stages

were not included in calculations of egg density (i.e. Blue Mackerel: Stages 1 and 8; Australian Sardine: Stage 1 and 12). This was done because eggs in the first stage may not be fully represented in samples and eggs in the last stage may have hatched.

### **3.2.4 Egg density**

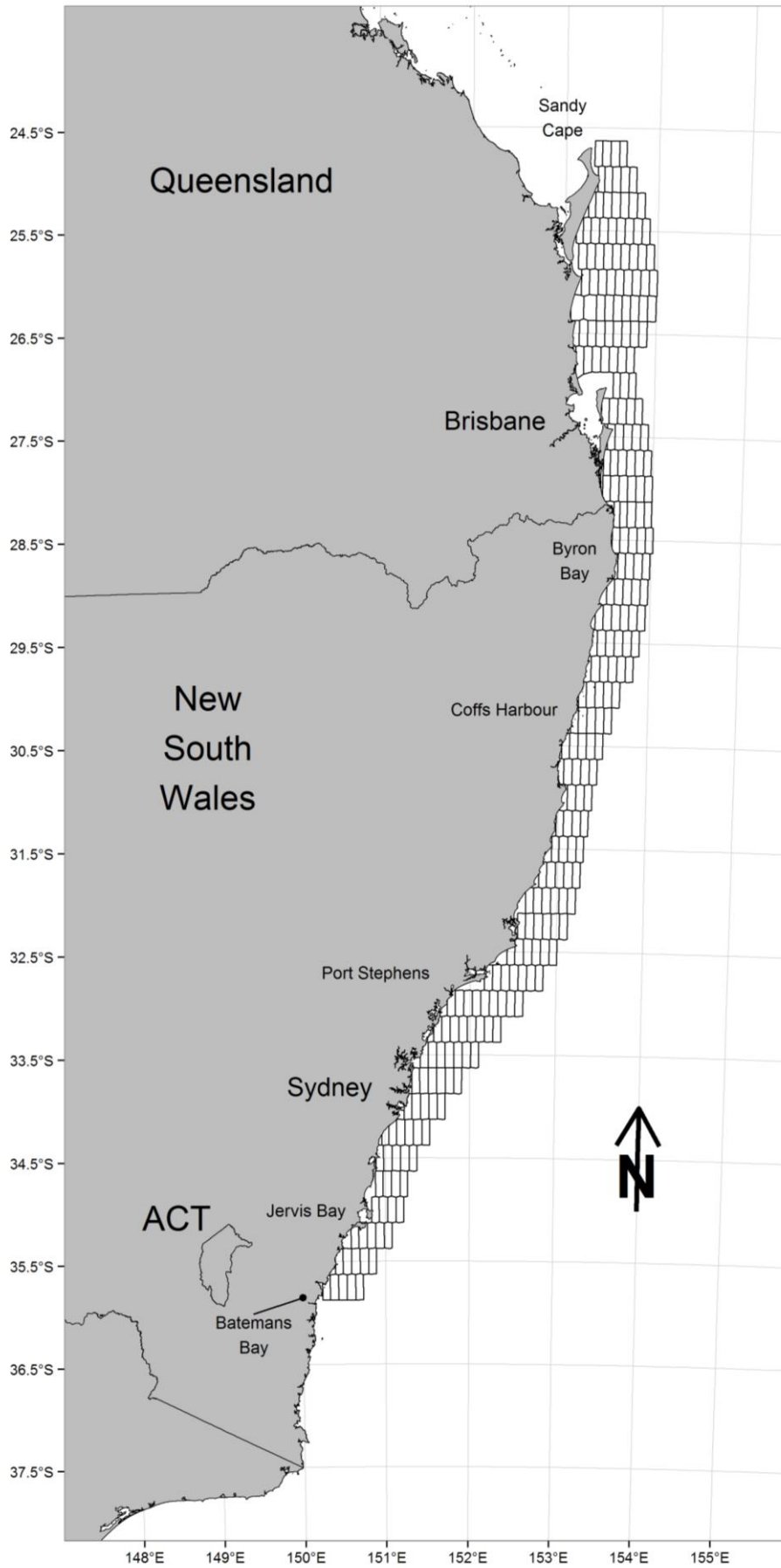
The number of eggs of each stage under one square metre of water ( $P_t$ ) was estimated at each site according to Equation 2:

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

where,  $C$  is the number of eggs of each age in each sample,  $V$  is the volume of water filtered ( $\text{m}^3$ ), and  $D$  is the depth (m) to which the net was deployed (Smith and Richardson 1977). Plots of egg distribution and abundance were prepared using ArcGIS® (Ver. 10.1).

### **3.2.5 Spawning area**

The Voronoi natural neighbour (VNN) method or Dirichlet tessellation (Watson 1981) was implemented using the 'deldir' function in the R package deldir (Turner 2015; R 3.2.0) and used to generate a polygon around each sampling site with the boundary as the midpoint equidistant between each sampling site (Fig. 4). The area represented by each station ( $\text{km}^2$ ) was then determined using the 'areaPolygon' function in the geosphere R package (Hijmans 2015). The spawning area ( $A$ ) was defined as the total area of grids where live Blue Mackerel or Australian Sardine eggs were found.



**Fig. 4.** Voronoi natural neighbour polygons used to estimate spawning area.

### 3.2.6 Daily egg production ( $P_0$ ) and egg mortality

Egg stages for Blue Mackerel and Australian Sardine were assigned an age in hours based on the Kernel Density Growth Profile (see Section 3.2.3).  $P_0$  was calculated using egg Stages 2 to 7 for Blue Mackerel (Fig. 3) and Stages 2 to 11 for Sardine.  $P_0$  can be estimated using the exponential model (Lasker 1985), a log-linear model (Picquelle and Stauffer 1985) or several generalised linear models (Ward et al. 2011a). Where the best fit to the density data predicted an increase in egg density over time (unphysical given the assumed exponentially declining model, likely due to sampling variability), assumed egg mortality rates of 0.2 to 0.6  $\text{day}^{-1} \cdot \text{m}^{-2}$  were applied to the exponential model, using the method of McGarvey and Kinloch (2001, Eq. 2). This resulted in a range of plausible estimates of mean daily egg production (Ward et al. 2009). These mortality rate values are reported for similar pelagic species (Bunn et al. 2000).

#### Model options

Non-linear least squares regression can be used to solve the exponential egg mortality model (Lasker 1985):

$$P_t = P_0 e^{-Z t} \quad \text{Equation 3}$$

where,  $P_t$  is density of eggs of age  $t$  and  $Z$  is the instantaneous rate of daily egg mortality.

Linear regression of ln-transformed estimates of egg density by age at each site can be used to calculate a (negatively) biased estimate of mean daily egg production ( $P_b$ ) (Picquelle and Stauffer 1985):

$$\ln P_{i,t} = \ln P_b - Z t \quad \text{Equation 4}$$

where,  $P_{i,t}$  is the density of eggs of age  $t$  at site  $i$  and  $Z$  is the instantaneous rate of daily egg mortality.

A bias correction factor can be applied to calculate unbiased estimates of Picquelle and Stauffer (1985):

$$P_0 = e^{\ln P_b + \sigma^2 / 2} \quad \text{Equation 5}$$

where,  $\sigma^2$  is the variance of the estimate of  $P_b$ .

Several types of Generalised Linear Models (GLMs) can also be used to estimate mean daily egg production (Wood 2006, Ward et al. 2011a). For observed egg densities ( $P_i$ ), the GLMs were of the form:

$$E[P_i] = g^{-1}(X b) \quad \text{Equation 6}$$

where,  $g$  is the (log) link function with an exponential family distribution, and  $X$  and  $b$  express the model. The covariate ( $X$ ) used in this multivariate GLM was egg density.

The two GLMs used in this study assumed a Quasi distribution with a log link function; variance was either proportional to the mean or the mean squared.

### *Choice of Model*

The results of the four models used to estimate egg production ( $P_0$ ) are presented in this report to provide insights into the uncertainty arising from the choice of statistical model. Best fits to the egg density data of Blue Mackerel predicted an increase in egg density over time and required assumed egg mortality rates of 0.2 to 0.6 day<sup>-1</sup>·m<sup>-2</sup> to be applied to the exponential model (McGarvey and Kinloch 2001). The mean value of egg production calculated from the four model fits was used to estimate spawning biomass for Australian Sardine. Model fits of the various methods used to estimate egg production ( $P_0$ ) are presented in Appendix 2a and 2b.

## 3.3 Adult Reproductive Parameters

### **3.3.1 Adult Sampling**

#### *Blue Mackerel*

A modified demersal trawl net was used to sample adult Blue Mackerel. This net was deployed from the *FV Hazel-K* between 21 August and 12 September 2014 in shelf and slope waters from Byron Bay to Stockton Beach, NSW (Fig. 2, Table 1). No adult Blue Mackerel were captured during trawling operations.

Adult Blue Mackerel were also targeted during 9 - 11 August 2015 from the *RV Tom Marshall* in shelf waters northeast of Moreton Island, Queensland. Surface and underwater lights were used to attract fish to the vessel for hook and line sampling. No samples of adult Blue Mackerel were collected.

#### *Australian Sardine*

Adult Australian Sardine were sampled using a modified demersal trawl net deployed from the *FV Hazel-K* between 21 August and 12 September 2014 in shelf and slope waters from Byron Bay to Stockton Beach, NSW (Fig. 2, Table 1). Ovaries of mature Australian Sardine were removed, labelled and fixed in a 10% formalin solution. Females (with ovaries removed) and mature males were labelled and frozen for later processing in the laboratory.

Additional samples of Australian Sardine were taken from commercial purse seine catches at Illuka, NSW during 11 - 18 September 2014. A maximum of 30 female Sardine was collected each day. Females were measured (mm fork length;FL) and the ovaries removed, labelled and individually preserved in 10% buffered formalin. Males were frozen for later processing in the laboratory, where they were dissected, weighed (g), and measured (mm FL). Weights for female Sardine were determined from the length-weight relationship for all commercial samples collected from Illuka, NSW (n = 1,380).

#### *Tailor*

Adult parameters of Tailor (fish length and sex) were collected from recreationally caught fish during two

routine fishery-dependent surveys by Fisheries Queensland on Fraser Island from 10-16 August 2014 and from 31 August to 6 September 2014. In addition, when freshly caught female Tailor were encountered, the fish were measured (FL to nearest 10 mm) and gonads removed. Ovaries were weighed (g) and samples were either fixed in a 5% buffered formalin solution immediately or kept on ice for a short period until they were able to be fixed in formalin. The approximate time between capture and fixation was recorded as <1 hr, 1-2 hrs or >2 hrs.

### 3.3.2 Adult Parameters

The following sections describe the methods used to estimate adult reproductive parameters for Australian Sardine and Tailor. Since no adult Blue Mackerel were collected during the present study, best estimates of adult parameters were obtained from South Australian DEPM surveys for Blue Mackerel conducted between 2001 and 2006 (see Section 3.4).

#### *Female weight (W)*

Mature females of Australian Sardine from each trawl sample were thawed and weighed ( $\pm 0.01$  g). This value was then adjusted by adding the corresponding preserved gonad weight. Fixation in formalin has a negligible effect on tissue weight (Lasker 1985). The weights of mature female Sardine collected from commercial samples were determined from the length-weight relationship for all commercial samples collected from Illuka, NSW ( $n = 1,380$ ). Fish weight (g) of Tailor was determined from an established length-weight relationship of  $1.176 \times 10^{-5} \cdot FL^{3.01}$  (Bade 1977). The mean weight of mature females in each population, for both Tailor and Australian Sardine, was calculated from the average of sample means weighted by proportional sample size:

$$W = \left[ \frac{\overline{W_i n_i}}{N} \right] \quad \text{Equation 7}$$

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

#### *Male weight*

Mature male Australian Sardine in each sample were thawed and weighed ( $\pm 0.01$  g). Male weights of Tailor were determined from an established length-weight relationship of  $\text{Weight} = 1.176 \times 10^{-5} \cdot FL^{3.01}$  (Bade 1977). The mean weight of mature males in each population, for both Tailor and Australian Sardine, was calculated from the average of sample means weighted by proportional sample size as described above for females.



### *Sex ratio (R)*

The mean sex ratios of mature individuals in the populations were calculated from the average of sample means weighted by sample size:

$$R = \left[ \frac{\overline{R_i n_i}}{N} \right] \quad \text{Equation 8}$$

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

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

where,  $F_i$  and  $M_i$  are the respective total weights of mature females and males in each sample  $i$ .

### *Batch fecundity (F)*

#### *Tailor*

Batch fecundity of Tailor was estimated from ovaries containing hydrated oocytes using the methods of Hunter et al. (1985). Both ovaries were weighed and the number of hydrated oocytes in three ovarian sub-sections were 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 gonad-free female weight and batch fecundity was determined by linear regression analysis and used to estimate the mean batch fecundity using the mean gonad-free weight (g) of all mature females collected.

#### *Australian Sardine*

Australian Sardine with hydrated oocytes were not collected during the current project. Therefore, the relationship between gonad-free female weight (g) and batch fecundity was calculated using linear regression analysis from data obtained during South Australian DEPM Australian Sardine surveys between 1998 and 2014. Mean gonad-free weight of mature female Sardine captured during the current project was used in this equation to estimate batch fecundity for the east coast.

### Spawning fraction (S)

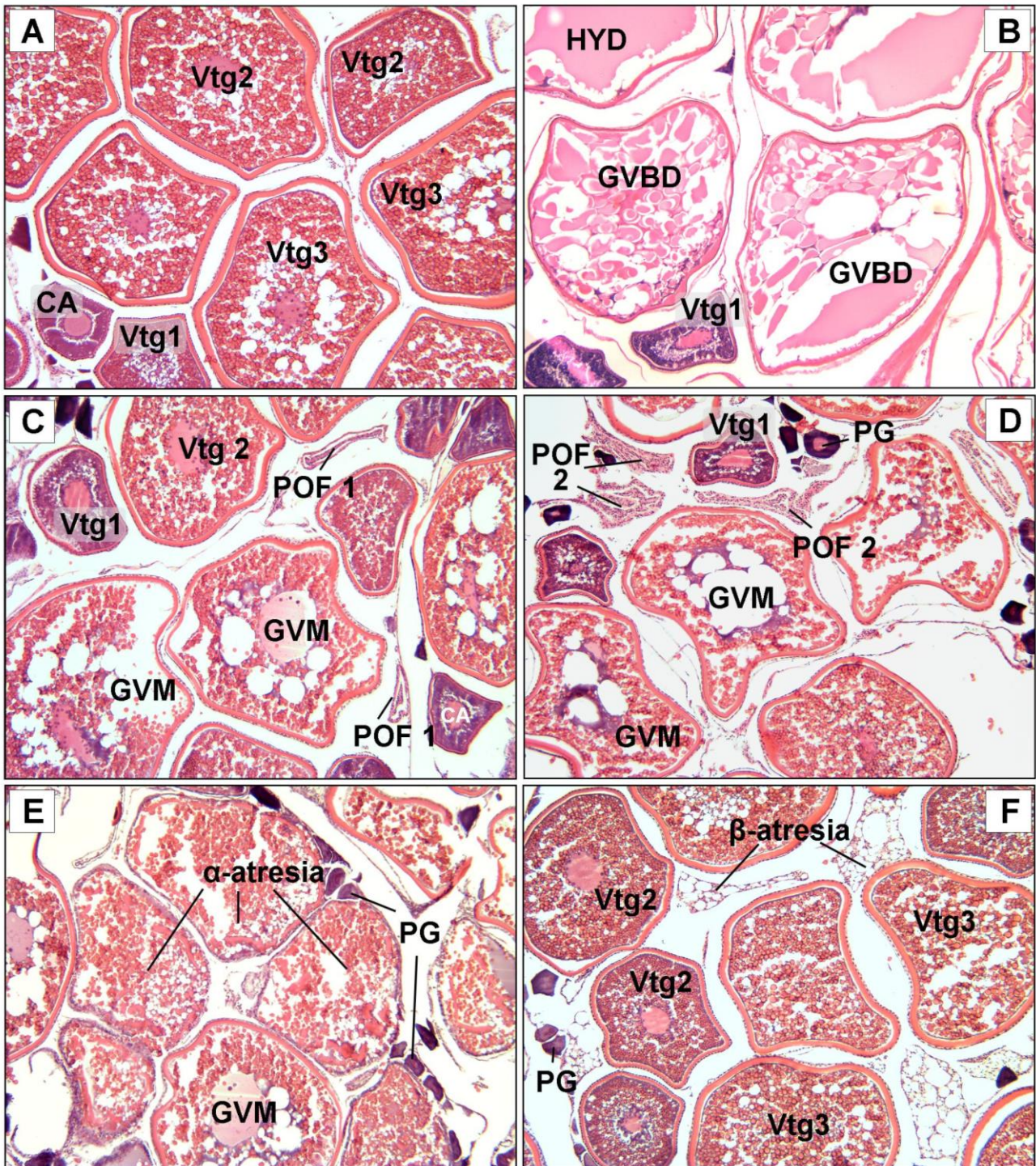
Ovaries of mature females of Tailor and Australian Sardine were sectioned and stained with haematoxylin and eosin (see Fig. 5 for examples of Tailor ovarian histology). Oocyte growth and maturation stages followed Lowerre-Barbieri et al. (2011), and fish reproductive phase terminology followed Brown-Peterson et al. (2011). Several sections from each ovary were examined to determine the presence/absence of post-ovulatory follicles (POFs). POFs were aged according to Ganas (2012). The spawning fraction of each sample with day-0 POFs ( $d_0$ ; assumed to be spawning or have spawned on the night of capture), day-1 POFs ( $d_1$ ; assumed to have spawned the previous night; Fig. 5c) and day-2 POFs ( $d_2$ ; assumed to have spawned two nights prior; Fig. 5d). The mean spawning fraction of the population was calculated from the average of sample means weighted by proportional sample size.

$$S = \left[ \frac{\overline{S_i} n_i}{N} \right] \quad \text{Equation 10}$$

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

$$\overline{S_i} = \frac{d_0 + d_1 + d_2}{3 n_i} \quad \text{Equation 11}$$

where,  $d_0$ ,  $d_1$  and  $d_2$  are the number of mature females with POFs in each sample and  $n_i$  is the total number of females within a sample.



**Fig. 5.** Photomicrographs of ovarian histology from spawning capable Tailor collected on Fraser Island, Queensland during August-September 2014. **A)** spawning capable phase showing asynchronous oocyte development; **B)** actively spawning sub-phase showing germinal vesicle break down and hydration; **C)** spawning capable phase with day-1 post-ovulatory follicles; **D)** spawning capable phase with day-2 post-ovulatory follicles; **E)** spawning capable phase with alpha atresia present; **F)** spawning capable phase with beta atresia present. Mid- to late-stages of atresia can sometimes be mistaken for post-ovulatory follicles. Oocyte development and maturation stages: PG = primary growth; CA = cortical alveolar; Vtg1 = primary vitellogenic; Vtg2 = secondary vitellogenic; Vtg3 = tertiary vitellogenic; GVM = germinal vesicle migration; GVBD = germinal vesicle breakdown; HYD = hydration; POF 1 = day-1 post-ovulatory follicles; POF 2 = day-2 post-ovulatory follicles;  $\alpha$ -atresia = early-stage atresia;  $\beta$ -atresia = mid-stage atresia.

## 3.4 Spawning Biomass and Bootstrapping Procedures

### 3.4.1 Spawning biomass

#### *Blue Mackerel*

Spawning biomass for Blue Mackerel was calculated according to Equation 1. This equation incorporated the best estimate of egg production ( $P_0$ ) obtained by assuming a fixed mortality of  $Z = 0.3 \text{ eggs m}^{-2} \text{ day}^{-1}$  as per Section 3.2.6. Best estimates of adult reproductive parameters were obtained from South Australian DEPM surveys for Blue Mackerel conducted between 2001 and 2006 (Table 2).

**Table 2.** Mean, minimum and maximum adult Blue Mackerel parameters determined in DEPM surveys in South Australia between 2001 and 2006 (Ward and Rogers 2007).

Adult Parameter	Mean 2001 to 2006 (min - max)
Female Weight ( $W$ , g)	452.0 (408.2 - 473.6)
Sex Ratio ( $R$ )	0.46 (0.36 - 0.63)
Fecundity ( $F$ , eggs·female <sup>-1</sup> )	52,182 (46,468 - 55,053)
Spawning Fraction ( $S$ )	0.14 (0.05 - 0.18)

#### *Australian Sardine*

Spawning biomass for Australian Sardine was calculated using the same approach with estimates of adult parameters obtained from samples collected in the trawl survey and purse seine catches obtained during the present study. The exception was batch fecundity, which was estimated by applying the relationship between gonad-free female weight (g) and batch fecundity from South Australian to the mean weight obtained in the present study (Section 3.3.1).

#### *Tailor*

There were insufficient numbers of Tailor eggs in ichthyoplankton samples to allow estimation of  $P_0$  or  $A$ .

### 3.4.2 Confidence intervals

Confidence intervals for the estimates of spawning biomass were obtained by bootstrapping egg densities and adult parameters separately. Egg age and density data for each station were resampled with replacement to generate 100,000 datasets. For each iteration, the selected model was used to estimate  $P_0$ . Resampling for all four adult parameters used a two-stage bootstrap with 100,000 bootstrap iterations (Efron and Tibshirani 1993). At the first adult resampling stage, the individual sample tows were resampled with



replacement. At the second stage, for each bootstrapped sample tow, the adult fish within the sample were resampled with replacement. The adult parameters  $W$ ,  $S$  and  $R$  were calculated from the bootstrapped sample of adult fish. Batch fecundity ( $F$ ) was calculated from the mean gonad-free weight using the batch relationship obtained by bootstrapping with replacement from females with hydrated oocytes. For each bootstrap iteration, the values of adult parameters  $W$ ,  $R$ ,  $F$ ,  $S$ , and mean gonad-free weight were calculated. To estimate 95% confidence intervals of spawning biomass, 100,000 estimates spawning of biomass were calculated using the spawning area ( $A$ ), 100,000 bootstrapped estimates of  $P_0$ , and adult parameters according to Equation 1. The percentile method was used to calculate the 95% confidence interval. Parameter estimates were calculated independently in Excel 2010 and using R 3.2.0 (for quality assurance). Confidence intervals were estimated using R 3.2.0.

### 3.5 Sensitivity Analysis

Sensitivity analyses were performed for Blue Mackerel and Australian Sardine to determine which parameters have the most influence on the estimates of spawning biomass. Each individual variable in Equation 1 was varied in turn, while holding all of the other variables constant at the value used to calculate spawning biomass. Sensitivity was measured by the magnitude of change in biomass that was produced by the analysis.

#### *Blue Mackerel*

A range of values reported for Blue Mackerel in Australia and Chub Mackerel in northern Pacific areas were used in the sensitivity analyses for the parameters of  $W$ ,  $R$ ,  $F$ , and  $S$  (Table 3). Chub Mackerel is a close relative of Blue Mackerel with a similar life history (Scoles et al. 1998, Takasuka et al. 2008, Catanese et al. 2010). The upper and lower values of spawning area ( $A$ ) used in the sensitivity analysis were  $\pm 20\%$  of the estimate obtained in the present study that was used to calculate spawning biomass. The range of values for  $P_0$  in the sensitivity analysis were the upper and lower 95% confidence intervals from bootstrapped egg densities calculated with a mortality rate ( $z$ ) of  $0.3 \text{ eggs}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ . The minimum values for  $W$ ,  $S$ ,  $F$  were sourced from east coast Blue Mackerel surveys from 2002 to 2005 (Table 3; Ward et al. 2009). The value of  $R$  from South Australia used to calculate spawning biomass was the minimum value for this parameter (Tables 2, 3). Maximum values were parameter estimates of  $W$ ,  $R$ ,  $F$  and  $S$  for northern Pacific Chub Mackerel (Table 3).

#### *Australian Sardine*

The sensitivity analyses for Australian Sardine were performed using the minimum and maximum of each of the five parameters –  $R$ ,  $W$ ,  $S$ ,  $F$ , and  $P$  – estimated for South Australian DEPM surveys between 1998 and 2014 (Table 4). The upper and lower values of spawning area ( $A$ ) used in the sensitivity analysis were  $\pm 20\%$  of the estimate obtained in the present study that was used to calculate spawning biomass.

### Tailor

Sensitivity analyses were not undertaken for Tailor, because the low abundance of eggs found in ichthyoplankton samples did not allow estimation of spawning biomass.

**Table 3.** Adult parameters of *Scomber* spp. sourced from available literature used to inform sensitivity analysis for female weight ( $W$ ), sex ratio ( $R$ ), spawning fraction ( $S$ ), and batch fecundity ( $F$ ). Values presented are study means. NSW: New South Wales; SA: South Australia; USA: United States of America

Species	Location	n	$W$ (g)	$R$	$F$ (eggs·female <sup>-1</sup> )	$S$	Source
<i>S. australasicus</i>	NSW, Australia	383	267.3	0.50	22,085	-	Ward et al. (2009)
<i>S. australasicus</i>	SA, Australia	1837	452.0	0.46	52,182	0.14	Ward et al. (2009)
<i>S. japonicus</i>	California, USA	271	-	-	68,400	0.087	Dickerson et al. (1992)
<i>S. japonicus</i>	Japan	137	-	-	-	0.169	Shiraishi et al. (2009)
<i>S. japonicus</i>	Japan	1159	491 <sup>#</sup>	0.60	33,780	0.39	Watanabe and Nishida (2002)
<i>S. japonicus</i>	Japan	192	562.1 <sup>#</sup>	-	89,200	0.174	Yamada et al. (1998)

<sup>#</sup> Gonad-free weight

**Table 4.** Adult parameters of Australian Sardine determined in DEPM surveys in South Australia between 1998 and 2014 used to inform sensitivity analysis for egg production ( $P_0$ , eggs·day<sup>-1</sup>·m<sup>-2</sup>), female weight ( $W$ , g), sex ratio ( $R$ ), spawning fraction ( $S$ ), and batch fecundity ( $F$ , eggs·female<sup>-1</sup>) (Ward et al. 2014b).

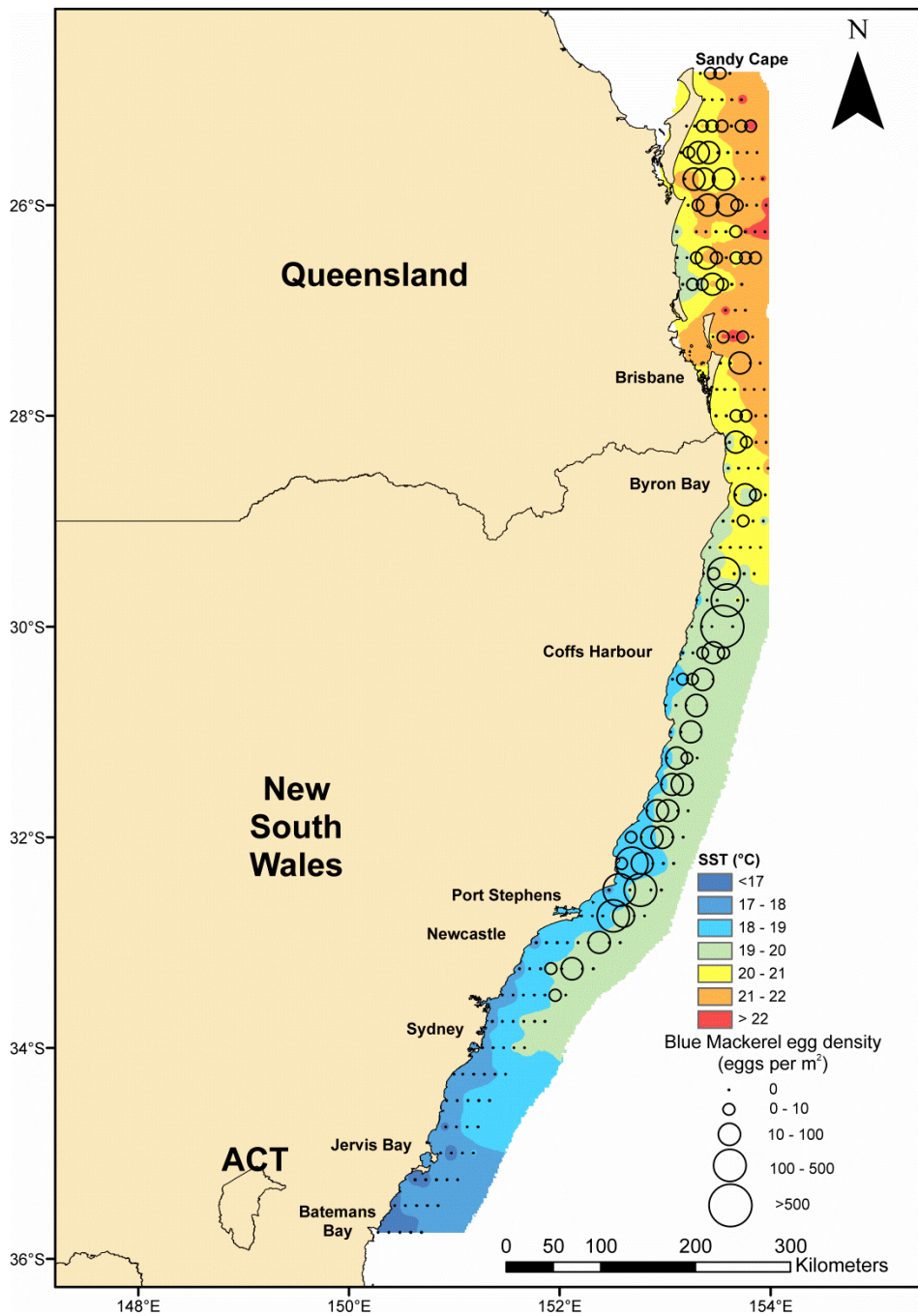
Adult Parameter	Mean 1998 to 2014 (min - max)
$P_0$	72.7 (38.1 - 120.9)
$W$	57.5 (45.2 - 78.7)
$R$	0.53 (0.36 - 0.68)
$F$	17,242 (10,904 - 24,790)
$S$	0.12 (0.04 - 0.18)

# 4. Results

## 4.1 Environmental Variables

### 4.1.1 Sea surface temperature

Sea surface temperatures (SSTs) ranged from 16.2 to 22.3°C (Fig. 6) during August-September 2014. Temperature varied latitudinally from high (north) to low (south).



**Fig. 6.** Blue Mackerel egg densities (eggs·m<sup>-2</sup>) and associated sea surface temperatures (SST, °C) along the east coast of Australia during August-September 2014. The northern-most transect is designated as number 1 and the southern-most is number 45.

## 4.2 Blue Mackerel

### **4.2.1 Distribution and Abundance of Eggs**

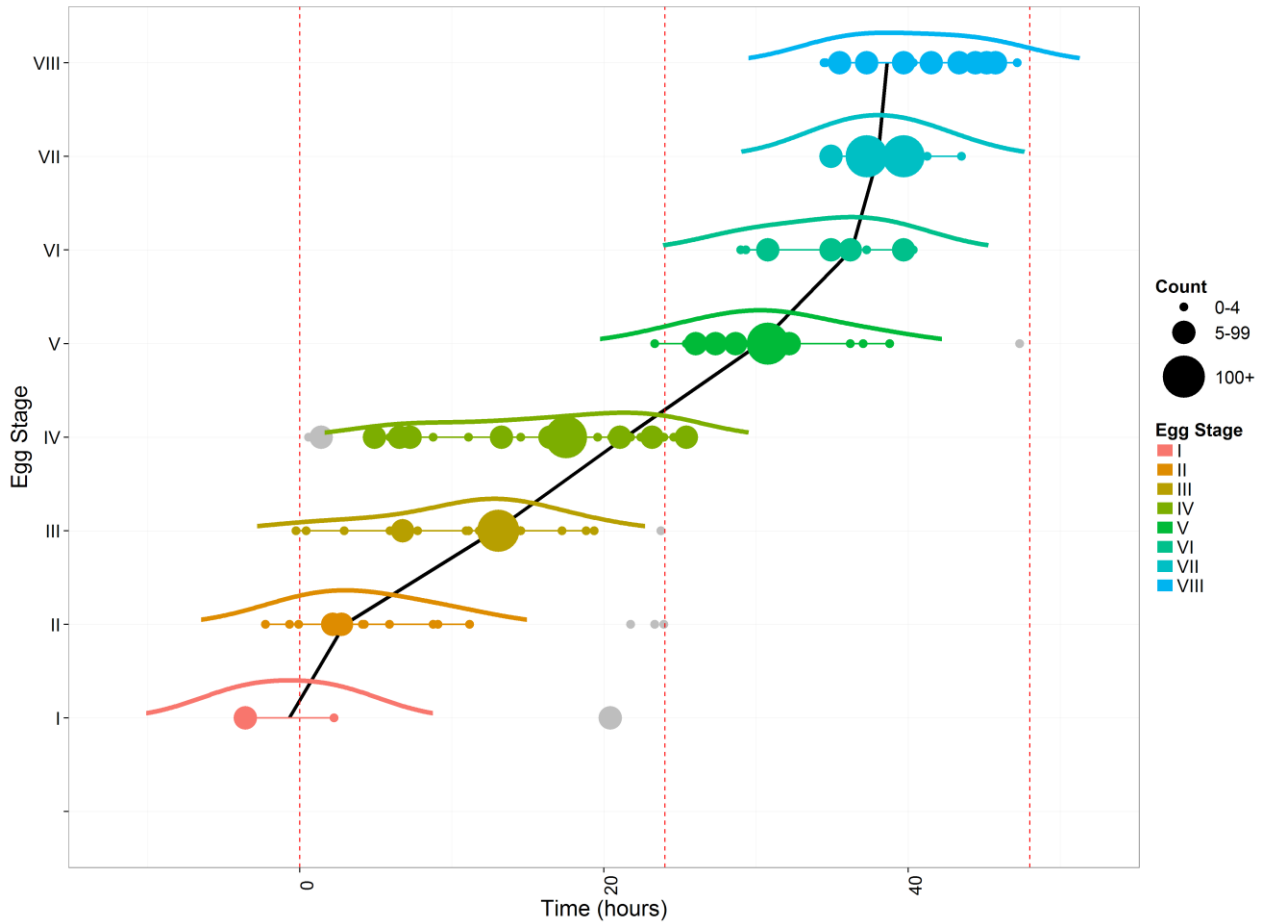
Ichthyoplankton samples were successfully collected from 262 sites located between Sandy Cape, Queensland and Batemans Bay, NSW. A total of 2,330 live Blue Mackerel eggs were collected from 70 of the 262 (26.7%) stations sampled. Blue Mackerel eggs were collected from waters between Sandy Cape to just south of Newcastle, where SSTs ranged between 18 and 22°C (Fig. 6). The highest densities of Blue Mackerel eggs were recorded in waters to the north of Coffs Harbour, NSW and off Port Stephens, NSW. Higher densities of Blue Mackerel eggs ( $>100$  eggs·m<sup>-2</sup>) were collected from sites with SSTs ranging between 18 and 20°C.

The distribution of eggs was influenced by depth, with most eggs collected from depths  $>50$  m. Ninety-four percent of eggs were collected in depths between 55 and 145 m, with the greatest number of eggs collected between 65 and 135 m (88% of eggs sampled).

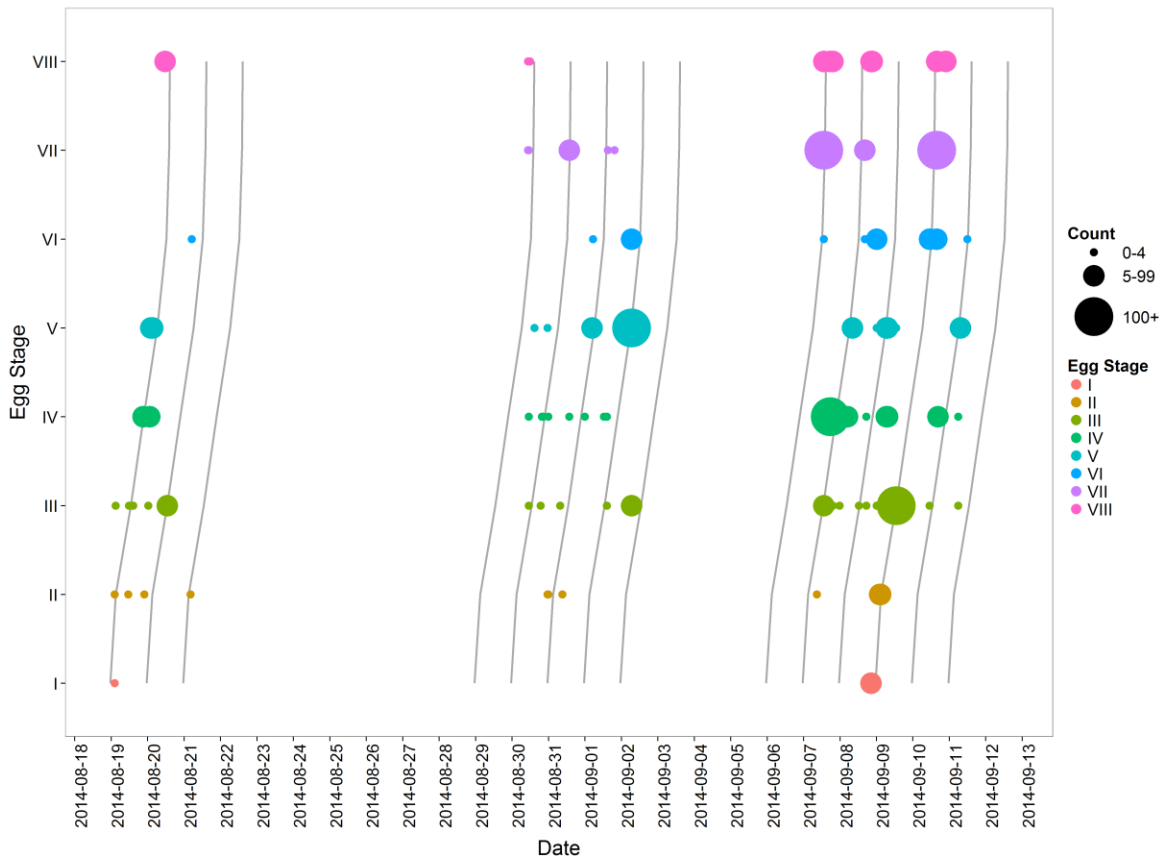
### **4.2.2 Egg Ageing**

The Kernel Density Growth Profile was used to establish the age of eggs in each stage (Fig. 7). The developmental progression of the egg from Stage 1 (just after spawning) to Stage 8 (just prior to hatching) took about 40 hours in water temperatures between 18 and 22°C. Peak spawning time was estimated to be around midnight based on the peak of the Stage 1 eggs (Fig. 7). The joined peaks for each egg stage provided a cohort-by-cohort profile of Blue Mackerel eggs through time and aligned well with corresponding egg stage/count data (Fig. 8).





**Fig. 7.** Kernel Density Growth Profile of each Blue Mackerel egg stage plotted by decimal time (hours) after spawning. Decimal time is based on time of sample collection. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample. Solid coloured lines represent the Kernel Density Smoothing and temporal extent of each egg stage. The solid black line is the modal time of developmental progression through the egg stages from post-spawn to pre-hatch. Grey points indicate eggs that were shifted  $\pm 24$  hours to assign egg stages to the correct timeframe (see Section 3.2.3). Red dashed lines specify midnight on the spawning night and 24 hr increments thereafter.



**Fig. 8.** A cohort-by-cohort profile of Blue Mackerel eggs over time. The joined peaks of the Kernel Density Smoothing for each Blue Mackerel egg stage (grey lines) are shown at 24 hours intervals from the estimated peak spawning time. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample.

#### 4.2.3 Spawning Area

The estimated spawning area for Blue Mackerel was 17,911 km<sup>2</sup>, comprising 27.3% of the total area sampled (65,528 km<sup>2</sup>, Table 5).

**Table 5.** Total survey area, spawning area (A), percent area containing eggs, and spawning biomass of Blue Mackerel.

Area sampled (km <sup>2</sup> )	Spawning area A (km <sup>2</sup> )	Area with eggs (%)	Spawning biomass (t)
65,528	17,911	27.3	83,300

#### 4.2.4 Daily Egg Production ( $P_0$ )

The distribution of egg density samples by egg stage, notably including several very high egg density samples for later egg stages, resulted in biologically unrealistic (negative) mortality estimates using the two exponential model and two GLM model fit methods (Table 6). Therefore, the estimate of mean daily egg production ( $P_0$ ) was implemented using the method of McGarvey and Kinloch (2001, Eq. 2), which uses the overall mean density of sampled eggs with an egg mortality rate of  $Z = 0.3 \text{ day}^{-1}$ . This gave the estimate for  $P_0$  of  $34.6 \text{ eggs} \cdot \text{day}^{-1} \cdot \text{m}^{-2}$  (14.6 - 69.1; Table 6). Examining the resultant estimates of  $P_0$  (Table 6) shows that they were not strongly dependent on the assumed value of  $Z$ .

**Table 6.** Mean daily egg production ( $P_0$ ) of Blue Mackerel estimated using assumed egg mortality rates ( $z$ ) ranging from 0 (mean) to  $0.6 \text{ day}^{-1}$ . The value used for biomass estimation is highlighted in bold. Ranges are 95% confidence intervals.

Model fit		$P_0$ (eggs·day <sup>-1</sup> ·m <sup>-2</sup> )
Mean	$Z = 0.0$	25.7
	$Z = 0.2$	31.5
	<b><math>Z = 0.3</math></b>	<b>34.6 (14.6 - 69.1)</b>
	$Z = 0.4$	37.9
	$Z = 0.6$	45.0

#### 4.2.5 Adult Reproductive Parameters

Samples of adult Blue Mackerel were not collected during the study. Only immature Blue Mackerel were captured during trawling operations, and the use of lights to attract fish for hook and line collections was unsuccessful. Therefore, data to provide the best estimates of reproductive parameters of adult Blue Mackerel were obtained from DEPM surveys from southern Australian conducted between 2001 and 2006 (Table 2) (Ward and Rogers 2007).

##### *Mean Female Weight ( $W$ )*

The mean weight of mature females ( $W$ ) obtained from South Australian DEPM surveys between 2001 and 2006 was 452.0 g (Table 2), with values ranging between 408.2 to 473.6 g (Ward and Rogers 2007).

##### *Sex ratio ( $R$ )*

The mean sex ratio ( $R$ ) of 0.46 was reported for Blue Mackerel South Australian waters between 2001 and 2006 (Ward and Rogers 2007). Values ranged from 0.36 to 0.63 (Table 2).

### *Batch fecundity (F)*

The mean batch fecundity ( $F$ ) obtained for South Australia was 52,182 eggs and ranged from 46,468 eggs to 55,053 eggs (Table 2).

### *Spawning fraction (S)*

The mean estimate of spawning fraction ( $S$ ) for Blue Mackerel in South Australia was 0.14, with values ranging from 0.05 to 0.18 (Table 2).

## **4.2.6 Spawning Biomass**

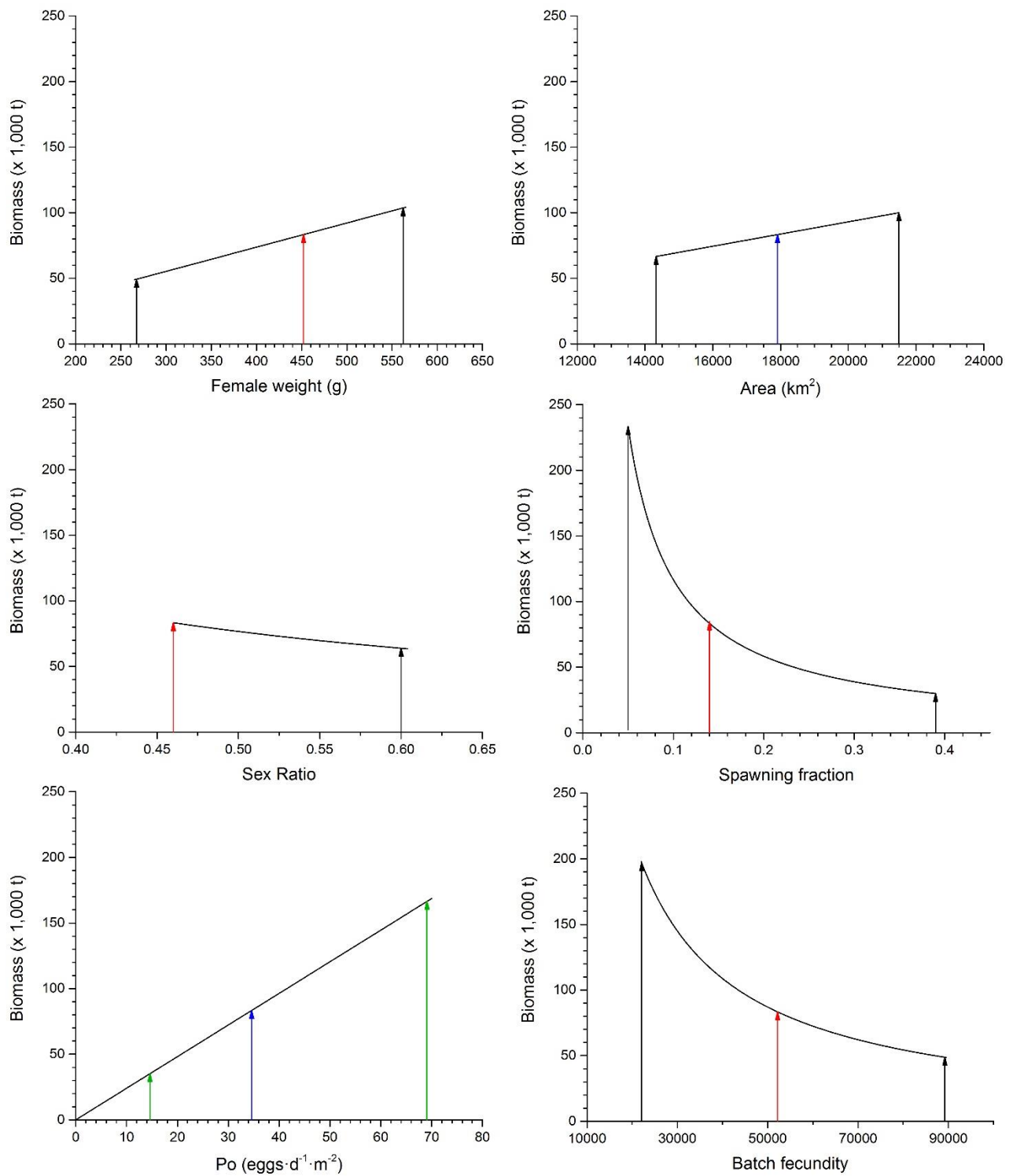
The estimate of Blue Mackerel spawning biomass calculated using data from the current survey and adult parameters from South Australia (Table 7) was 83,300 t (Table 5). Using the minimum and maximum 95% CI values for  $P_0$ , spawning biomass estimates ranged between 35,100 t and 165,000 t, respectively.

**Table 7.** Parameters used in the calculations of spawning biomass of Blue Mackerel in 2014. #Range is 95% CI for  $P_0$  at  $Z = 0.3 \text{ eggs}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ . \*Source: Ward and Rogers (2007)

Parameter	Mean (min - max)
Egg Production ( $P_0$ , $\text{eggs}\cdot\text{day}^{-1}\cdot\text{m}^{-2}$ )	34.6 (14.6 - 69.1)#
Sex Ratio ( $R$ )	0.46 (0.36 - 0.63)*
Fecundity ( $F$ , $\text{eggs}\cdot\text{female}^{-1}$ )	52,182 (46,468 - 55,053)*
Spawning Fraction ( $S$ )	0.14 (0.05 - 0.18)*
Female Weight ( $W$ , g)	452.0 (408.2 - 473.6)*
Spawning Area ( $A$ , $\text{km}^2$ )	17,911

## **4.2.7 Sensitivity Analysis**

The relative sensitivity of estimates of Blue Mackerel spawning biomass to realistic variations in each parameter showed that estimates of spawning biomass were mainly between ~ 50,000 t and 100,000 t (Fig. 9). The exceptions were the lower estimates of spawning fraction (0.05) and batch fecundity (22,085  $\text{eggs}\cdot\text{female}^{-1}$ ) and the higher estimate of daily egg production (69.1  $\text{eggs}\cdot\text{day}^{-1}\cdot\text{m}^{-2}$ ). These variations produced estimates of spawning biomass between 150,000 to 250,000 t.

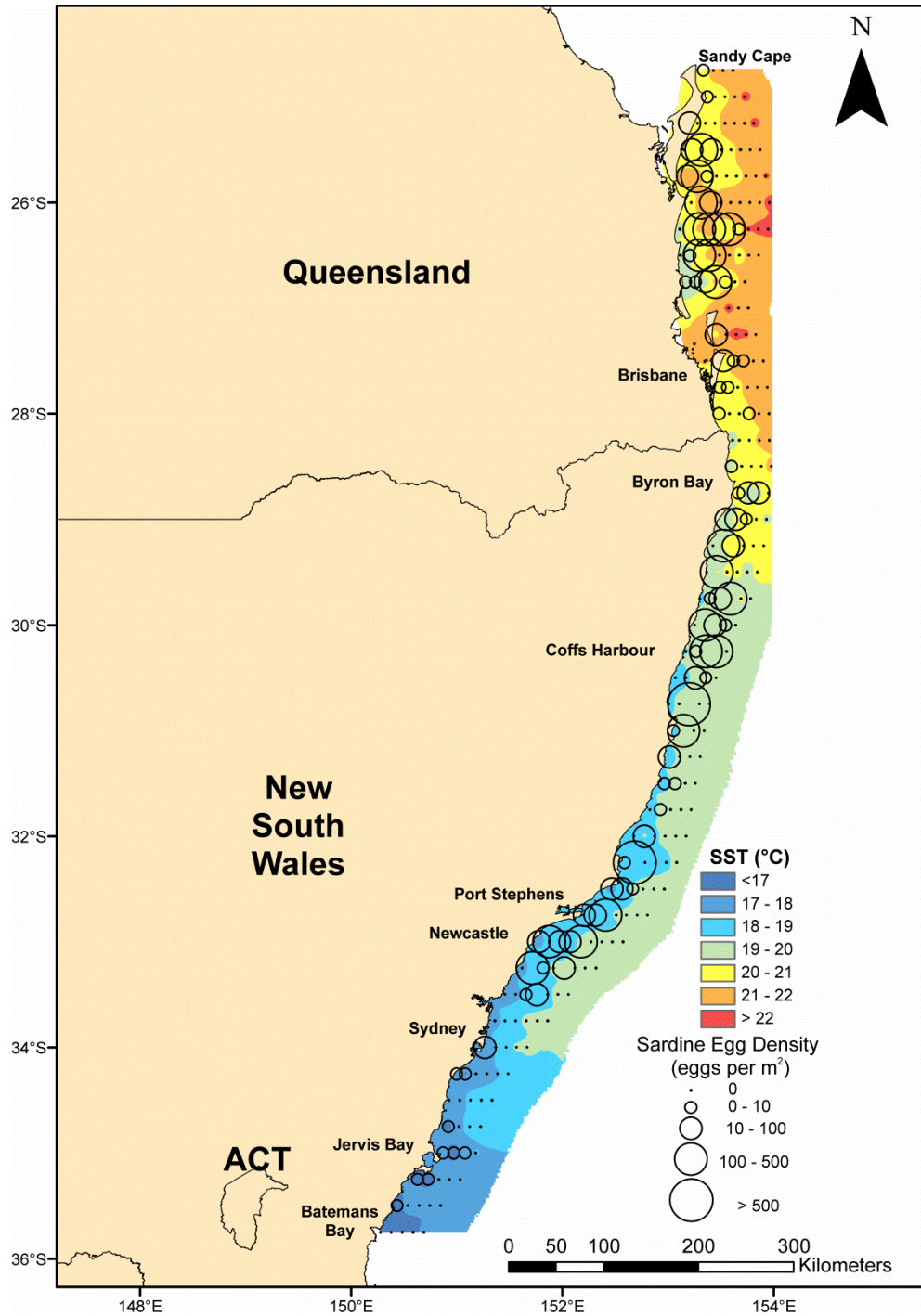


**Fig. 9.** Sensitivity analyses of the effects of individual parameters on estimates of spawning biomass of Blue Mackerel. Blue arrows are values estimated in the current survey, green arrows are values representing 95% CI for that parameter, and red and black arrows are mean, minimum and maximum values sourced from the literature as described in Table 2.

## 4.3 Australian Sardine

### 4.3.1 Distribution and Abundance of Eggs

A total of 3,461 live Australian Sardine eggs were collected from 89 of the 262 (34.0%) stations sampled. Most Australian Sardine eggs were collected from waters between Sandy Cape, Queensland and just south of Newcastle, NSW where SSTs ranged between 17 and 22°C (Fig. 10).

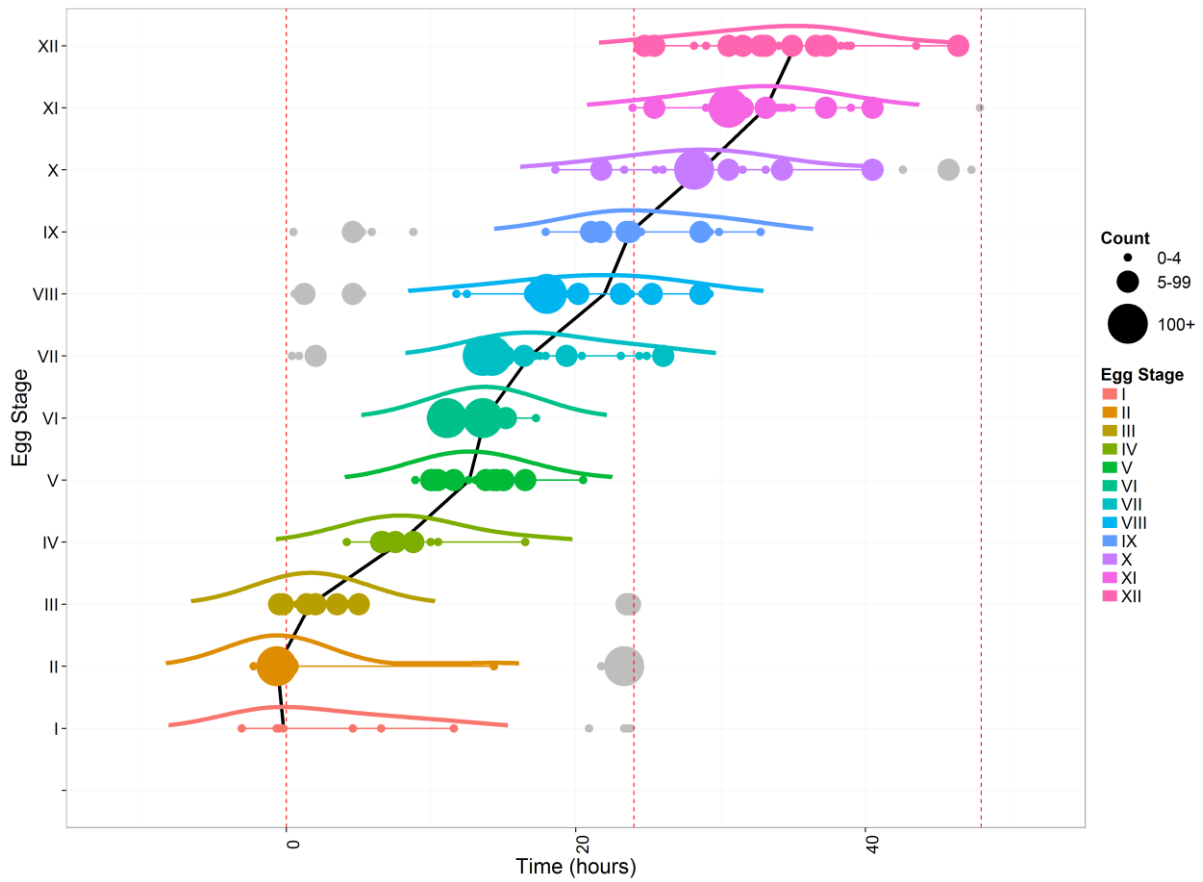


**Fig. 10.** Australian Sardine egg densities (eggs·m<sup>-2</sup>) and associated sea surface temperatures (SST, °C) along the east coast of Australia during August-September 2014. Note: The northern-most transect is designated as number 1 and the southern-most is number 45.

The highest densities of Australian Sardine eggs ( $>100$  eggs·m<sup>-2</sup>) were collected from sites with SSTs ranging between 18 and 21°C. The distribution of eggs was influenced by depth with 94% of eggs collected between 40 and 120 m. Eighty-eight percent of eggs were collected at depths between 45 and 100 m. Geographically, egg densities were greatest in southern Queensland from Fraser Island to Caloundra (positive station mean on Transects 4 to 9: 117 eggs·m<sup>-2</sup>; 21°C) compared to northern NSW (positive station mean on Transects 17 to 27: 68 eggs·m<sup>-2</sup>; 20°C) or mid NSW (positive station mean on Transects 30 to 36: 92 eggs·m<sup>-2</sup>; 19°C).

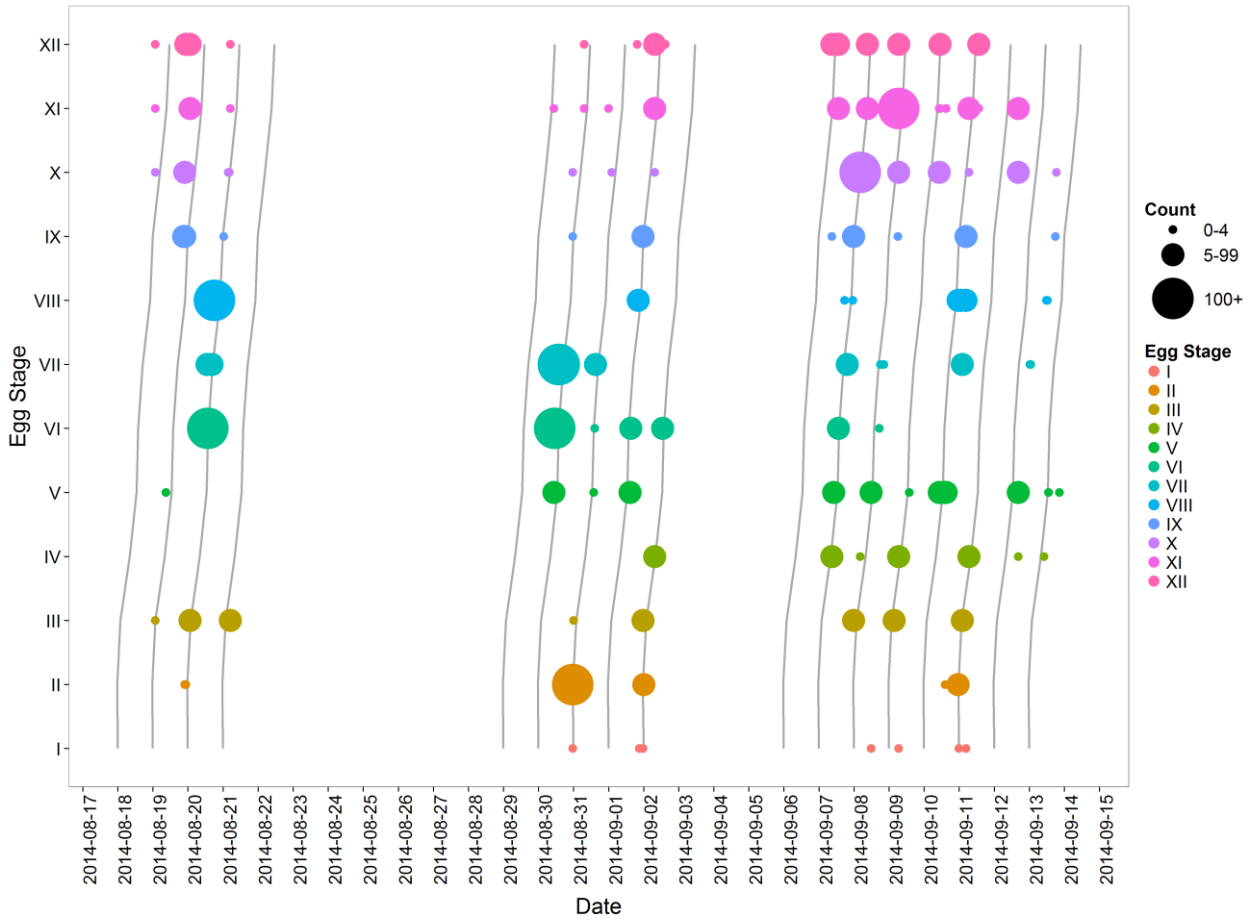
### **4.3.2 Egg Ageing**

The Kernel Density Growth Profile was used to establish the age of eggs in each stage (Fig. 11). The developmental progression from Stage 1 (just after spawning) to Stage 12 (just prior to hatching) took about 40 hours in water temperatures between 17 and 22°C. Peak spawning time was estimated to be around midnight based on the peak for Stage 1 eggs (Fig. 11). The joined peaks for each egg stage provided a cohort-by-cohort profile of Australian Sardine eggs through time and aligned well with corresponding egg stage/count data (Fig. 12). The modal age of each egg stage estimated with the Kernel Density Growth Profile was comparable to temperature-developments curves for Australian Sardine at similar temperatures (White and Fletcher 1998) (Fig. 13).

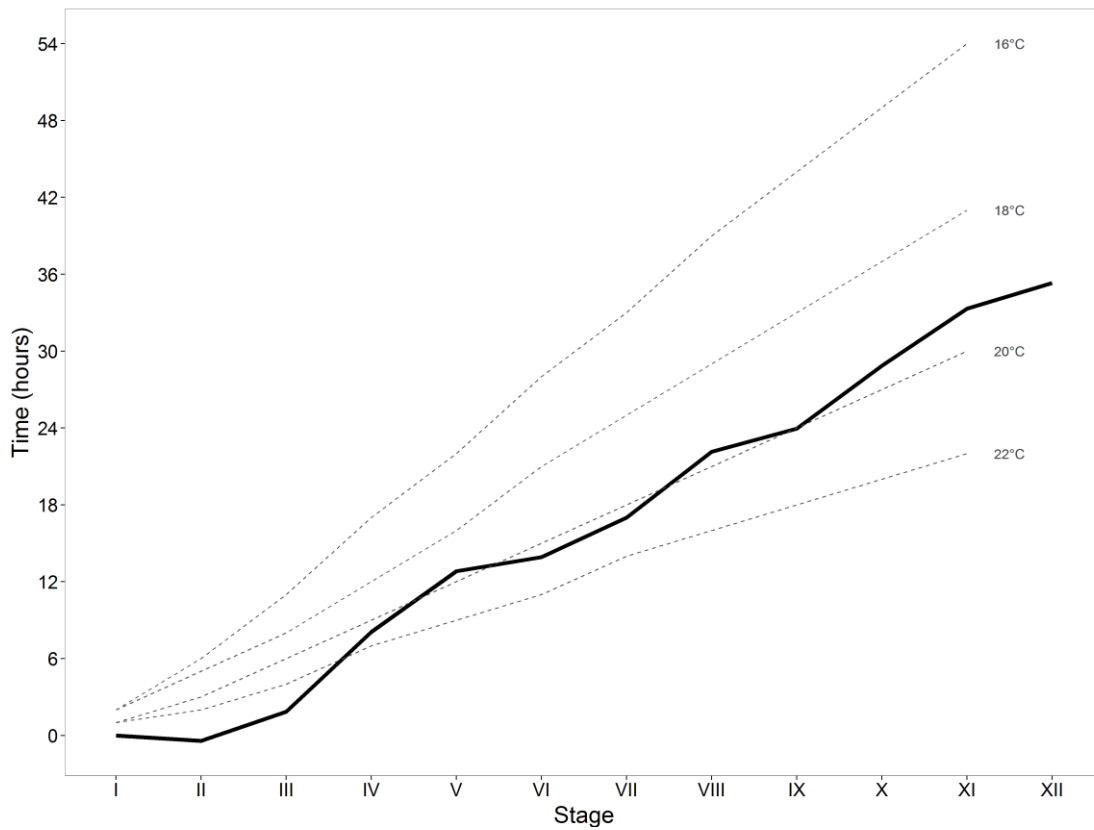


**Fig. 11.** Kernel Density Growth Profile of each Australian Sardine egg stage plotted by decimal time (hours) after spawning. Decimal time is based on time of sample collection. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample. Solid coloured lines represent the Kernel Density Smoothing and temporal extent of each egg stage. The solid black line is the modal time of developmental progression through the egg stages from post-spawn to pre-hatch. Grey points indicate eggs that were shifted  $\pm 24$  hours to assign egg stages to the correct timeframe (see Section 3.2.3). Red dashed lines specify midnight on the spawning night and 24 hr increments thereafter.





**Fig. 12.** A cohort-by-cohort profile of Australian Sardine eggs as they age. The joined peaks of the Kernel Density Smoothing for each Australian Sardine egg stage (grey lines) are shown at 24 hour separations from the estimated peak spawning time. Each colour represents an egg stage, and the size of each point indicates the count of eggs in that stage in a given sample (binned).



**Fig. 13.** Modal age of each egg stage (hours; estimated with the Kernel Density Growth Profile; solid line) plotted against temperature-development curves for Australian Sardine (White and Fletcher 1998; dashed lines). The mean *in situ* temperature encountered during the current survey was 20°C (range: 17 to 22°C).

#### 4.3.3 Spawning Area

The estimated spawning area for the entire survey area was 22,400 km<sup>2</sup>, comprising 34.2% of the total area sampled (65,528 km<sup>2</sup>, Table 8).

**Table 8.** Total survey area, spawning area (*A*), percent area containing eggs, and spawning biomass of Australian Sardine.

Area sampled (km <sup>2</sup> )	Spawning area <i>A</i> (km <sup>2</sup> )	Area with eggs (%)	Spawning biomass (t)
65,528	22,400	34.2	49,575

#### 4.3.4 Daily Egg Production ( $P_0$ )

The estimate of mean daily egg production ( $P_0$ ), obtained by averaging the estimates from the four model fits, was 52.6 eggs·day<sup>-1</sup>·m<sup>-2</sup> (95% CI = 39.1 - 78.1; Table 9).

**Table 9.** Mean daily egg production ( $P_0$ ) of Australian Sardine estimated using four alternate models. The value used for biomass estimation is highlighted in bold. Ranges are 95% confidence intervals.

Model fit	$P_0$ (eggs·day <sup>-1</sup> ·m <sup>-2</sup> )
Exponential model, $\rho \sim \exp(\text{age})$ , NLS	43.6 (36.2 - 58.8)
Linear version of exponential model, $(\ln)\rho \sim \text{age}$ , corrected NLS	61.7 (50.5 - 92.5)
GLM, $\rho \sim \text{age}$ , Quasi family, log link, $\text{var}(y)=\mu(y)$	47.0 (35.2 - 65.1)
GLM, $\rho \sim \text{age}$ , Quasi family, log link, $\text{var}(y)=\mu(y)^2$	58.0 (32.9 - 104.8)
<b>Mean of all model fits</b>	<b>52.6 (39.1 - 78.1)</b>

#### 4.3.5 Adult Reproductive Parameters

A sample of 124 Australian Sardine taken at site 1f (off Patches Beach, NSW) on the *FV Hazel-K* and five samples from purse seine catches off Illuka, NSW were used to estimate adult reproductive parameters. A total of 478 adult Australian Sardine were collected, including 247 females and 231 males (Table 10), with an average fork length of 160 mm (range: 130 - 197 mm FL). Estimates of the adult female reproductive parameters used in calculations of spawning biomass are provided in Table 10 and 11. Bootstrapped parameter estimates with 95% confidence intervals are shown in Table 12.

**Table 10.** Total number, mean weights (g) by sex, and sex ratio ( $R$ ; proportion of females by weight) of adult Australian Sardine collected off the east coast in 2014. Values in bold are sums (\*) or weighted means (#). Refer to Fig. 2 for location of Site 1f. NSW: New South Wales

Location	Collection Method	Date	Male n	Female n	Mean Male Weight	Mean Female Weight ( $W$ , g)	Sex Ratio ( $R$ )
Site 1f	Trawl	22/08/2014	24	100	36.7	33.3	0.48
Illuka, NSW	Purse seine	11/09/2014	40	30	30.5	32.9	0.52
Illuka, NSW	Purse seine	12/09/2014	29	30	34.1	36.3	0.52
Illuka, NSW	Purse seine	15/09/2014	46	30	28.6	31.6	0.53
Illuka, NSW	Purse seine	16/09/2014	54	27	35.8	36.7	0.51
Illuka, NSW	Purse seine	18/09/2014	38	30	35.8	40.8	0.53
<b>Grand Total</b>			<b>231*</b>	<b>247*</b>	<b>33.3#</b>	<b>38.8#</b>	<b>0.54#</b>

### Mean female weight (*W*)

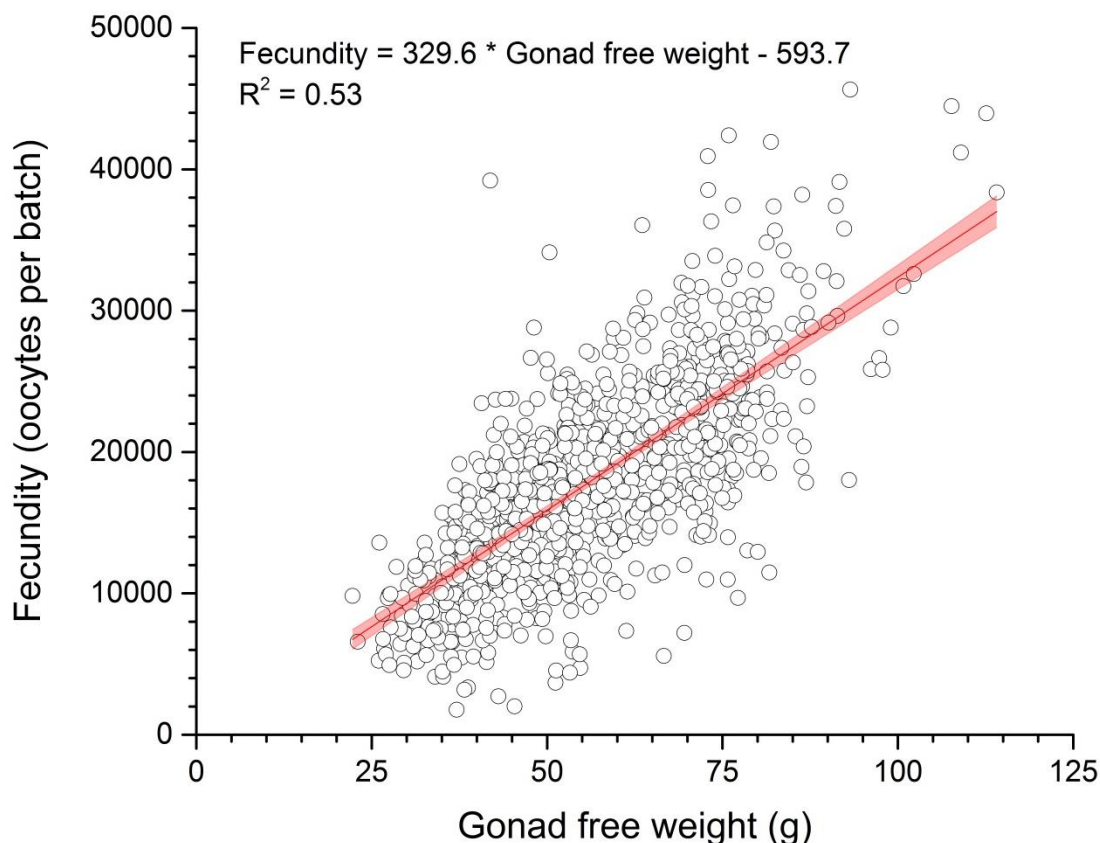
The mean weight of mature females in samples ranged from 31.6 to 40.8 g (Table 10). The weighted mean weight of mature females was 38.8 g (95% CI = 36.8 - 42.1, Tables 10, 12).

### Sex ratio (*R*)

The sex ratio calculated from the survey was 0.54 (95% CI = 0.40 - 0.70, Tables 10, 12).

### Batch fecundity (*F*)

Of the 247 mature Australian Sardine females collected during the current survey, none contained hydrated ovaries. Therefore, a linear relationship between gonad-free female weight and batch fecundity of Australian Sardine from 1998 to 2014 in South Australia ( $n = 1052$ ) was used to calculate batch fecundity of mature east coast Australian Sardine (Batch Fecundity =  $329.6 \times$  Gonad-free Female Weight - 593.74;  $R^2 = 0.53$ ; Fig. 14). Using mean gonad free weight for all east coast Australian Sardine samples collected (38.0g), the estimate of mean batch fecundity (*F*) was 11,942 hydrated oocytes per batch (95% CI = 11,148 - 13,036; Table 12).



**Fig. 14.** Relationship between gonad-free female weight and batch fecundity of Australian Sardine from 1998 to 2014 in South Australia ( $n = 1052$ ) used to calculate batch fecundity of mature east coast Australian Sardine. Red shaded area = 95% CI.

### Spawning fraction (*S*)

Of the 247 ovaries examined during the current survey, 7 had day-0 POFs, 29 had day-1 POFs and 70 day-2 POFs (Table 11). The spawning fraction of females in samples from the current survey ranged from 0.01 to 0.23. The weighted mean spawning fraction for all 2014 data was 0.14 (95% CI = 0.04 - 0.21).

### 4.3.6 Spawning Biomass

The estimate of Australian Sardine spawning biomass calculated using data from the 2014 survey (Table 12) was 49,600 t (95% CI = 24,200 - 213,300; Table 8).

**Table 11.** Total number of post ovulatory follicles (POF) and estimates of spawning fraction (*S*) for samples of female Australian Sardine collected along the East Australian Coast in 2014. Refer to Fig. 2 for location of Site 1f. Values in the bottom row are sums (\*) or a weighted mean (#). NSW: New South Wales

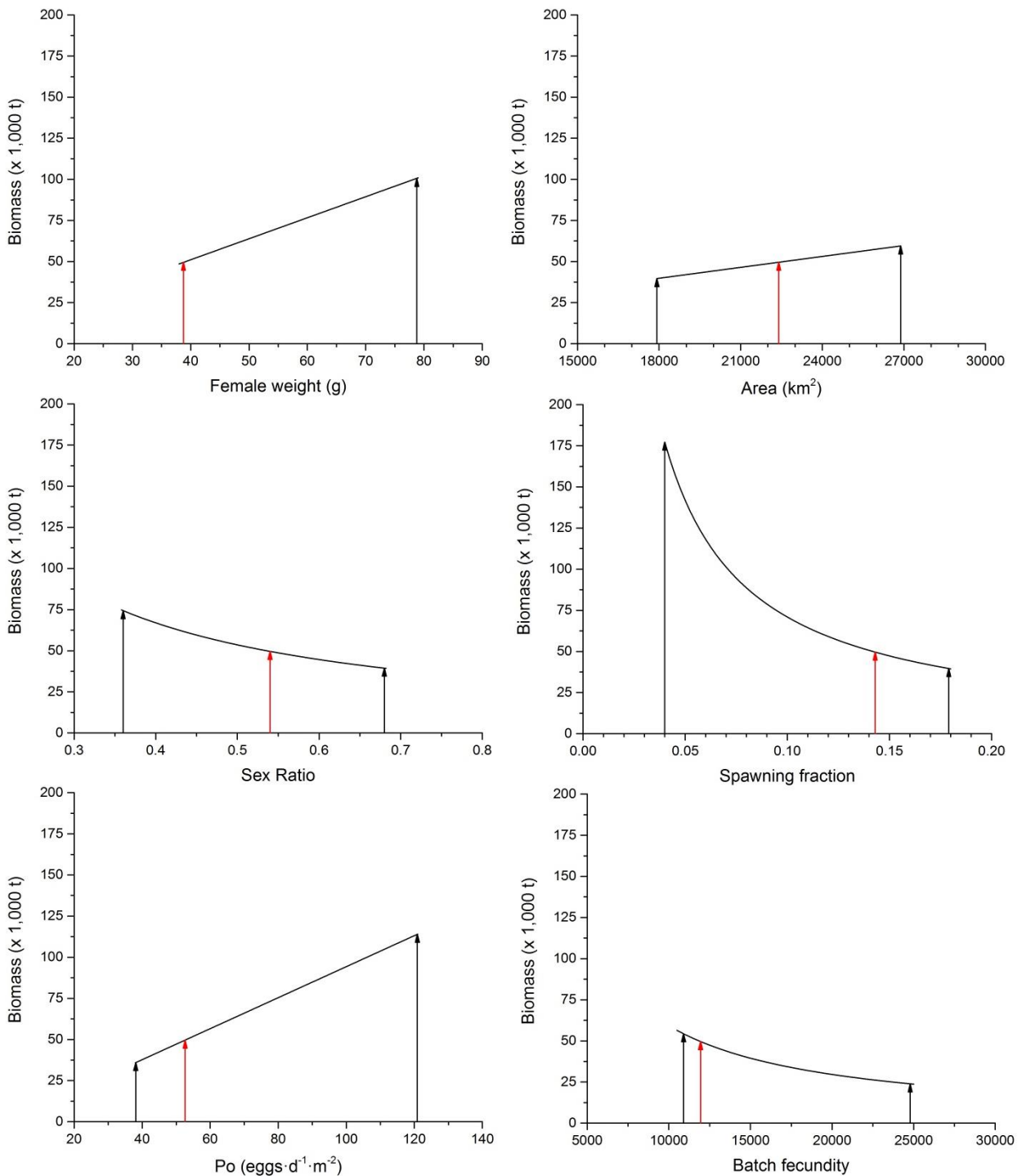
Location	Collection Method	Date	POF 0	POF 1	POF 2	Total Females	Spawning Fraction ( <i>S</i> )
Site 1f	Trawl	22/08/2014	0	14	56	100	0.23
Illuka, NSW	Purse seine	11/09/2014	0	1	0	30	0.01
Illuka, NSW	Purse seine	12/09/2014	0	0	2	30	0.02
Illuka, NSW	Purse seine	15/09/2014	0	3	1	30	0.04
Illuka, NSW	Purse seine	16/09/2014	2	7	5	27	0.17
Illuka, NSW	Purse seine	18/09/2014	5	4	6	30	0.17
<b>Grand Total</b>			<b>7*</b>	<b>29*</b>	<b>70*</b>	<b>247*</b>	<b>0.14#</b>

**Table 12.** Parameters used in the calculations of spawning biomass of Australian Sardine in 2014.

Parameter	Number (95% CI)
Egg Production ( $P_0$ , eggs·day <sup>-1</sup> ·m <sup>-2</sup> )	52.6 (39.1 - 78.1)
Sex Ratio ( <i>R</i> )	0.54 (0.40 - 0.70)
Fecundity ( <i>F</i> , eggs·female <sup>-1</sup> )	11,942 (11,148 - 13,036)
Spawning Fraction ( <i>S</i> )	0.14 (0.04 - 0.21)
Female Weight ( <i>W</i> , g)	38.8 (36.8 - 42.1)
Spawning Area ( <i>A</i> , km <sup>2</sup> )	22,400

#### **4.3.7 Sensitivity Analysis**

The sensitivity of estimates of spawning biomass to reasonable variations in each parameter returned spawning biomass estimates of Australian Sardine on the east coast of Australia in the range of ~ 30,000 to 110,000 t (Fig. 15). Credible values for only one parameter (spawning fraction,  $S$ ; 0.04) provide results that fell outside that range (i.e. ~ 175,000 t). The mean estimate of female weight (38.8 g;  $W$ ) in this study is low compared to estimates of this parameter obtained from Australian Sardine during DEPM surveys in South Australia from 1998 to 2014 (mean: 57.5 g; Table 4). The estimate of batch fecundity used in the analysis was correspondingly low.



**Fig. 15.** Sensitivity analysis of the effects of individual parameters on estimates of spawning biomass of Australian Sardine. Red arrows are values estimated in the current survey, and black arrows are the minimum and maximum values estimated for South Australian DEPM surveys between 1998 and 2014 as described in Table 4.

## 4.4 Tailor

### 4.4.1 Distribution and Abundance of Eggs

Of the 262 stations sampled (Fig. 1), no Tailor eggs were collected in formalin-preserved samples. One egg was positively identified during genetic analyses on Transect 4 (perpendicular to Fraser Island, Queensland; Appendix 1). Therefore,  $P_0$  and  $A$  could not be calculated, and spawning biomass could not be estimated.

### 4.4.2 Spawning Area

Tailor eggs were not collected in formalin-preserved samples. One Tailor egg was positively identified during genetic analyses on Transect 4 perpendicular to Fraser Island, and Tailor larvae were found in ichthyoplankton samples off Fraser Island. Therefore, spawning area of Tailor was not estimated (Table 13).

**Table 13.** Total survey area, spawning area ( $A$ ), percent area containing eggs, and spawning biomass of Tailor.

Area sampled (km <sup>2</sup> )	Spawning area $A$ (km <sup>2</sup> )	Area with eggs (%)	Spawning biomass (t)
65,528	-	0	-

### 4.4.3 Daily Egg Production ( $P_0$ )

Mean daily egg production,  $P_0$ , could not be estimated due to the low abundance of Tailor eggs collected in ichthyoplankton samples.

### 4.4.4 Adult Reproductive Parameters

A total of 878 adult Tailor ( $n = 278$  males and 600 females) were sampled on Fraser Island, Queensland during two recreational fishing surveys in August-September 2014 for estimation of mean female weight and sex ratio. Average fork length was 359 mm (range: 240 - 560 mm FL). Ovaries were collected from a further 206 females for histological analyses (average length: 375 mm FL; range: 310 - 520 mm FL). Estimates of female reproductive parameters for use in calculations of spawning biomass are provided in Table 14 and Table 15. Bootstrapped parameter estimates with 95% confidence intervals are shown in Table 16.



**Table 14.** Total number, mean weights (g) by sex, and sex ratio (*R*; proportion of females by weight) of adult Tailor measured on Fraser Island, Queensland during two recreational fishing surveys in August -September 2014. Values in bold are sums (\*) or weighted means (#). QLD: Queensland

Location	Date	Male n	Female n	Mean Male Weight	Mean Female Weight ( <i>W</i> , g)	Sex Ratio ( <i>R</i> )
Fraser Island, QLD	11 - 14 Aug 2014	167	356	495	539.0	0.42
Fraser Island, QLD	30 Aug to 5 Sept 2014	111	244	664	721.5	0.29
<b>Grand Total</b>		<b>278*</b>	<b>600*</b>	<b>562#</b>	<b>613#</b>	<b>0.70#</b>

#### *Mean female weight (W)*

The mean weight of mature females in samples ranged from 539 to 722 g (Table 14). The weighted mean weight of mature females was 613 g (95% CI = 534 - 734; Tables 14, 16).

#### *Sex ratio (R)*

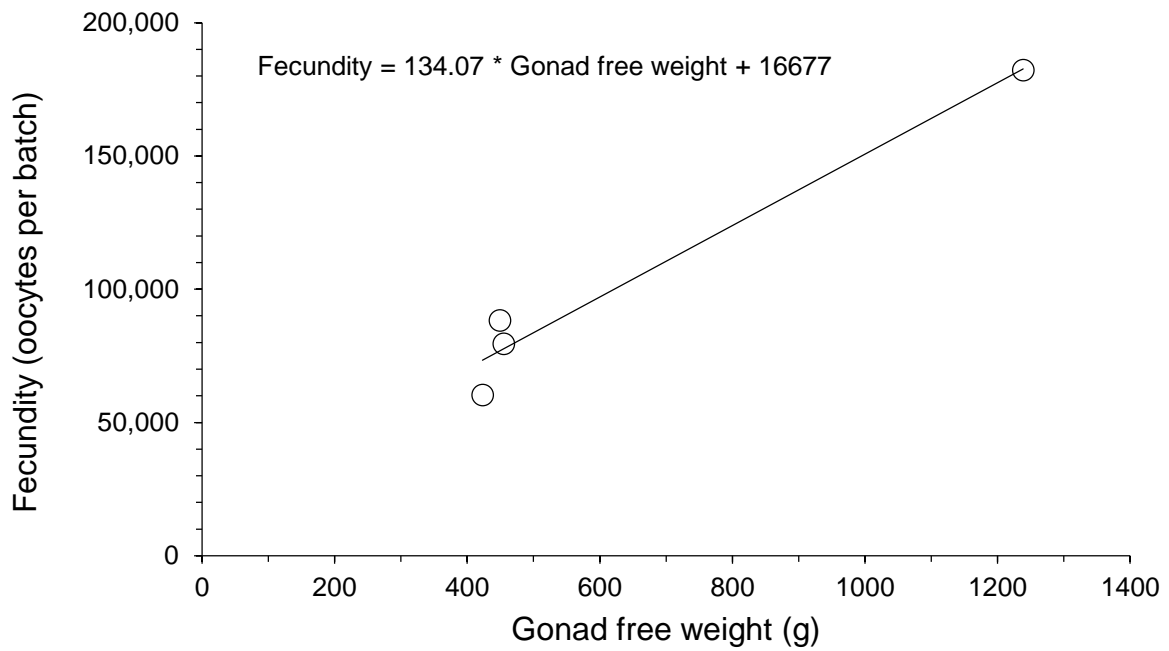
The sex ratio calculated from the survey was 0.70 (95% CI = 0.67 - 0.73; Tables 14, 16).

#### *Batch fecundity (F)*

Of the 206 mature Tailor females collected for histological analyses, four contained hydrated ovaries (e.g. Fig. 5b). Batch fecundity ranged from 60,447 to 182,269 (mean = 102,656) hydrated oocytes. The linear relationship between gonad-free female weight and batch fecundity of these fish was: Batch Fecundity =  $134.07 \times \text{Gonad-free Female Weight} + 16677$  (Fig. 16). Using this relationship to estimate mean batch fecundity (*F*) of all mature Tailor collected (mean gonad free weight = 639.1 g) produced an estimate of 102,361 hydrated oocytes per batch (95% CI = -189,404 - 290,749).

#### *Spawning fraction (S)*

Of the 206 Tailor ovaries examined, 4 had hydrated oocytes and/or day-0 POFs, 10 had day-1 POFs and 71 day-2 POFs (Fig. 5, Table 15). The spawning fraction of females in samples ranged from 0.12 to 0.30. The weighted mean spawning fraction for all 2014 data was 0.14 (95% CI = 0.11 - 0.32).



**Fig. 16.** Relationship between gonad-free female weight and batch fecundity of Tailor collected during the 2014 survey on Fraser Island, Queensland (n = 4).

**Table 15.** Total number of post ovulatory follicles (POF) and estimates of spawning fraction (S) for samples of female Tailor collected on Fraser Island, Queensland during two recreational fishing surveys in August - September 2014. Values in the bottom row are sums (\*) or a weighted mean (#). \*Includes hydrated females. QLD: Queensland

Location	Date	POF 0+	POF 1	POF 2	Total Females	Spawning Fraction (S)
Fraser Island, QLD	11 - 15 Aug 2014	0	1	17	20	0.30
Fraser Island, QLD	31 Aug to 5 Sept 2014	4	9	54	186	0.12
<b>Grand Total</b>		<b>4*</b>	<b>10*</b>	<b>71*</b>	<b>206*</b>	<b>0.14#</b>

#### 4.4.5 Spawning Biomass

Spawning biomass estimates could not be calculated, because the low abundance of eggs found in ichthyoplankton samples did not allow application of the DEPM.

**Table 16.** Parameters estimated for use in potential future spawning biomass calculations of Tailor.

Parameter	Number (95% CI)
Sex Ratio ( $R$ )	0.70 (0.67 - 0.73)
Fecundity ( $F$ , eggs·female <sup>-1</sup> )	102,361 (-189,404 - 290,749)
Spawning Fraction ( $S$ )	0.14 (0.11 - 0.32)
Female Weight ( $W$ , g)	613 (534 - 734)

#### 4.4.6 Sensitivity Analysis

Sensitivity analyses were not undertaken for Tailor because the low abundance of eggs found in ichthyoplankton samples did not allow spawning biomass to be estimated with the DEPM.

# 5. Discussion

## 5.1. Blue Mackerel

### ***Spawning patterns***

The distribution of Blue Mackerel eggs during the present study (Sandy Cape, Queensland to Newcastle, NSW) was similar to that observed during a survey conducted in late July 2004 (Ward and Rogers 2007, Neira and Keane 2008, Ward et al. 2009). However, the mean egg density estimated in the present study (25.7 eggs·m<sup>-2</sup>) was much higher than previously recorded (12.4 eggs·m<sup>-2</sup>), which suggests that the July 2004 survey may have preceded the peak spawning season. Eggs collected during surveys in October 2002 and 2003 (Ward and Rogers 2007, Neira and Keane 2008, Ward et al. 2009) were found over much smaller areas than in the present study (i.e. 2002/03: ≤ 10,078 km<sup>2</sup>; current: 17,911 km<sup>2</sup>). In addition, fewer eggs and a lower proportion of positive stations were recorded compared to the current survey. This information suggests that the October 2002 and 2003 surveys may have been conducted after the peak spawning season. The August-September 2014 survey appears to have been completed close to the peak spawning season of Blue Mackerel off the east coast because of: i) the large area over which eggs were collected, ii) the large number of eggs obtained (2,330), and iii) the high egg densities recorded.

The highest densities of Blue Mackerel eggs in the present study were recorded in waters just to the north of Coffs Harbour, NSW and off Port Stephens, NSW. During previous studies, the highest egg densities were recorded off Diamond Head, NSW, which lies between these two locations (Ward and Rogers 2007, Neira and Keane 2008). In the present study, most Blue Mackerel eggs were collected from sites with SSTs ranging from 18 to 22°C, and the highest densities (>100 eggs·m<sup>-2</sup>) were found between 18 to 20°C. Neira and Keane (2008) also obtained most eggs from SSTs between 18 and 21°C. In the present study, most Blue Mackerel eggs were collected from depths >50 m which is consistent with the results of Ward and Rogers (2007). Furthermore, 94% of eggs in the current survey were collected from depths between 55 and 145 m and 88% from 65 to 135 m.

### ***DEPM Parameters***

Estimates of egg production obtained in the present study were obtained from a relatively large number of sites (70 stations) compared to Ward and Rogers (2007) and Ward et al. (2009), who reported collecting Blue Mackerel eggs at 29 and 45 sites in 2003 and 2004, respectively. The current survey appeared to cover the main spawning area, since large numbers of eggs were not collected from the ends of transects or at the northern and southern limits of the survey area. However, as is often the case in DEPM studies, mortality could not be estimated directly from samples (e.g. Bernal et al. 2011). Hence, assumed rates of egg mortality were used to convert the estimate of mean egg density to an estimate of  $P_0$ . This approach is considered to be conservative and unlikely to result in over-estimation of  $P_0$  (McGarvey and Kinloch 2001, Ward and Rogers 2007).

The high  $P_0$  recorded in the present study (34.6) probably reflects the timing of the survey to coincide with the peak spawning season, whereas the July 2004 survey (8.2; Ward and Rogers 2007) probably occurred

before peak spawning. A similar statistical approach (i.e. applying an assumed mortality ( $z$ ) of 0.3 to mean egg density) was used to estimate egg production for 2004 (Ward and Rogers 2007).

The spawning area estimated in the current study (17,911 km<sup>2</sup>) is comparable to the spawning area estimated in July 2004 (i.e. 17,503 km<sup>2</sup>). However, it is important to note that the previous estimate of spawning area was based on a relatively small number of large grids compared to the present study (e.g. 85 versus 262). Therefore, the relatively high estimate of spawning area for July 2004 needs to be viewed with some caution (as noted in Ward et al. 2009), as it could in part reflect the large size of individual grids used to calculate spawning area (i.e. reducing the precision of the estimate of spawning area).

The method used to estimate egg age and spawning time in the present study (Kernel Density Growth Profiling) builds on the approach used for Australian Sardine and Jack Mackerel by Ward et al. (2014b, 2015a). This approach has important implications for the application of the DEPM to new species for which egg temperature-development keys have not been established. Our estimates of the egg duration obtained using Kernel Density Growth Profiling were similar to those obtained by Lockwood et al. (1981), Mendiola et al. (2006) and Hunter and Kimbell (1980) for other species of *Scomber* at similar temperatures. This finding confirmed that i) the developmental rates of Blue Mackerel eggs are similar to those of other scombrids, and ii) Kernel Density Growth Profiling is a suitable method for estimating egg age and spawning time. Importantly, this approach is based on samples obtained in the field and not subject to tank effects that may influence temperature-development keys based on eggs reared under laboratory conditions.

Attempts to collect adult Blue Mackerel during the present study were unsuccessful. Only immature fish were captured during the trawl survey and efforts to jig spawning adults from offshore reefs in southern Queensland were not productive. In contrast, demersal trawling during the day-time was successfully used to sample adult Jack Mackerel off the east coast during January 2014 (Ward et al. 2015a). Daylight trawling may not be suitable for sampling adult Blue Mackerel, because adults swim faster (He 1993; Mr David Guillot, pers. comm.) and have a higher capacity to avoid trawl nets than Jack Mackerel. In addition, adult Blue Mackerel may primarily spawn at deeper depths than those sampled during the present study or may be associated with hard-bottom, deep-water habitats as opposed to the mainly soft bottom habitat that was sampled in the current survey (Dr J. Stewart, pers. comm.). Other scombrids in spawning condition have been caught effectively from deep water using large trawl nets (see Priede et al. 1995). Future studies should attempt to sample spawning Blue Mackerel from deep water habitats using both hook and line and trawl nets.

### ***Spawning biomass and sensitivity analyses***

The best estimate of the spawning biomass of Blue Mackerel off the east coast obtained in the present study was ~83,300 t (95% CI = 35,100 - 165,000 t) values for  $P_0$ , spawning biomass estimates ranged between, which is considerably higher than the previous best estimate of ~23,000 t (Ward and Rogers 2007). This difference largely reflects the higher  $P_0$  (34.6 eggs·day<sup>-1</sup>·m<sup>-2</sup>) of the current survey compared to 2004 and is likely associated with improved timing of the survey to coincide with the peak spawning season. Since adult samples were not collected during the present study, data from South Australia were used to estimate adult parameters and provide the estimate of spawning biomass for Blue Mackerel off eastern Australia.

Therefore, the current estimate of spawning biomass of Blue Mackerel off the east coast, i.e. ~83,300 t (95% CI = 35,100 - 165,000 t), should be used with some caution. The sensitivity analyses show that estimates of spawning biomass are particularly sensitive to variation in estimates of spawning fraction.

## 5.2 Australian Sardine

### ***Spawning patterns***

The presence of large numbers of eggs and larvae of Australian Sardine between Sandy Cape, Queensland and Newcastle, NSW during the present study supports the hypothesis that the main spawning season off the east coast occurs in northern NSW and southern Queensland during late winter and early spring (Ward and Staunton-Smith 2002, Ward et al. 2015a). This was further reinforced with the collection of spawning adult Australian Sardine off northern NSW during the current survey in August-September 2014.

Most eggs of Australian Sardine were collected from stations with SSTs between 17 and 22°C, which is also comparable with findings of other broad scale surveys off both the east coast (e.g. Ward and Staunton-Smith 2002, Ward et al. 2007, 2015a) and southern Australia (e.g. Ward et al. 2011b, 2014b). However, in southern areas (i.e. off Tasmania and southern Australia) eggs and larvae have also been found at locations with lower SSTs than those recorded in the present study (e.g. Ward and Staunton-Smith 2002, Ward et al. 2014b, 2015a).

Egg densities in the current survey were highest in southern Queensland from Fraser Island to Caloundra compared to northern and mid coast of NSW. A preliminary DEPM survey in July 2004 on the east coast found the greatest densities of Australian Sardine eggs to be along the mid to northern NSW coast (Ward et al. 2007) compared to southern Queensland. In southern Queensland, egg densities of Australian Sardine were highest during August-September (Ward and Staunton-Smith 2002). Our results support these findings and the hypothesis of Ward et al. (2003) that Australian Sardine spawn in southern Queensland during late winter-early spring when regional water temperatures are low.

### ***DEPM parameters***

This report presents the results of the first dedicated application of the DEPM to Australian Sardine off the east coast (noting that it was conducted in conjunction with a dedicated survey for Blue Mackerel). The study provides the most robust estimate of the spawning biomass that has been obtained for this species off eastern Australia (i.e. ~ 49,575 t), because it is based on robust estimates of each DEPM parameter.

Ages of Australian Sardine egg stages obtained from Kernel Density Growth Profiling were comparable to those reported from temperature-development keys of White and Fletcher (1998). The similarity between the two methods confirm the suitability of Kernel Density Growth Profiling for estimating egg age and spawning time from samples collected during surveys.

The estimate of egg production ( $P_0$ ) obtained in the present study was collected from a relatively large number of samples ( $n = 70$ ). However, as is the case in most DEPM studies, the model fits used to estimate  $P_0$  were relatively poor. We used the average value obtained from four models to estimate spawning biomass. The estimate of egg production obtained in this study ( $52.6 \text{ eggs}\cdot\text{day}^{-1}\cdot\text{m}^{-2}$ ) was lower than those presented by both Ward et al. (2007) and Staunton-Smith and Ward (2000). It is important to note that the scarcity of eggs at the southern end of the survey area provides a high degree of confidence that the entire spawning area was sampled, which was not achieved in previous surveys (e.g. Staunton-Smith and Ward 2000, Ward et al. 2007). It is important to cover the entire spawning area because this parameter is strongly correlated with spawning biomass (Gaughan et al. 2004). The estimate of spawning area ( $22,400 \text{ km}^2$ ) provided in the present study is larger than those previously estimated by Staunton-Smith and Ward (2000) and Ward et al. (2007), i.e.  $\sim 3,400 - 6,400 \text{ km}^2$  and  $\sim 9,400 \text{ km}^2$ , respectively.

The collection of adult samples for estimation of reproductive parameters from the current survey addressed one of the key uncertainties associated with previous estimates of spawning biomass of Australian Sardine off eastern Australia (Ward et al. 2007). The mean size of fish collected was smaller than those obtained by Staunton-Smith and Ward (2000) and off southern Australia, and estimates of batch fecundity for this survey are correspondingly lower. However, the sensitivity analyses show that estimates of spawning biomass are relatively unaffected by concurrent variations in these two parameters (i.e. mean female weight and batch fecundity). Estimates of spawning biomass are more strongly influenced by variations in estimates of spawning fraction. The estimate of mean spawning fraction obtained in the present study (0.14) is similar to the estimate obtained for 1998 (0.15) by Staunton-Smith and Ward (2000), and the mean value for South Australia between 1998 and 2014 (0.12). These values are consistent with global means for this species during the peak spawning season, and the estimate of this parameter is considered robust.

Although all DEPM parameters were estimated with a high degree of confidence in this study, one question remains unresolved: what proportion of the adult population occurred outside the spawning area during the survey period? A survey conducted during January 2014 (Ward et al. 2015a) obtained a smaller estimate of Australian Sardine spawning biomass between southern Tasmania and Port Stephens, NSW ( $\sim 11,000 \text{ t}$ ). This suggests that the proportion of the total east coast population that was present in this southern area even during the peak spawning period in that area was relatively small. In addition, there is substantial anecdotal evidence (Ward and Staunton-Smith 2002, Ward et al. 2003) for northerly movement of small pelagic fishes, including Australian Sardine, along the east coast into northern NSW and southern Queensland during winter. This movement pattern of predominately temperate species into sub-tropical waters to spawn has also been observed for pelagic fishes in other western boundary current systems (see Ward et al. 2003). These observations support the hypothesis of Ward and Staunton-Smith (2002) that the majority of the adult population of Australian Sardine off the east coast occurs in northern NSW and southern Queensland during August-September.

### ***Spawning biomass and sensitivity analyses***

The estimate of spawning biomass obtained in the present study, i.e.  $\sim 49,600 \text{ t}$  (95% CI =  $24,200 - 213,300 \text{ t}$ ), is larger than the estimates of  $\sim 25,000 \text{ t}$  obtained by Staunton-Smith and Ward (2000) and  $\sim 28,800 \text{ t}$  by

Ward et al. (2007). This difference mainly reflects the larger spawning area observed in the present study compared to both Staunton-Smith and Ward (2000) and Ward et al. (2007). The estimate provided here is also much larger than the estimate of the spawning biomass of Sardine (~11,000 t) between northern Tasmania and southern Victoria during January 2014 (Ward et al. 2015a). The relative proportion of this southern spawning aggregation that moves northwards (and potentially spawns) during winter is unknown. This survey is considered to provide a reliable (albeit probably conservative) estimate of the total adult population of Australian Sardine off the east coast because it includes the entire area spawning where spawning occurs during the peak spawning season and most of the adult stock is likely to have been in the survey area during the sampling period.

## 5.3 Tailor

### ***Spawning patterns***

Adult Tailor in spawning condition were successfully collected from Fraser Island, Queensland during August-September 2014. Tailor are reported to spawn in early winter to late spring along the east coast with peak spawning off Fraser Island occurring in October (Bade 1977). Adult females collected during the survey were either in spawning condition or actively spawning. High percentages of atresia were not present in any of the ovaries examined, which indicated that the peak spawning season had not yet occurred in the area. Bade (1977) reported Tailor with gonads in the 'spent' condition (equates to 'regressing phase' in Brown-Peterson et al. 2011) were most abundant off Fraser Island in November.

A surprising outcome of the current survey was the lack of Tailor eggs and larvae collected during the ichthyoplankton surveys. This raises concerns about the accuracy of estimates of Tailor eggs and larvae reported by Ward et al. (2003) in southern Queensland over the same seasonal time period. Blue Mackerel and Tailor eggs and larvae have similar pigment patterns and features, although the size of the eggs and oil globules are diagnostic (e.g. Neira et al. 1998). It is possible that at least some Blue Mackerel eggs and larvae were misidentified as Tailor by Ward et al. (2003).

Previous investigations have found Tailor eggs in surface waters (<3 m) off Indian Head and Waddy Point, Fraser Island during September/October, suggesting Tailor off Fraser Island may spawn in nearshore waters (Halliday 1990, Zeller et al. 1996). Bade (1977) also suggested that Tailor in Australia spawn close to the coastline rather than offshore. In addition, the adult ovary samples collected during the current survey were taken from shore-based, recreational fishers. Hydrated ovaries and day 1 POFs were found in these samples, indicating imminent or recent spawning activity. However, in other parts of its global range, Tailor are reported to spawn in offshore, continental shelf waters (Norcross et al. 1974, Juanes et al. 1996, Fahay et al. 1999). Future DEPM surveys for Tailor should include intensive sampling of nearshore waters off Fraser Island.



## ***DEPM parameters***

Adult reproductive parameters of Tailor estimated during the current survey were comparable to previous studies. The mean fecundity estimate (102,361 eggs·female<sup>-1</sup>) was lower than those of Bade (1977) and Robillard et al. (2008), but within the reported range. The sex ratio from the current survey (0.7) was similar to that calculated from fishery catches of Tailor in Western Australia (0.6 biased towards females; Smith et al. 2013). Bade (1977) reported a sex ratio of 0.5 for Tailor in southern Queensland. Mean female weight has not been reported for Tailor in other studies, but length at 50% maturity is estimated to be 320 mm TL in Western Australia (Smith et al. 2015) and 451 mm TL on the east coast of the USA (Robillard et al. 2008), with 100% maturity reported to occur at 300 mm FL in southern Queensland (Bade 1977). Tailor examined in the present study matured at a comparable length (mean: 359 mm FL). The spawning fraction presented in the current study of 0.14 is the first estimate of this parameter for Tailor. Collectively, adult parameters specifically for use with the DEPM have not been previously estimated for Tailor.

Although the eggs of Tailor were not collected in the present study, Tailor is a good candidate for the DEPM because: i) Tailor are batch spawners with indeterminate fecundity, ii) genetic verification of the eggs has been achieved (see Appendix 1), and iii) estimates of adult reproductive parameters were obtained. However, future egg surveys should sample inshore areas, and the timing of the survey should be shifted to September-October in southern Queensland/northern NSW to better capture the peak of the spawning season. Our results provide a foundation for future application of the DEPM to Tailor.

## **6. Conclusions**

This study has provided estimates of Blue Mackerel spawning biomass for eastern Australian, but since no adult samples were collected during this study and data from South Australia were used to estimate adult parameters, the estimate of spawning biomass of 83,300 t should be treated with caution. During the present study, sampling intensity for estimates of egg production in the region was higher than in exploratory surveys conducted in 2003 and 2004 by Ward and Rogers (2007). Therefore, current estimates of egg production and spawning area are considered to be more robust than those previously reported for the region.

The estimate of spawning biomass of Australian Sardine in the present study (49,575 t) is larger than preliminary estimates for eastern Australia in 2004. This survey is likely to provide a reliable estimate of the total adult population of Australian Sardine off the east coast because it includes the entire spawning area and most of the adult stock is likely to have been in the area surveyed during the survey period.

Adult parameters related to the DEPM were estimated for Tailor, but eggs were not collected during the current survey. As a result, egg production, spawning area and spawning biomass for Tailor could not be estimated. Tailor is a good candidate for the DEPM, but egg surveys should focus on inshore areas and be conducted during September-October to capture the peak of the spawning season.

## 7. Implications and recommendations

- The estimate of spawning biomass of Blue Mackerel off eastern Australia (83,300 t) is based on robust estimates of egg production and spawning area, but should be treated with caution because estimates of adult parameters were not obtained during the present study. Techniques for sampling adults should be developed before the DEPM is re-applied to Blue Mackerel off eastern Australia.
- The estimate of spawning biomass of Australian Sardine (49,575 t) is considered robust as it is based on reliable estimates of all DEPM parameters, but is likely to be negatively biased because some proportion of the (non-spawning) adult population is likely to have occurred outside the survey area (i.e. further south) during the survey period.
- Tailor may be a suitable candidate for application of the DEPM. However, the next step would be to conduct a relatively small study 1) to assess the feasibility of conducting a plankton survey close to shore and 2) to establish effective methods for estimating batch fecundity.

## 8. Further development

This study made some crucial technical developments (e.g. established a robust method for ageing wild fish eggs) and filled several key knowledge gaps (e.g. provided estimates of adult reproductive parameters for Australian Sardine and Tailor along the east coast). However, further work is needed to address remaining gaps including 1) a method for sampling adult Blue Mackerel, 2) the feasibility of conducting a near shore plankton survey for Tailor, and 3) alternative methods for estimating batch fecundity of Tailor.

## 9. Extension and Adoption

The need for this study was identified by the Resource Assessment Group (RAG) for the SPF. Progress reports have been provided to the RAG and other stakeholders during the course of the study. The final report will be provided to AFMA to provide a basis for setting recommended biological catches for Blue Mackerel and Australian Sardine in the Eastern sub-area of the SPF. Findings for Tailor will be presented to Fisheries Queensland. Results of this project will also be presented at stakeholder fora as requested by FRDC, AFMA or Fisheries Queensland.

### Planned Outcomes and Benefits

1. Enhanced sustainability of Blue Mackerel and Australian Sardine fisheries off the east coast of Australia. Estimates of spawning biomass will be used to establish recommended biological catches

for Blue Mackerel and Australian Sardine in the Eastern sub-area of the SPF and to inform sustainable management of other fisheries for Australian Sardine along the east coast. Methods developed will improve the reliability of future estimates of spawning biomass of both species. Beneficiaries include: AFMA, Fisheries Queensland, NSW DPI, Tas DPIPWE, commercial and recreational fishers in all jurisdictions along the east coast, conservation groups and the broader community.

2. Enhanced sustainability of Tailor fisheries off the east coast of Australia. Beneficiaries include: Fisheries Queensland, NSW DPI, commercial and recreational fishers in all jurisdictions along the east coast, conservation groups and the broader community.

## 10. Project materials developed

A manuscript describing Kernel Density Growth Profiling to estimate ages of eggs and infer the time of peak spawning is currently being prepared for submission to a scientific journal.

Manuscripts that document other aspects of the study will also be developed.

### Outputs and outcomes

The primary outputs and outcomes of the project are:

1. Estimates of the spawning area and total mean daily egg production of Blue Mackerel and Australian Sardine off eastern Australia during winter/spring.
2. The first estimates of DEPM-related adult reproductive parameters (e.g. spawning fraction, batch fecundity, sex ratio, female weight) of Tailor.
3. Estimates of spawning biomass of Blue Mackerel and Australian Sardine off the east coast during winter/spring.
4. Improved scientific basis for assessment and management of the SPF and fisheries for Australian Sardine off eastern Australia.

# 11. References

- AFMA. 2009. Small Pelagic Fishery Management Plan 2009. Australian Fisheries Management Authority, Canberra, Federal Register of Legislative Instruments F2010L00081. 51 pp.
- AFMA. 2015. Small Pelagic Fishery Harvest Strategy. Australian Fisheries Management Authority, Canberra. April 2015. 9 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.
- Anonymous. 2014. Fisheries Fact Sheet: Tailor. Pages 1-4 in G. o. W. A. Department of Fisheries, editor.
- Bade, T. M. 1977. The biology of tailor (*Pomotomus saltatrix* Linn.) from the east coast of Australia. Unpublished M Sc. Thesis. University of Queensland.
- Beckley, L. E., and C. D. van der Lingen. 1999. Biology, fishery and management of sardines (*Sardinops sagax*) in southern African waters. *Marine and Freshwater Research* **50**:955-978.
- Bernal, M., Y. Stratoudakis, S. Wood, L. Ibaibarriaga, L. Valdés, and D. Borchers. 2011. A revision of daily egg production estimation methods, with application to Atlanto-Iberian sardine. 2. Spatially and environmentally explicit estimates of egg production. *ICES Journal of Marine Science: Journal du Conseil* **68**:528-536.
- Brown-Peterson, N. J., D. M. Wyanski, F. Saborido-Rey, B. J. Macewicz, and S. K. Lowerre-Barbieri. 2011. A Standardized Terminology for Describing Reproductive Development in Fishes. *Marine and Coastal Fisheries* **3**:52-70.
- Bunn, N. A., C. J. Fox, and T. Webb. 2000. A literature review of studies on fish egg mortality: implications for the estimation of spawning stock biomass by the annual egg production method. 111, Centre for Environment, Fisheries and Aquaculture Science.
- Catanese, G., M. Machado, and C. Infante. 2010. Evolutionary relatedness of mackerels of the genus *Scomber* based on complete mitochondrial genomes: Strong support to the recognition of Atlantic *Scomber colias* and Pacific *Scomber japonicus* as distinct species. *Gene* **452**:35-43.
- Collette, B. B., and C. E. Nauen. 1983. FAO Species Catalogue. Vol. 2. Scombrids of the world. An annotated and illustrated catalogue of Tunas, Mackerels, Bonitos and related species known to date. *FAO Fisheries Synopsis* **2**:137.
- Cunha, M. E., C. Vendrell, and P. Gonçalves. 2008. Experimental study of the dependence of embryonic development of *Trachurus trachurus* eggs on temperature. *ICES Journal of Marine Science: Journal du Conseil* **65**:17-24.
- Deuel, D. G., J. R. Clark, and A. J. Mansueti. 1966. Description of embryonic and early larval stages of bluefish, *Pomatomus saltatrix*. *Transactions of the American Fisheries Society* **95**:264-271.
- Dickerson, T. L., B. J. Macewicz, and J. R. Hunter. 1992. Spawning frequency and batch fecundity of chub mackerel, *Scomber japonicus*, during 1985. *California Cooperative Oceanic Fisheries Investigations Report* **33**:130-140.
- Dimmlich, W. F., T. M. Ward, and B. W.G. 2009. Spawning dynamics and biomass estimates of Australian anchovy (*Engraulis australis*) in gulf and shelf waters of South Australia. *Journal of Fish Biology* **75**:1560-1576.
- Efron, B., and R. J. Tibshirani. 1993. *An Introduction to the Bootstrap*. Chapman and Hall, Inc., New York.
- Fahay, M. P., P. L. Berrien, D. L. Johnson, and W. W. Morse. 1999. Essential Fish Habitat Source Document: Bluefish, *Pomatomus saltatrix*, Life History and Habitat Characteristics. NOAA Technical Memorandum NMFS-NE-144. 78 p.
- 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.
- Flood, M., I. Stobutzki, J. Andrews, C. Ashby, G. Begg, R. Fletcher, C. Gardner, L. Georgeson, S. Hansen, K. Hartmann, P. Hone, P. Horvat, L. Maloney, B. McDonald, A. Moore, A. Roelofs, K. Sainsbury, T. Saunders, T. Smith, C. Stewardson, J. Stewart, and B. e. Wise. 2014. Status of key Australian fish stocks reports 2014, Fisheries Research and Development Corporation, Canberra.
- Ganias, K. 2012. Thirty years of using the postovulatory follicles method: Overview, problems and alternatives. *Fisheries Research* **117–118**:63-74.
- 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.
- Georgeson, L., I. Stobutzki, and R. Curtotti. 2014. Fishery status reports 2013–14. Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra.
- Goldsworthy, S. D., B. Page, P. J. Rogers, C. Bulman, A. Wiebkin, L. J. McLeay, L. Einoder, A. M. M. Baylis, M. Braley, R. Caines, K. Daly, C. Huvneers, K. Peters, A. D. Lowther, and T. M. Ward. 2013.

- Trophodynamics of the eastern Great Australia Bight ecosystem: ecological change associated with the growth of Australia's largest fishery. *Ecological Monitoring* **255**.
- Gomon, M., D. Bray, and R. H. Kuitert. 2008. *Fishes of the Australia's South Coast*. Museum of Victoria. New Holland Publishers (Australia).
- Halliday, I. 1990. Tailor tagging project; summary of 1988 and 1989 results. Queensland Department of Primary Industries. 8 p.
- He, P. 1993. Swimming speeds of marine fish in relation to fishing gears. *ICES Marine Science Symposium* **196**:183-189.
- Hijmans, R. J. 2015. geosphere: Spherical Trigonometry. R package version 1.3-13. <http://CRAN.R-project.org/package=geosphere>.
- Hunter, J. R., and C. A. Kimbell. 1980. Early life history of Pacific mackerel, *Scomber japonicus*. *Fishery Bulletin* **78(1)**:89-101.
- 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., N. C. H. Lo, and J. H. L. Roderick. 1985. Batch fecundity in multiple spawning fishes. National Marine Fisheries Services. National Oceanic and Atmospheric Administration Technical Report.
- Hutchins, B., and R. Swainston. 1986. *Sea Fishes of Southern Australia*. Swainston Publishing, Perth.
- Juanes, F., J. Hare, and A. Miskiewicz. 1996. Comparing early life history strategies of *Pomatomus saltatrix*: a global approach. *Marine and Freshwater Research* **47**:365-379.
- Kramer, D. 1960. Development of eggs and larvae of Pacific mackerel and distribution and abundance of larvae 1952-1956. *Fishery Bulletin* **174**:60.
- 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.
- Leigh, G. M., and M. F. O'Neill. 2004. Stock Assessment of the Queensland-New South Wales Tailor Fishery (*Pomatomus saltatrix*). Department of Primary Industries and Fisheries, The State of Queensland.
- Lenanton, R., S. Ayvazian, A. Pearce, R. Steckis, and G. Young. 1996. Tailor (*Pomatomus saltatrix*) off Western Australia: where does it Spawn and how are the Larvae Distributed? *Marine and Freshwater Research* **47**:337-346.
- Litherland, L., J. Andrews, P. Lewis, and J. Stewart. 2014. Tailor *Pomatomus saltatrix*. In M. Flood, I. Stobutzki, J. Andrews, C. Ashby, G. Begg, R. Fletcher, C. Gardner, L. Georgeson, S. Hansen, K. Hartmann, P. Hone, P. Horvat, L. Maloney, B. McDonald, A. Moore, A. Roelofs, K. Sainsbury, T. Saunders, T. Smith, C. Stewardson, J. Stewart, and B. Wise, editors. Status of key Australian fish stocks reports 2014. Fisheries Research and Development Corporation, Canberra.
- Lockwood, S. J., J. H. Nichols, and W. A. Dawson. 1981. The estimate of a mackerel (*Scomber scombrus* L) spawning stock size by plankton survey. *Journal of Plankton Research* **3**:217-233.
- Lowerre-Barbieri, S. K., N. J. Brown-Peterson, H. Murua, J. Tomkiewicz, D. M. Wyanski, and F. Saborido-Rey. 2011. Emerging Issues and Methodological Advances in Fisheries Reproductive Biology. *Marine and Coastal Fisheries* **3**:32-51.
- Marks, R. E., and D. O. Conover. 1993. Ontogenetic shift in the diet of young-of-year bluefish *Pomatomus saltatrix* during the oceanic phase of the early life history. *Fishery Bulletin* **91**:97-106.
- 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.
- Mendiola, D., P. Alvarez, U. Cotano, E. Etxebeste, and A. M. de Murguia. 2006. Effects of temperature on development and mortality of Atlantic mackerel fish eggs. *Fisheries Research* **80**:158-168.
- Miskiewicz, A., B. Bruce, and P. Dixon. 1996. Distribution of Tailor (*Pomatomus saltatrix*) Larvae along the Coast of New South Wales, Australia. *Marine and Freshwater Research* **47**:331-336.
- 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). 31pp.
- Neira, F. J., and J. P. Keane. 2008. Ichthyoplankton-based spawning dynamics of blue mackerel (*Scomber australasicus*) in south-eastern Australia: links to the East Australian Current. *Fisheries Oceanography* **17**:281-298.
- Neira, F. J., J. M. Lyle, and J. P. Keane. 2009. Shelf spawning habitat of *Emmelichthys nitidus* in south-east Australia - Implications and suitability for egg-based biomass estimation. *Estuarine Coastal and Shelf Science* **81**:521-532.
- Neira, F. J., A. Miskiewicz, and T. Trnski. 1998. Larvae of temperate Australian fishes: laboratory guide for larval fish identification. University of Western Australia Press, Nedlands, W.A.

- Norcross, J. J., S. L. Richardson, W. H. Massmann, and E. B. Joseph. 1974. Development of Young Bluefish (*Pomatomus saltatrix*) and Distribution of Eggs and Young in Virginian Coastal Waters. *Transactions of the American Fisheries Society* **103**:477-497.
- Nurthen, R., R. Cameron, and D. Briscoe. 1992. Population genetics of tailor, *Pomatomus saltatrix* (Linnaeus) (Pisces: Pomatomidae) in Australia. *Marine and Freshwater Research* **43**:1481-1486.
- Parker, K. 1980. A direct method for estimating northern anchovy, *Engraulis mordax*, spawning biomass. *Fisheries Bulletin* **84**:541-544.
- 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.
- Pikitch, E., P. D. Boersma, I. L. Boyd, D. O. Conover, P. Cury, T. Essington, S. S. Heppell, E. D. Houde, M. Mangel, D. Pauly, É. Plagányi, K. Sainsbury, and R. S. Steneck. 2012. Little Fish, Big Impact: Managing a Crucial Link in Ocean Food Webs. Lenfest Ocean Program. Washington, DC. 108 pp. <http://www.oceanconservationscience.org/foragefish/>.
- Priede, I. G., T. Raid, and J. J. Watson. 1995. Deep-Water Spawning of Atlantic Mackerel *Scomber Scombrus*, West of Ireland. *Journal of the Marine Biological Association of the United Kingdom* **75**:849-855.
- Queensland Department of Agriculture, F. a. F. 2013. Stock status of Queensland's fisheries resources 2012. Queensland DAFF, Brisbane.
- Queensland Department of Agriculture, F. a. F. 2014. Status Report 2011 - 2014: Queensland East Coast Inshore Fin Fish Fishery. Queensland DAFF, Brisbane.
- Robillard, E., C. S. Reiss, and C. M. Jones. 2008. Reproductive biology of bluefish (*Pomatomus saltatrix*) along the East Coast of the United States. *Fisheries Research* **90**:198-208.
- Rogers, P. J., T. M. Ward, L. J. McLeay, M. Lowry, and D. Williams. 2009. Reproductive biology of blue mackerel, *Scomber australasicus*, off southern and eastern Australia: suitability of the Daily Egg Production Method for stock assessment. *Marine and Freshwater Research* **60**:187-202.
- 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.
- Scoles, D. R., B. B. Collette, and J. E. Graves. 1998. Global phylogeography of mackerels of the genus *Scomber*. *Fishery Bulletin* **96**:823-842.
- Shiraishi, T., S. Ketkar, Y. Katoh, M. Nyuji, A. Yamaguchi, and M. Matsuyama. 2009. Spawning frequency of the Tsushima Current subpopulation of chub mackerel *Scomber japonicus* off Kyushu, Japan. *Fisheries Science* **75**:649-655.
- Smith, A. D. M., C. J. Brown, C. M. Bulman, E. A. Fulton, P. Johnson, I. C. Kaplan, H. Lozano-Montes, S. Mackinson, M. Marzloff, L. J. Shannon, Y.-J. Shin, and J. Tam. 2011. Impacts of fishing low-trophic level species on marine ecosystems. *Science* **333**:1147-1150.
- 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. Hobart.
- Smith, K., P. Lewis, J. Brown, C. Dowling, A. Howard, L. R., and B. Molony. 2013. Status of nearshore finfish stocks in south-western Western Australia Part 2: Tailor. Department of Fisheries, Western Australia.
- Smith, P. E., and S. L. Richardson. 1977. Standard techniques for pelagic fish egg and larva surveys. Food and Agriculture Organisation. Fisheries Technical Paper No.
- Staunton-Smith, J., and T. M. Ward. 2000. Stock assessment of pelagic bait fishes in southern Queensland with special reference to pilchards (*Sardinops sagax*). FRDC Project 95/043, Department of primary Industries, Queensland, Brisbane.
- 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.
- Stewart, J., and D. J. Ferrell. 2001. Age, growth, and commercial landings of yellowtail scad (*Trachurus novaezelandiae*) and blue mackerel (*Scomber australasicus*) off the coast of New South Wales, Australia. *New Zealand Journal of Marine and Freshwater Research* **35**:541-551.
- Stratoudakis, Y., M. Bernal, K. Ganas, and A. Uriarte. 2006. The daily egg production method: recent advances, current applications and future challenges. *Fish and Fisheries* **7**:35-57.
- Takasuka, A., Y. Ozeki, and H. Kubota. 2008. Multi-species regime shifts reflected in spawning temperature optima of small pelagic fish in the western North Pacific. *Marine Ecology Progress Series* **360**:211-217.
- Taylor, S., J. Webley, and K. McInnes. 2012. 2010 Statewide Recreational Fishing Survey. Queensland Department of Agriculture, Fisheries and Forestry, Brisbane.

- Turner, R. 2015. deldir: Delaunay Triangulation and Dirichlet (Voronoi) Tessellation. R package version 0.1-9. <http://CRAN.R-project.org/package=deldir>.
- Ward, T., B. Molony, J. Stewart, J. Andrews, and A. Moore. 2014a. Australian Sardine *Sardinops sagax*, in M. Flood, I. Stobutzki, J. Andrews, C. Ashby, G. Begg, R. Fletcher, C. Gardner, L. Georgeson, S. Hansen, K. Hartmann, P. Hone, P. Horvat, L. Maloney, B. McDonald, A. Moore, A. Roelofs, K. Sainsbury, T. Saunders, T. Smith, C. Stewardson, J. Stewart & B. Wise (eds) 2014, *Status of key Australian fish stocks reports 2014*, Fisheries Research and Development Corporation, Canberra.
- Ward, T. M., P. Burch, and A. R. Ivey. 2012. South Australian Sardine (*Sardinops sagax*) Fishery: Stock Assessment Report 2012. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.
- Ward, T. M., P. Burch, L. J. McLeay, and A. R. Ivey. 2011a. 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. 2015a. 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., 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., A. R. Ivey, and P. Burch. 2011b. Spawning biomass of sardine, *Sardinops sagax*, in waters off South Australia in 2011. Final Report to PIRSA Fisheries. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Aquatic Sciences Publication No. F2007/000566-4, SARDI Research Report Series No. 584.
- Ward, T. M., A. R. Ivey, and J. D. Carroll. 2014b. Spawning biomass of sardine, *Sardinops sagax*, in waters off South Australia in 2014. Report to PIRSA Fisheries and Aquaculture. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2007/000566-6. SARDI Research Report Series No. 807. 33 pp.
- Ward, T. M., A. R. Ivey, and J. Earl. 2014c. Commonwealth Small Pelagic Fishery: Fishery Assessment Report 2013. Report to the Australian Fisheries Management Authority., South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2010/000270-5. SARDI Research Report Series No. 778. 105 pp.
- Ward, T. M., A. R. Ivey, and J. Earl. 2015b. Commonwealth Small Pelagic Fishery: Fishery Assessment Report 2014. Report to the Australian Fisheries Management Authority. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2010/000270-6. SARDI Research Report Series No. 847. 113 pp.
- Ward, T. M., M. Kinloch, J. G.K., and F. J. Neira. 1998. A Collaborative Investigation of the Usage and Stock Assessment of Baitfish in Southern and Eastern Australian Waters, with Special Reference to Pilchards (*Sardinops sagax*). South Australian Research and Development Institute (Aquatic Sciences). Final Report to Fisheries Research and Development Corporation.
- 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., and P. J. Rogers, editors. 2007. Development and evaluation of egg-based stock assessment methods for blue mackerel *Scomber australasicus* in southern Australia. Final report to the Fisheries Research and Development Corporation Project 2002/061. SARDI Aquatic Science, Adelaide.
- 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 austrasicus*. *Journal of Marine and Freshwater Research* **62**:112-128.
- Ward, T. M., D. W. Schmarr, L. J. McLeay, P. J. Rogers, and A. Ivey. 2007. A preliminary investigation of the spawning biomass of sardine (pilchard, *Sardinops sagax*) off eastern Australia. Final report for the New South Wales Department of Primary Industry. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.
- 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.

- Ward, T. M., J. Staunton-Smith, S. Hoyle, and I. A. Halliday. 2003. Spawning patterns of four species of predominantly temperate pelagic fishes in the sub-tropical waters of southern Queensland. *Estuarine, Coastal and Shelf Science* **56**:1125-1140.
- Watanabe, C., and H. Nishida. 2002. Development of assessment techniques for pelagic fish stocks: applications of daily egg production method and pelagic trawl in the Northeastern Pacific Ocean. *Fisheries Science* **68**:97-100.
- Watson, D. F. 1981. Computing the n-dimensional Delaunay tessellation with application to Voronoi polytopes. Pages 167-172 *The Computer Journal*.
- White, K. V., and W. J. Fletcher. 1998. Identifying the developmental stages for eggs of the Australian pilchard, *Sardinops sagax*. Fisheries Western Australia. Fisheries Research Report No.103.
- Wilks, S. J. 1977. Biological and fisheries data on bluefish, *Pomatomus saltatrix* (Linnaeus). National Oceanic and Atmospheric Administration, Sandy Hook Laboratory. Technical Series Report No. 11. 56 p.
- Wood, S. N. 2006. *Generalized Additive Models An Introduction with R*. Chapman and Hall/CRC.
- Yamada, T., I. Aoki, and I. Mitani. 1998. Spawning time, spawning frequency and fecundity of Japanese chub mackerel, *Scomber japonicus* in the waters around the Izu Islands, Japan. *Fisheries Research* **38**:83-89.
- Zeller, B., B. Pollock, and L. Williams. 1996. Aspects of Life History and Management of Tailor (*Pomatomus saltatrix*) in Queensland. *Marine and Freshwater Research* **47**:323-329.



# 12. Appendices

## Appendix 1. Genetic identification of Blue Mackerel (*Scomber australasicus*) and Tailor (*Pomatomus saltatrix*) eggs.

Keane, J.P.

### **A1.1 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.

Blue Mackerel (*Scomber australasicus*) and Tailor (*Pomatomus saltatrix*) occur along the south east Australian coast and spawn in late winter and spring (Ward et al. 2003; Neira and Keane, 2008). Eggs of Blue Mackerel spawned in Australian waters have been previously described (Neira and Keane 2008). Conversely, eggs of Tailor have only been described from international waters, namely the Black Sea (Salekhova, 1959) and western regions of the North and South Atlantic Oceans (Deuel et al., 1966; Connell, 2012). Previous studies have found eggs of Blue Mackerel have very similar morphological features to those of *Lepidotrigla spp.*, particularly at the early stages (Neira and Keane, 2008; Connell, 2012). It is uncertain as to what species may pose similar morphological characters to Tailor in Australian waters due to the limited number of egg studies in this region.

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

In this study, we employed a molecular approach to identify and validate ethanol preserved eggs of Blue Mackerel and Tailor, as well as eggs that possess similar morphological characteristics to these two species. Results of these analyses were used to validate morphological identifications of simultaneously caught eggs preserved in formalin.

## **A1.2 Methods**

### *A1.2.1 Samples*

Replicate plankton hauls (see Section 3.2) were completed at 57 stations between Fraser Island (24°45' S) and Batemans Bay (33°45' S) (Fig. A1). Replicate hauls were conducted at two station (on the shelf break) and every second station (10 nm) shoreward of the shelf break on every second transect (30 nm) (Fig. A1). Ichthyoplankton samples were collected using a bongo sampler equipped with 500 µm mesh, two 3 m long plankton nets enclosed in a purpose built, weighted stainless steel frame to facilitate vertical drops. The mouth of each net (0.6 m diameter) was fitted with a General Oceanics flowmeter to estimate the 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. 0.3 ms<sup>-1</sup>. Captured plankton was immediately drained of excess seawater and preserved in 96% ethanol.

### *A1.2.2 Morphometric identification*

Ethanol preserved eggs were rehydrated in distilled water to better reflect diameters of fresh eggs, and measured to 0.01 mm under a stereomicroscope. Eggs were identified using a combination of morphological characters described for Blue Mackerel and Tailor eggs (Salekhova, 1959; Deuel et al., 1966; Connell, 2012; Neira and Keane, 2008).

The main diagnostic features of Blue Mackerel eggs are: (a) spherical, 1.05 to 1.30 mm in diameter; (b) smooth chorion; (c) small perivitelline space; (d) prominent, unsegmented yolk sac; (e) single oil globule, 0.26 to 0.31 mm in diameter, which becomes pigmented mid-stage in development, and that is posteriorly located in the yolk of late-stage eggs and yolk-sac larvae; and (f) embryo pigment consisting of a paired row of melanophores along the dorsal surface of trunk and tail, and no pigment over the nape region.

Tailor eggs have not been described from Australian waters, however, egg descriptions exist for the species from North America, South Africa and the Black Sea (Salekhova, 1959; Deuel et al., 1960; Connell, 2012). Variations exist in the egg characteristics between each region/description, particularly in relation to egg diameter and the level of yolk segmentation (Table A1). The main diagnostic features of Tailor eggs are: (a) spherical, 0.80 to 1.20 mm in diameter; (b) smooth chorion; (c) narrow perivitelline space; (d) single oil globule, 0.22 to 0.30 mm in diameter, which becomes pigmented mid-stage in development, and that is posteriorly located in the yolk of late-stage eggs and yolk-sac larvae; and (e) dark embryo pigment forming a border around the head and thorax and scattered on the yolk ventrally, adjacent to the embryo.

**Table A1.** Variation in some key characters of Tailor eggs reported in different studies

Egg diameter (mm)	Oil globule Diameter (mm)	Yolk texture	Region	Reference
0.80-1.05	0.27-0.30	n/a	Black Sea	Salekhova (1959)
0.90-1.20	0.22-0.30	Partial segmentation only visible at late morula stage.	Western North Atlantic	Dueul et al. (1960)
0.84-0.96	0.22-0.27	segmented	Western South Atlantic	Connell (2012)

### A1.2.3 Molecular identification

A molecular approach of Mitochondrial DNA (mtDNA) extraction, amplification, and sequencing established by Ward *et al.* (2005) was employed to genetically identify ethanol preserved fish eggs. DNA extractions from eggs were carried out using the QIAamp DNA Micro Kit (QIAGEN, USA), following the manufacturer's protocol for tissue extraction. Amplification by polymerase chain reactions (PCRs) were performed using MyTaq HSTM DNA Polymerase (Bioline) with PCR product purification and bi-directional sequencing performed by MacroGen Inc. (Seoul, Republic of Korea). The primers FishF2 (5'TCGACTAATCATAAAGATATCGGCAC3') and FishR2 (5'ACTTCAGGGTGACCGAAGAATCAGAA3') were used in the PCRs to amplify an approximately 655 bp fragment from the 5' region of the cytochrome oxidase subunit 1 (*cox1*) gene. Sequences were aligned to reference data in the Fish Barcode of Life Database (BOLD) stored within GenBank® via Basic Local Alignment Search Tool (BLAST) searches. The gene (reference species) returning the highest percent similarity for both the forward (FishF2) and reverse (FishR2) sequences are listed. Similarity is the percent sequence identity between the extracted egg mtDNA sequence and the voucher (reference) specimen sequence. Eggs that did not sequence in either the forward or reverse directions are not listed.

## A1.3 Results

### A1.3.1 Blue Mackerel

A total of 89 eggs identified morphologically as Blue Mackerel, as well as 33 eggs possessing similar characteristics to Blue Mackerel, were subjected to mtDNA analysis. Of the suspected Blue Mackerel eggs, a total of 85 (96%) yielded quality mtDNA to enable sequencing (Tables A2, A3). Alignment of the *cox1* sequences revealed a total of 78 Blue Mackerel and 6 Gemfish eggs, while 4 eggs had insufficient DNA to facilitate sequencing. Eggs of Gemfish were later identified in formalin preserved samples and deemed distinguishable from those of Blue Mackerel as being marginally smaller in diameter, having a larger perivitelline space, stouter embryo and a distinctive 'H' shaped pigment pattern on the embryo nape in the latter stages. The eggs of Gemfish resembled those of the closely related Barracouta (*Thyrstites atun*) which were partially described by Robertson (1975). From the 33 suspected non-Blue Mackerel eggs subjected to molecular analyses, 31 yielded quality mtDNA to enable sequencing. All these sequences aligned to species other than Blue Mackerel indicating no misidentification of non- Blue Mackerel eggs (Tables A2, A4).

Eggs of Blue Mackerel were confirmed to be present in samples via mtDNA analyses along 450 nm of coastline between Fraser Island, Queensland (Transect 4; 25°30' S) and Newcastle, southern NSW (Transect 34; 33°0' S) (Fig. A1). Eggs of Gemfish were only detected at the shelf break on two transects near Smoky Cape (Transect 24, 30°30' S; Transect 26, 31°00' S).

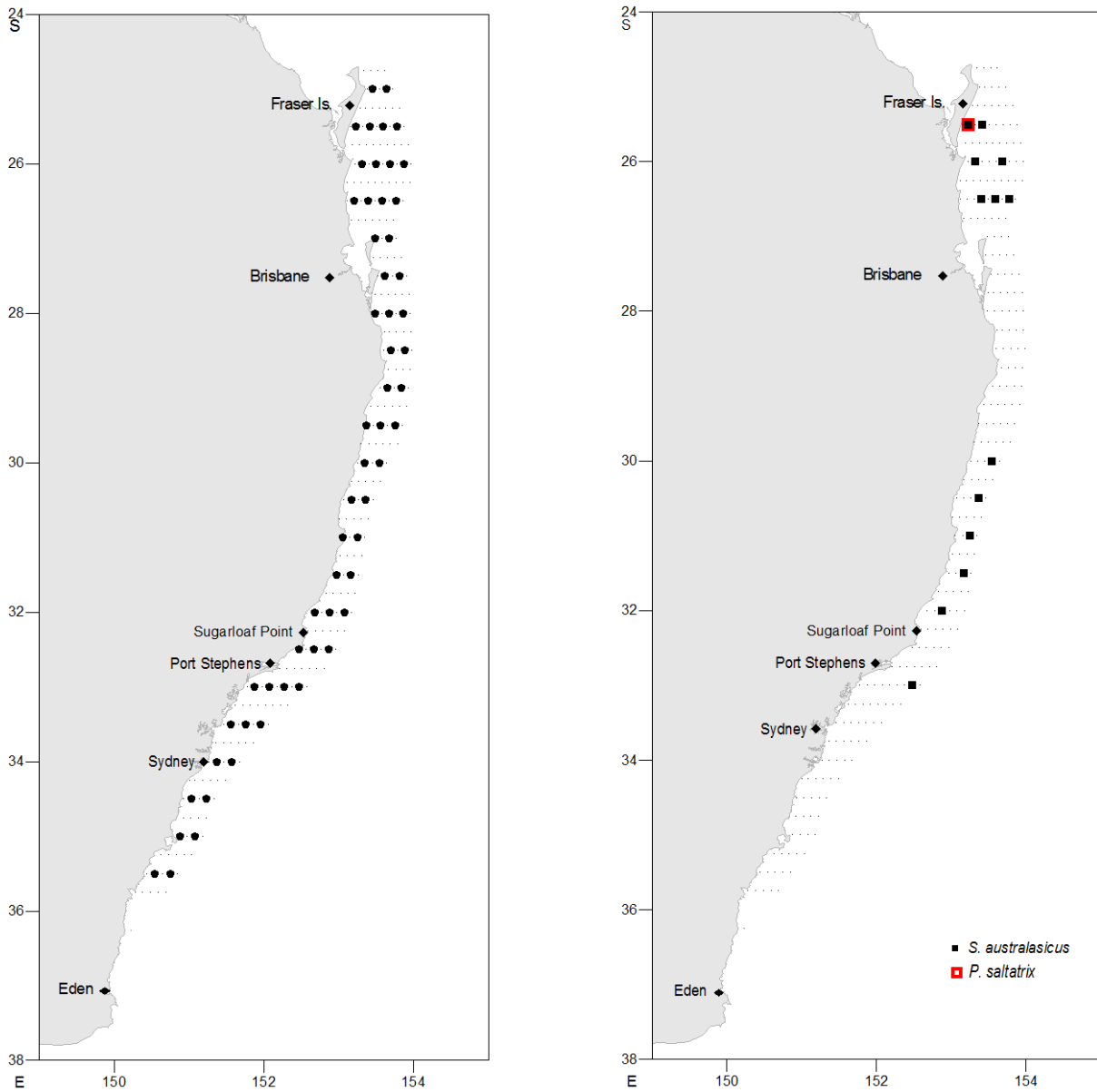
**Table A2.** *Cox1*-based specific identifications of morphologically identified Blue Mackerel and similar eggs collected during the August-September 2014 survey along eastern Australia.

Morphological identification	n tested	Genetic Identification			Notes
		<i>S. australasicus</i>	Other	No DNA	
Blue Mackerel <i>S. australasicus</i>	89	79	6	4	Misidentified eggs aligned with <i>Rexea solandri</i> * (93-99%)
Not Blue Mackerel, but possessing similar characteristics	34	0	32	2	Sequences aligned with <i>Centrolophus niger</i> (n=3; 99%), <i>Lepidotrigla</i> spp. (n=18; 87-94%), <i>Saurida</i> spp. (n=5; 90-95%) Other (n=6 <90%)

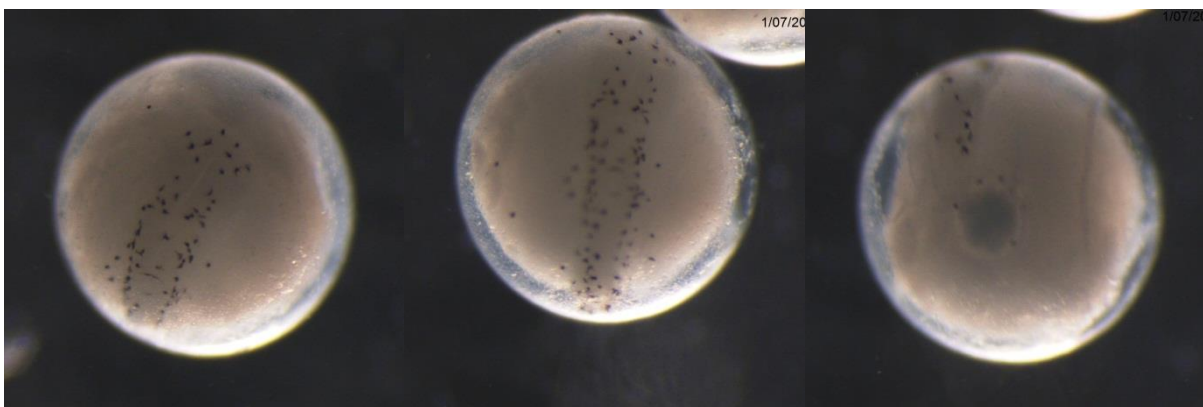
\* *Rexea solandri* was separated morphologically in formalin preserved specimens and resembled that of *Thyrsites atun* (Robertson 1975); see text.

### A1.3.2 Tailor

Examination of ethanol preserved eggs yielded no eggs that could be confidently identified as Tailor based on the morphological descriptions from North America, South Africa and the Black Sea and the expectation that high numbers of Tailor eggs would be present in the samples. Following this a variety of eggs with similar morphological characters to that of Tailor were subjected to mtDNA testing. Of a total of 61 eggs were tested 44 yielded quality mtDNA to enable sequencing (Table A5). One egg gene sequence aligned with that of Tailor, while most of the other 43 aligned with a variety of other perciform species. The one Tailor egg (Figs. A1, A2) captured in this study was 0.90 mm in diameter and resembled the descriptions of Tailor from international waters.



**Fig. A1.** Maps of south eastern Australia showing location of replicate plankton hauls (large dots) for molecular analyses (left) and location of molecular confirmed eggs of Blue Mackerel (*S. australasicus*) and Tailor (*P. saltatrix*) (right).



**Fig. A2.** Photographs of the sole Tailor egg captured of Fraser Island (Qld) and identified from mtDNA analysis. Images (L to R) show head, body and tail (plus oil globule) of embryo.

## **A 1.4 Discussion**

Molecular analyses successfully validated eggs identified as *S. australasicus* and facilitated the separation of morphologically similar pelagic eggs from co-occurring taxa. Morphologically similar eggs to those of *S. australasicus* included *R. solandri*, *C. niger*, *Lepidotrigla spp.* and *Saurida spp.* with the latter two taxa previously shown to co-occur with *S. australasicus* off eastern Australia (Neira and Keane, 2007). Previous experience by the author allowed for the morphological separation of *Lepidotrigla spp.* and *Saurida spp.* eggs from those of *S. australasicus*, while eggs of *C. niger* were separated morphologically by having a marginally larger diameter. These morphological separations were validated by the mtDNA analyses.

A total of six eggs of *R. solandri* that were morphologically misidentified as *S. australasicus* were highlighted by molecular analyses. Post molecular examination of formalin preserved eggs from the same stations showed *R. solandri* eggs differ from *S. australasicus* eggs by having a marginally smaller diameter, larger perivitelline space, stouter embryo and a distinctive 'H' shaped pigment pattern on the embryo nape in the latter stages. These morphological features allowed eggs of *R. solandri* to be distinguishable from those of *S. australasicus* in formalin preserved samples. The eggs of *R. solandri* resembled the partial description of the closely related *Thyrsites atun* in New Zealand waters (Robertson, 1975). The eggs of *R. solandri* occurred at the shelf break in waters near the EAC separation point, a result that supports previous findings that adults migrate to that region to spawn during late winter (Prince and Griffin, 2001).

Misidentifications in ethanol preserved eggs is not uncommon given ethanol causes fish eggs to shrink and become opaque, thus masking key morphological characters (Goodsir *et al.*, 2008). The difficulty in visually identifying or assigning developmental stages to ethanol preserved eggs infers that ethanol should not be used as a primary fixative when morphological identification and staging is required. Rather ethanol fixation should complement formalin fixed samples to facilitate genetic validation of formalin fixed samples, as has been conducted in this study. Formalin fixation results in good preservation of morphological characters, however, formaldehyde interacts with DNA making genetic identification problematic (Steedman, 1976; Karaïskou *et al.*, 2007; Goodsir *et al.*, 2008).

The one Tailor egg that was identified in this study was morphologically similar to *P. saltatrix* eggs described from international waters. This result also indicates that Tailor eggs can be identified molecularly. However, from this one egg it cannot be determined as to if Tailor eggs can be morphologically separated from morphologically similar eggs of co-occurring taxa in Australian waters. The wide variety of taxa identified by the molecular analyses is reflective of the wide scoping approach taken in an attempt to detect Tailor eggs given no confident morphological identifications and the fact that Tailor eggs are yet to be formally described in Australian waters.

## A 1.5 References

- Ahlstrom, E.H. and Moser, H.G. (1980) Characters Useful in Identification of Pelagic Marine Fish Eggs. *CA COOP. Oceanic Fisheries Investment Reports* 21:131pp.
- Connell, D. (2012) Marine fish eggs and larvae from the east coast of South Africa. The South African Institute for Aquatic Biodiversity. <http://fisheggs-and-larvae.saiab.ac.za/> (Accessed 07/09/2015)
- Deuel D., Clark, J.R. and Mansueti, A.J. (1966) Description of Embryonic and Early Larval Stages of Bluefish, *Pomatomus saltatrix*. *Transactions of the American Fisheries Society*, 95(3): 264-271.
- 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.
- Neira, F.J. and Keane, J.P. (2008). Ichthyoplankton-based spawning dynamics of blue mackerel (*Scomber australasicus*) in south-eastern Australia: links to the East Australian Current. *Fisheries Oceanography* 17(4): 281-298.
- Prince, J.D. and Griffin, D.A. (2001) Spawning dynamics of the eastern gemfish (*Rexea solandri*) in relation to regional oceanography in south-eastern Australia. *Marine and Freshwater Research*, 52: 611–622.
- Salekhova, L. P. (1959) On the development of the bluefish (*Pomatomus saltatrix*) Linne. *Trudy Sevastopol's Key Biologicheskoy Stantsii* 11: 182-188.
- Steedman H. F. (1976) Examination, sorting and observation fluids. In: Steedman H. F., editor. *Zooplankton Fixation and Preservation*. a. Paris. 350: Monographs on Oceanographic Methodology, 4. UNESCO. p. 182-183.
- Ward, T.M., Staunton-Smith, J., Hoyle, S. and Halliday, I.A. (2003) Spawning patterns of four species of predominantly temperate pelagic fishes in the sub-tropical waters of southern Queensland. *Estuarine Coastal and Shelf Science*, 56: 1125–1140.
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D.N. (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 360: 1847-1857.

**Table A3. (Next Page)** Results of sequence alignment of extracted mtDNA from pelagic fish eggs morphological identified as Blue Mackerel with reference sequences on GenBank® via BLAST searches. Best aligned match (species; highest % similarity) for Forward and Reverse sequences are listed. Similarity is the percent sequence identity between the extracted egg mtDNA sequence and the voucher (reference) specimen sequence.

Egg ID	Transect-Station	Primer	GenBank sequence alignment (BLAST)	Similarity (%)	Diameter (mm)
BMK14-21	4-6	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.26
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-22	4-6	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.08
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-23	4-6	FishF2	Scomber australasicus voucher BW-A802	99	1.18
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-24	4-6	FishF2	Scomber australasicus voucher BW-A802	99	1.10
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-94	4-4	FishF2	Scomber australasicus voucher BW-A805	98	1.40
		FishR2	Scomber australasicus voucher BW-A805	94	
BMK14-102	4-8	FishF2	Scomber australasicus voucher BW-A802	99	1.24
		FishR2	Scomber australasicus voucher BW-A802	93	
BMK14-201	4-6	FishF2	Scomber australasicus voucher BW-A805	99	1.32
		FishR2	Scomber australasicus voucher BW-A805	87	
BMK14-202	4-6	FishF2	Scomber australasicus voucher BW-A802	99	1.20
		FishR2	Scomber australasicus voucher BW-A802	86	
BMK14-203	4-6	FishF2	Scomber colias mitochondrial DNA, complete genome	98	1.24
		FishR3	Scomber australasicus voucher BW-A802	90	
BMK14-208	4-6	FishF3	Scomber australasicus mitochondrial DNA, complete genome	99	1.08
		FishR4	Scomber australasicus mitochondrial DNA, complete genome	86	
BMK14-62	6-4	FishF4	Scomber australasicus voucher BW-A805	97	1.26
		FishR5	Scomber australasicus voucher BW-A805	99	
BMK14-63	6-4	FishF5	Scomber australasicus voucher BW-A805	98	1.26
		FishR6	Scomber australasicus voucher BW-A805	99	
BMK14-66	6-8	FishF6	Scomber australasicus voucher BW-A802	99	1.28
		FishR7	Scomber australasicus voucher BW-A802	96	
BMK14-67	6-8	FishF7	Scomber australasicus voucher BW-A805	99	1.26
		FishR8	Scomber australasicus voucher BW-A805	99	
BMK14-68	6-8	FishF8	Scomber australasicus voucher BW-A802	99	1.20
		FishR9	Scomber australasicus voucher BW-A802	99	
BMK14-70	6-8	FishF9	Scomber sp. TY mitochondrial COI gene	100	1.08
		FishR10	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-71	6-8	FishF10	Scomber australasicus mitochondrial DNA, complete genome	99	1.16
		FishR11	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-25	8-6	FishF11	Scomber colias mitochondrial DNA, complete genome	99	1.24
		FishR12	Scomber colias mitochondrial DNA, complete genome	99	
BMK14-26	8-6	FishF12	Scomber australasicus voucher BW-A805	100	1.10
		FishR13	Scomber australasicus voucher BW-A805	99	
BMK14-27	8-6	FishF13	Scomber sp. TY mitochondrial COI gene	99	1.14
		FishR14	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-28	8-6	FishF14	Scomber australasicus mitochondrial DNA, complete genome	99	1.14
		FishR15	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-29	8-6	FishF15	Scomber australasicus mitochondrial DNA, complete genome	97	1.06
		FishR16	-		
BMK14-30	8-6	FishF16	Scomber australasicus voucher BW-A802	99	1.16
		FishR17	Scomber australasicus voucher BW-A802	99	
BMK14-221	8-4	FishF17	Scomber australasicus voucher BW-A802	100	1.14
		FishR18	Scomber australasicus voucher BW-A802	99	
BMK14-226	8-2	FishF18	Scomber australasicus voucher BW-A805	99	1.32
		FishR19	Scomber australasicus voucher BW-A805	87	
BMK14-227	8-2	FishF19	Scomber australasicus voucher BW-A805	99	1.24
		FishR20	Scomber australasicus voucher BW-A805	87	
BMK14-228	8-2	FishF20	Scomber australasicus voucher BW-A802	99	1.30
		FishR21	Scomber australasicus voucher BW-A802	87	
BMK14-229	8-2	FishF21	Scomber australasicus mitochondrial DNA, complete genome	99	1.18
		FishR22	Scomber australasicus mitochondrial DNA, complete genome	87	
BMK14-1	22-2	FishF22	Scomber australasicus voucher BW-A805	99	1.24
		FishR23	Scomber australasicus voucher BW-A805	99	
BMK14-2	22-2	FishF23	Scomber japonicus mitochondrial DNA, complete genome	99	1.36
		FishR24	Scomber australasicus voucher BW-A802	100	
BMK14-3	22-2	FishF24	Scomber australasicus mitochondrial DNA, complete genome	99	1.26
		FishR25	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-4	22-2	FishF25	Scomber australasicus voucher BW-A805	100	1.30
		FishR26	Scomber australasicus voucher BW-A805	99	
BMK14-5	22-2	FishF26	Scomber australasicus voucher BW-A802	100	1.18
		FishR27	Scomber australasicus voucher BW-A802	99	
BMK14-6	22-2	FishF27	Scomber japonicus mitochondrial DNA, complete genome	98	1.16
		FishR28	Scomber australasicus voucher BW-A802	100	
BMK14-7	22-2	FishF28	Scomber australasicus voucher BW-A802	99	1.14
		FishR29	Scomber australasicus voucher BW-A802	99	
BMK14-8	22-2	FishF29	Scomber australasicus voucher BW-A802	99	1.18
		FishR30	Scomber australasicus voucher BW-A802	95	
BMK14-9	22-2	FishF30	Scomber australasicus mitochondrial DNA, complete genome	99	1.22
		FishR31	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-10	22-2	FishF31	Scomber australasicus mitochondrial DNA, complete genome	99	1.18
		FishR32	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-49	24-2	FishF32	Scomber australasicus mitochondrial D	99	1.18
		FishR33	Scomber australasicus mitochondrial D	98	
BMK14-51	24-2	FishF33	Scomber australasicus mitochondrial D	91	1.26
		FishR34	Scomber australasicus mitochondrial D	92	
BMK14-54	24-2	FishF34	-		1.48
		FishR35	Scomber sp. TY mitochondrial COI gene	90	
BMK14-55	24-2	FishF35	Scomber australasicus mitochondrial DNA, complete genome	99	1.18
		FishR36	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-57	24-2	FishF36	Scomber australasicus voucher BW-A805	99	1.18
		FishR37	Scomber australasicus voucher BW-A805	99	
BMK14-58	24-2	FishF37	Scomber australasicus voucher BW-A802	99	1.22
		FishR38	Scomber australasicus voucher BW-A802	99	
BMK14-59	24-2	FishF38	Scomber australasicus voucher BW-A802	92	1.16
		FishR39	Scomber australasicus voucher BW-A802	98	



Table A3. Continued.

Egg ID	Transect-Station	Primer	GenBank sequence alignment (BLAST)	Similarity (%)	Diameter (mm)
BMK14-162	24-2	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.40
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-164	24-2	FishF2	Scomber australasicus voucher BW-A805	99	1.26
		FishR2	Scomber australasicus voucher BW-A805	100	
BMK14-165	24-2	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.20
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-166	24-2	FishF2	Scomber australasicus voucher BW-A802	99	1.22
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-167	24-2	FishF2	Scomber australasicus voucher BW-A802	100	1.20
		FishR2	Scomber australasicus voucher BW-A802	95	
BMK14-168	24-2	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.18
		FishR2	-	-	
BMK14-169	24-2	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.14
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-170	24-2	FishF2	Scomber australasicus voucher BW-A802	99	1.10
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-52	24-2	FishF2	Rexea solandri mitochondrion, complete genome	98	1.16
		FishR2	Rexea solandri mitochondrion, complete genome	99	
BMK14-56	24-2	FishF2	Rexea solandri mitochondrion, complete genome	99	1.20
		FishR2	Rexea solandri mitochondrion, complete genome	99	
BMK14-11	26-2	FishF2	Scomber australasicus voucher BW-A805	100	1.22
		FishR2	Scomber australasicus voucher BW-A805	100	
BMK14-14	26-2	FishF2	Scomber australasicus voucher BW-A802	99	1.06
		FishR2	Scomber australasicus voucher BW-A802	100	
BMK14-15	26-2	FishF2	Scomber japonicus mitochondrial DNA, complete genome	99	1.22
		FishR2	Scomber australasicus voucher BW-A802	100	
BMK14-20	26-2	FishF2	-	88	1.00
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	95	
BMK14-180	26-2	FishF2	Scomber australasicus voucher BW-A802	97	1.00
		FishR2	-	81	
BMK14-183	26-4	FishF2	Scomber australasicus voucher BW-A802	99	<i>Damaged</i>
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-16	26-2	FishF2	Rexea solandri mitochondrion, complete genome	99	1.14
		FishR2	Rexea solandri mitochondrion, complete genome	99	
BMK14-12	26-2	FishF2	Rexea solandri mitochondrion, complete genome	93	1.18
		FishR2	-	-	
BMK14-18	26-2	FishF2	Rexea solandri mitochondrion, complete genome	99	1.10
		FishR2	Rexea solandri mitochondrion, complete genome	98	
BMK14-19	26-2	FishF2	Rexea solandri mitochondrion, complete genome	99	1.14
		FishR2	Rexea solandri isolate REX3 COI	98	
BMK14-41	28-2	FishF2	Scomber australasicus voucher BW-A805	100	1.16
		FishR2	Scomber australasicus voucher BW-A805	99	
BMK14-42	28-2	FishF2	Scomber australasicus mitochondrial DNA, complete genome	82	1.36
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-43	28-2	FishF2	Scomber japonicus mitochondrial DNA, complete genome	98	1.32
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-44	28-2	FishF2	Scomber australasicus voucher BW-A805	99	1.24
		FishR2	Scomber australasicus voucher BW-A805	100	
BMK14-45	28-2	FishF2	Scomber australasicus voucher BW-A802	99	1.26
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-46	28-2	FishF2	Scomber australasicus voucher BW-A802	93	1.26
		FishR2	Scomber colias voucher MLFPI124 cytoc	80	
BMK14-185	28-2	FishF2	Scomber australasicus voucher BW-A802	99	1.30
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-186	28-2	FishF2	Scomber australasicus voucher BW-A802	99	1.34
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-31	30-4	FishF2	-	-	1.24
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	96	
BMK14-32	30-4	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.28
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-33	30-4	FishF2	Scomber australasicus voucher BW-A802	99	1.32
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-34	30-4	FishF2	Scomber australasicus voucher BW-A802	99	1.18
		FishR2	Scomber australasicus voucher BW-A802	98	
BMK14-35	30-4	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.28
		FishR2	Scomber australasicus voucher BW-A805	88	
BMK14-36	30-4	FishF2	Scomber australasicus voucher BW-A802	100	1.34
		FishR2	Scomber australasicus voucher BW-A802	99	
BMK14-37	30-4	FishF2	Scomber australasicus mitochondrial DNA, complete genome	99	1.34
		FishR2	Scomber australasicus mitochondrial DNA, complete genome	99	
BMK14-38	30-4	FishF2	Scomber australasicus voucher BW-A805	88	1.34
		FishR2	Scomber australasicus voucher BW-A805	97	
BMK14-39	30-4	FishF2	Scomber australasicus voucher BW-A805	90	1.34
		FishR2	Scomber australasicus voucher BW-A805	82	
BMK14-40	30-4	FishF2	Scomber australasicus voucher BW-A805	99	1.34
		FishR2	Scomber australasicus voucher BW-A805	99	
BMK14-144	34-2	FishF2	Scomber australasicus voucher BW-A805	98	1.24
		FishR2	Scomber australasicus voucher BW-A805	94	
BMK14-145	34-2	FishF2	Scomber australasicus voucher BW-A802	85	1.26
		FishR2	Scomber australasicus voucher BW-A802	97	

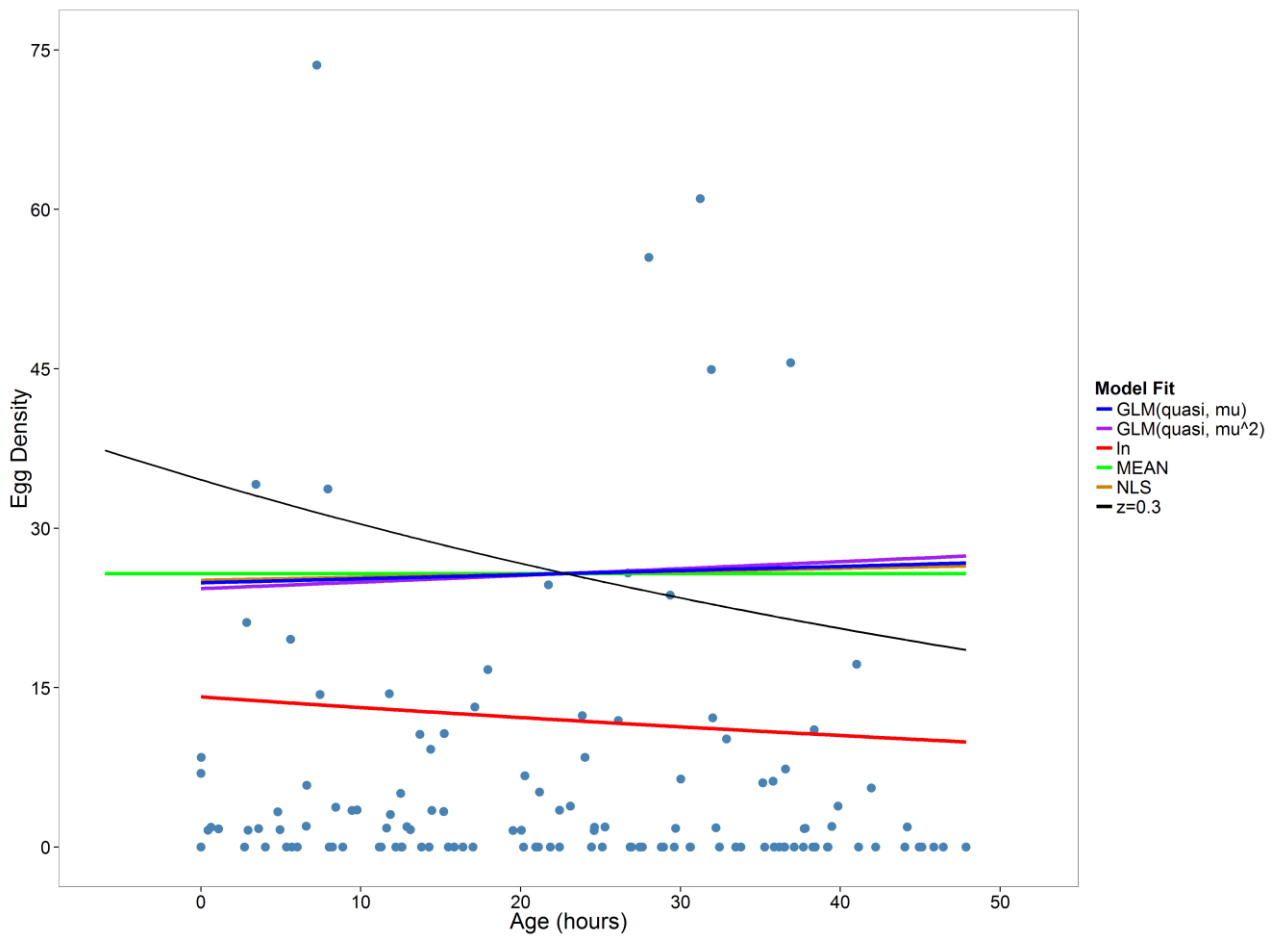
**Table A4.** Results of sequence alignment of extracted mtDNA from pelagic fish eggs possessing morphological characteristics similar to, but not, Blue Mackerel, with reference sequences on GenBank® via BLAST searches. Best aligned match (species; highest % similarity) for Forward and Reverse sequences are listed. Similarity is the percent sequence identity between the extracted egg mtDNA sequence and the voucher (reference) specimen sequence.

Egg ID	Transect-Station	Primer	GenBank sequence alignment (BLAST)	Similarity (%)	Diameter (mm)
BMK14-199	2-4	FishF2	Lepidotrigla sp. FNSIC086-11	93	1.26
		FishR2	Procetichthys krefftii isolate: ZMUB 19724	74	
BMK14-204	4-6	FishF2	Lepidotrigla sp. FNSIC086-11	92	1.18
		FishR2	Lepidotrigla multispinosa voucher Smith 157.5-2 cytochr	80	
BMK14-108	4-8	FishF2	Apistus carinatus voucher HACH5	86	1.12
		FishR2	-		
BMK14-103	4-8	FishF2	Saurida macrolepis COI; partial cds	91	1.12
		FishR2	Saurida macrolepis COI; partial cds	91	
BMK14-104	4-8	FishF2	Saurida macrolepis COI; partial cds	93	1
		FishR2	Saurida macrolepis COI; partial cds	88	
BMK14-105	4-8	FishF2	Saurida macrolepis COI; partial cds	95	1.02
		FishR2	Saurida undosquamis voucher TR1317EK	90	
BMK14-106	4-8	FishF2	Saurida macrolepis COI; partial cds	85	1.08
		FishR2	Saurida undosquamis voucher TR1317EK	90	
BMK14-107	4-8	FishF2	Saurida macrolepis COI; partial cds	93	1.1
		FishR2	Saurida macrolepis COI; partial cds	92	
BMK14-69	6-8	FishF2	-		1.2
		FishR2	Antigonia capros voucher MLFPI106	82	
BMK14-212	8-8	FishF2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	93	1.22
		FishR2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	85	
BMK14-213	8-8	FishF2	Lepidotrigla sp. FNSIC086-11	92	1.1
		FishR2	Lepidotrigla multispinosa voucher Smith 157.5-2	80	
BMK14-214	8-8	FishF2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	93	1.2
		FishR2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	83	
BMK14-215	8-8	FishF2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	93	1.12
		FishR2	Chaenomugil proboscideus mitochondrion, complete genome	79	
BMK14-216	8-8	FishF2	Lepidotrigla sp. FNSIC086-11	91	1.1
		FishR2	Lepidotrigla multispinosa voucher Smith 157.5-2	77	
BMK14-217	8-8	FishF2	Lepidotrigla sp. FNSIC086-11	93	1.08
		FishR2	Lepidotrigla vanessa voucher BIOUG&lt;CAN&gt;;BW-A456	79	
BMK14-218	8-8	FishF2	Lepidotrigla sp. FNSIC086-11	93	1.12
		FishR2	Procetichthys krefftii complete genome	75	
BMK14-126	14-6	FishF2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	93	1.1
		FishR2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	92	
BMK14-127	14-6	FishF2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	93	1.1
		FishR2	Lepidotrigla mulhalli voucher BIOUG&lt;CAN&gt;;BW-A453	94	
BMK14-142	14-4	FishF2	Lepidotrigla sp. FNSIC086-11	88	1.2
		FishR2	Lepidotrigla dieuzeidei voucher FCFOPS-137	92	
BMK14-143	14-4	FishF2	Lepidotrigla sp. FNSIC086-11	89	1.24
		FishR2	Lepidotrigla dieuzeidei voucher FCFOPB076-04	87	
BMK14-125	14-6	FishF2	Hemitripterus villosus voucher IOCAFV_Hv_001	87	1.36
		FishR2	-		
BMK14-134	16-4	FishF2	Lepidotrigla multispinosa voucher Smith 157.5-3	82	0.98
		FishR2	-		
BMK14-77	18-2	FishF2	Lepidotrigla sp. FNSIC086-11	89	1.1
		FishR2	Lepidotrigla dieuzeidei voucher FCFOPS-137	92	
BMK14-50	24-2	FishF2	Lepidotrigla sp. FNSIC086-11	93	1.24
		FishR2	Lepidotrigla dieuzeidei voucher FCFOPB076-04	87	
BMK14-47	28-2	FishF2	Lepidotrigla sp. FNSIC086-11	93	1.18
		FishR2	Lepidotrigla sp. FNSIC086-11	91	
BMK14-189	28-4	FishF2	Lepidotrigla sp. FNSIC086-11	92	1.18
		FishR2	Lepidotrigla sp. FNSIC086-11	88	
BMK14-190	28-4	FishF2	Stereolepis gigas voucher MFC162	86	1.16
		FishR2	Stereolepis gigas voucher MFC162	87	
BMK14-191	28-4	FishF2	Lithognathus aureti voucher ADC09_183.19#7	89	1.12
		FishR2	Stereolepis gigas voucher MFC162	87	
BMK14-48	28-2	FishF2	-		1.36
		FishR2	Diaphus holti voucher CSFOM-035	77	
BMK14-146	34-2	FishF2	Centrolophus niger voucher US&lt;ZAF&gt;;BRBM1	99	1.34
		FishR2	Centrolophus niger mitochondrial COI	99	
BMK14-147	34-2	FishF2	Centrolophus niger voucher US&lt;ZAF&gt;;BRBM1	99	1.4
		FishR2	Centrolophus niger voucher US&lt;ZAF&gt;;BRBM1	99	
BMK14-148	34-2	FishF2	Centrolophus niger voucher US&lt;ZAF&gt;;BRBM1	99	1.44
		FishR2	Centrolophus niger voucher US&lt;ZAF&gt;;BRBM1	99	

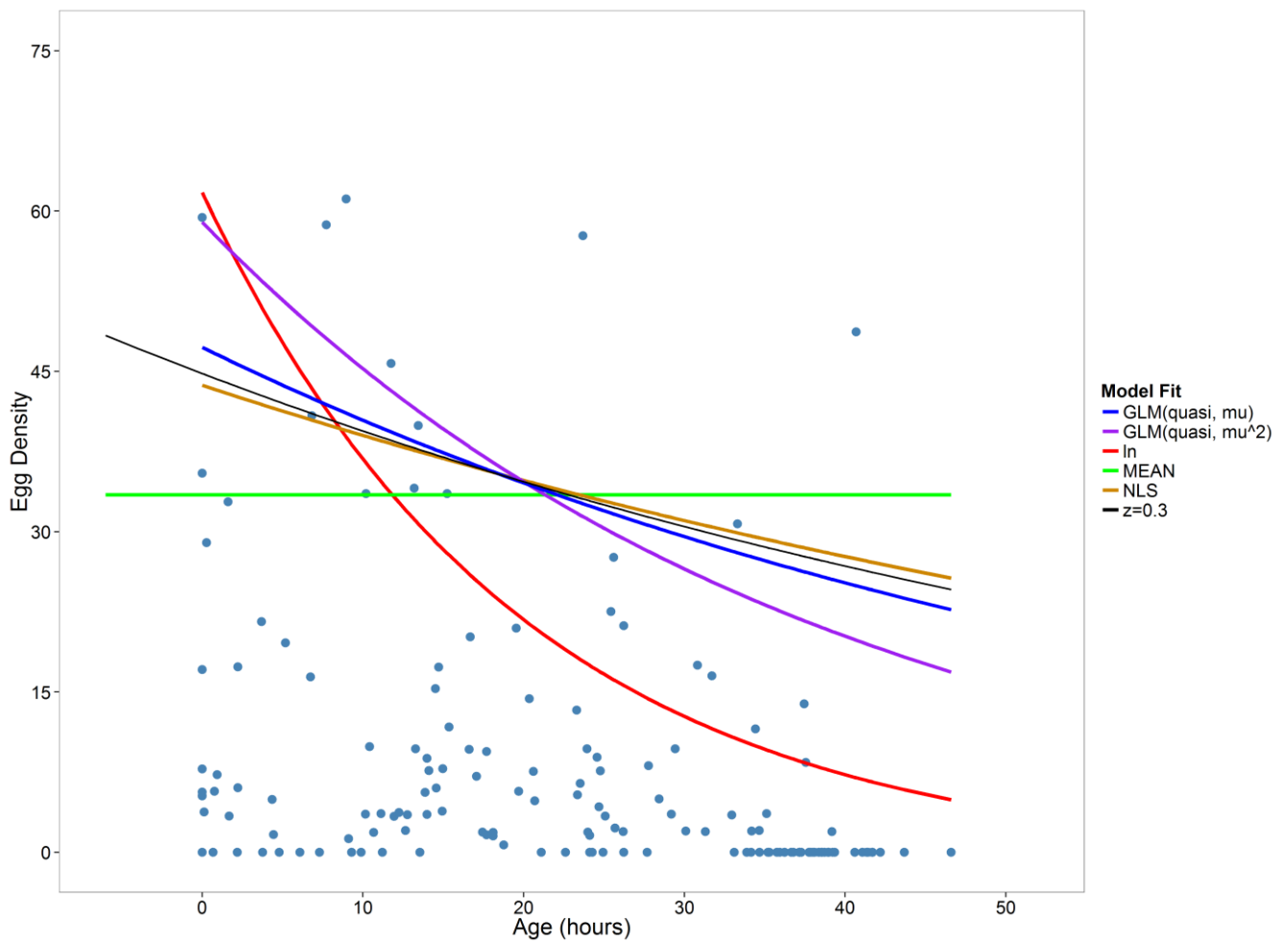
**Table A5. (Next Page)** Results of sequence alignment of extracted mtDNA from pelagic fish eggs possessing morphological characteristics similar to Tailor, with reference sequences on GenBank® via BLAST searches. Best aligned match (species; highest % similarity) for Forward and Reverse sequences are listed. Similarity is the percent sequence identity between the extracted egg mtDNA sequence and the voucher (reference) specimen sequence.

Egg ID	Transect-Station	Primer	GenBank sequence alignment (BLAST)	Similarity (%)	Diameter (mm)
<b>Tailor (<i>Pomatomus saltatrix</i>)</b>					
BMK14-	4-8	FishF2	Pomatomus saltatrix voucher BIOUG&It;CAN&gt;;BW-A1508	99	0.90
		FishR2	Pomatomus saltatrix voucher BIOUG&It;CAN&gt;;BW-A1508	98	
<b>Other species</b>					
BMK14-2	2-4	FishF2	Parupeneus spilurus voucher BIOUG&It;CAN&gt;;BW-A716	99	0.88
		FishR2	Parupeneus spilurus voucher BIOUG&It;CAN&gt;;BW-A716	89	
BMK14-46	4-4	FishF2	Neoscombrops annectens voucher Smith 176.3 #1_05	85	0.84
		FishR2	Neoscombrops annectens voucher Smith 176.3 #1_05	85	
BMK14-47	4-4	FishF2	Neoscombrops annectens voucher Smith 176.3 #1_05	86	0.88
		FishR2	Neoscombrops annectens voucher Smith 176.3 #1_05	85	
BMK14-48	4-4	FishF2	Neoscombrops annectens voucher Smith 176.3 #1_05	86	0.82
		FishR2	Neoscombrops annectens voucher Smith 176.3 #1_05	85	
BMK14-49	4-4	FishF2	Neoscombrops annectens voucher Smith 176.3 #1_05	86	0.86
		FishR2	Neoscombrops annectens voucher ACD07_176.3 #2	86	
BMK14-51	4-4	FishF2	Caprodon unicolor voucher BPBM:FR 352	99	0.88
		FishR2	Caprodon unicolor voucher BPBM:FR 352	99	
BMK14-52	4-4	FishF2	Pseudanthias mica voucher MB073601	87	0.72
		FishR2	Pseudanthias mica voucher MB073601	83	
BMK14-7	4-6	FishF2	Pseudorhombus natalensis voucher ADC 259.19#1	88	1.12
		FishR2	Electrona risso isolate 3794	79	
BMK14-8	4-6	FishF2	Pseudorhombus natalensis voucher ADC 259.19#1	88	1.10
		FishR2	-		
BMK14-64	4-8	FishF2	Centrolophus niger voucher BW-2034	95	0.92
		FishR2	Centrolophus niger voucher US&It;ZAF&gt;;BRBM1	96	
BMK14-66	4-8	FishF2	Ambiserrula jugosa voucher I.44770-002	99	0.86
		FishR2	Ambiserrula jugosa voucher I.44770-002	98	
BMK14-67	4-8	FishF2	Ambiserrula jugosa voucher I.44770-002	99	0.80
		FishR2	Ambiserrula jugosa voucher I.44770-002	100	
BMK14-68	4-8	FishF2	Cynoglossus lighti	81	0.80
		FishR2	Cynoglossus semilaevis voucher MBCSC:Fish:TCL116479	84	
BMK14-69	4-8	FishF2	Cynoglossus semilaevis voucher MBCSC:Fish:TCL116479	84	0.78
		FishR2	Platybelone argala	79	
BMK14-70	4-8	FishF2	Pelates quadrilineatus voucher PGN175	90	0.78
		FishR2	Pelates quadrilineatus voucher PGN175	90	
BMK14-71	4-8	FishF2	Pelates quadrilineatus voucher PGN175	82	0.82
		FishR2	-		
BMK14-11	6-6	FishF2	Pagrus auratus voucher CAUR2	99	1.00
		FishR2	Pagrus auratus voucher CAUR2	88	
BMK14-12	6-8	FishF2	Platycephalus caeruleopunctatus voucher BIOUG&It;CAN&gt;;BW-7	100	0.90
		FishR2	Platycephalus caeruleopunctatus voucher BIOUG&It;CAN&gt;;BW-7	88	
BMK14-13	6-8	FishF2	Sphyraena japonica complete genome	93	1.00
		FishR2	Sphyraena barracuda voucher MX968	78	
BMK14-24	8-4	FishF2	Caprodon longimanus voucher BIOUG&It;CAN&gt;;BW-A606	88	0.98
		FishR2	Acanthistius sp. BOLD:AAF8832 voucher Smith 166.2-3	81	
BMK14-25	8-4	FishF2	Caprodon longimanus voucher BIOUG&It;CAN&gt;;BW-A606	88	0.94
		FishR2	Acanthistius sp. BOLD:AAF8832 voucher Smith 166.2-3	83	
BMK14-26	8-4	FishF2	Neoscombrops annectens voucher Smith 176.3 #1_05	86	0.88
		FishR2	Acanthistius sp. BOLD:AAF8832 voucher Smith 166.2-3	78	
BMK14-27	8-4	FishF2	Neoscombrops annectens voucher Smith 176.3 #1_05	86	0.90
		FishR2	Acanthistius joanae voucher ADC10_166.2 #4	80	
BMK14-4	14-6	FishF2	-	93	1.16
		FishR2	Rhodymenichthys dolichogaster isolate 1458	78	
BMK14-5	14-6	FishF2	Platycephalus caeruleopunctatus voucher BIOUG&It;CAN&gt;;BW-7	99	0.82
		FishR2	Platycephalus caeruleopunctatus voucher BIOUG&It;CAN&gt;;BW-7	100	
BMK14-7	14-6	FishF2	Platycephalus longispinis voucher BIOUG&It;CAN&gt;;BW-A530	99	0.74
		FishR2	Platycephalus longispinis voucher BIOUG&It;CAN&gt;;BW-A530	99	
BMK14-18	18-2	FishF2	Scorpius lineolata voucher BIOUG&It;CAN&gt;;BW-A742	99	0.98
		FishR2	-		
BMK14-19	18-2	FishF2	Sebastes mentella voucher SWFSC12-36	85	1.00
		FishR2	Polyprion oxygeneios voucher BIOUG&It;CAN&gt;;BW-A599	87	
BMK14-20	18-2	FishF2	Callanthias legras voucher ADC09_168.1#6	87	0.86
		FishR2	Callanthias legras voucher ADC09_168.1#6	88	
BMK14-21	18-2	FishF2	Callanthias legras voucher ADC09_168.1#6	87	0.84
		FishR2	Callanthias legras voucher ADC09_168.1#6	86	
BMK14-23	18-2	FishF2	Parapercis sp. 2 CHC-2011 isolate rubr2	88	0.74
		FishR2	Parapercis sp. 2 CHC-2011 isolate rubr2	88	
BMK14-12	18-4	FishF2	Amblyceps arunchalensis voucher NBFGR:AA8101A	76	0.84
		FishR2	Upeneichthys vlamingii voucher BW-A728	89	
BMK14-13	18-4	FishF2	Rhabdosargus sarba voucher BIOUG&It;CAN&gt;;BW-A685	92	0.98
		FishR2	Rhabdosargus sarba voucher BIOUG&It;CAN&gt;;BW-A685	92	
BMK14-14	18-4	FishF2	Sillago flindersi voucher BIOUG&It;CAN&gt;;BW-A1560	100	0.74
		FishR2	Sillago flindersi voucher BIOUG&It;CAN&gt;;BW-A1560	100	
BMK14-16	18-4	FishF2	Platycephalus longispinis voucher BIOUG&It;CAN&gt;;BW-A526	99	0.80
		FishR2	Platycephalus longispinis voucher BIOUG&It;CAN&gt;;BW-A526	100	
BMK14-64	24-2	FishF2	Parapercis allporti voucher CSIRO:H.7482-01	97	0.92
		FishR2	Parapercis allporti voucher AMS:I.40809-002	98	
BMK14-71	24-2	FishF2	Atypichthys strigatus voucher BIOUG&It;CAN&gt;;BW-A746	99	1.02
		FishR2	Atypichthys strigatus voucher BIOUG&It;CAN&gt;;BW-A746	99	
BMK14-27	28-2	FishF2	Atypichthys strigatus voucher BIOUG&It;CAN&gt;;BW-A747	99	1.00
		FishR2	Atypichthys strigatus voucher BIOUG&It;CAN&gt;;BW-A746	99	
BMK14-25	34-2	FishF2	Macroramphosus scolopax voucher FCFOP70-15	97	0.92
		FishR2	Macroramphosus scolopax voucher FCFOP70-15	98	
BMK14-26	34-2	FishF2	Macroramphosus scolopax voucher CSFOM-376	97	0.92
		FishR2	Macroramphosus scolopax voucher FCFOP70-15	97	
BMK14-27	34-2	FishF2	Macroramphosus scolopax voucher CSFOM-376	99	0.94
		FishR2	Macroramphosus scolopax voucher FCFOP70-15	98	
BMK14-28	34-2	FishF2	Plectranthias japonicus isolate FNSIC185-11	85	0.92
		FishR2	Emmelichthyidae sp. BOLD:AAG1166 voucher MFL1496	86	
BMK14-29	34-2	FishF2	Vinciguerria attenuata voucher CSFOM-109	89	0.84
		FishR2	Vinciguerria attenuata voucher CSFOM-109	92	

Appendix 2a: Egg density (eggs·m<sup>-2</sup>) versus egg age (hours) for Blue Mackerel. Lines represent methods used to estimate mean daily egg production ( $P_0$ ) of Blue Mackerel on the east coast of Australia. Shown are the fit of two GLMs applied to the data, a log-linear fit (ln), an exponential fit (NLS), mean egg density (mean), and predicted egg density with the assumed egg mortality rate ( $z$ ) of 0.3. Egg densities >75 eggs·m<sup>-2</sup> are not shown (equates to 7 points; range: 132 to 1363 eggs·day<sup>-1</sup>·m<sup>-2</sup>).



Appendix 2b: Egg density (eggs·m<sup>-2</sup>) versus egg age (hours) for Australian Sardine. Lines represent methods used to estimate mean daily egg production ( $P_0$ ) of Australian Sardine on the east coast of Australia. Shown are the fit of two GLMs applied to the data, a log-linear fit (ln), an exponential fit (NLS), mean egg density (mean), and predicted egg density with the assumed egg mortality rate ( $z$ ) = 0.3. Egg densities >75 eggs·m<sup>-2</sup> are not shown (equates to 15 points; range: 92 to 896 eggs·m<sup>-2</sup>).



## Appendix 3: Project Staff

### *Authors*

Associate Professor Tim Ward (SARDI)

Dr Gretchen Grammer (SARDI)

Mr Alex Ivey (SARDI)

Dr Jonathan Carroll (SARDI)

Dr John Keane (IMAS, University of Tasmania)

Dr John Stewart (NSW DPI)

Dr Lenore Litherland (Fisheries Queensland, DAF)

### *Other Technical staff*

Mr Lorenzo Andreacchio (SARDI)

Dr Owen Burnell (SARDI)

Mr Joshua Fredberg (SARDI)

Mr Graham Hooper (SARDI)

Mr Matt Lloyd (SARDI)

Mr Leonardo Mantilla (SARDI)

Mr Nathavong Navong (SARDI)

### *Owners of FV Dell Richie II and FV Hazel-K*

Mr Stuart Richie and Mr Russel Kerr

### *Skippers and crews*

Mr John Richey *FV Dell Richey II*

Mr Ian Rule

Mr Jerrod McDermott

Mr William Rouwhoust

Mr Dion Lodum

Mr Michael Burns *FV Hazel-K*

Mr Jake Latham

Mr Sean Maberly *RV Tom Marshall*

Mr Mark McLennan