Summer spawning patterns and preliminary Daily Egg Production Method survey of Jack Mackerel and Australian Sardine off the East Coast

Tim M. Ward, Owen Burnell, Alex Ivey, Jonathan Carroll, John Keane, Jeremy Lyle, Stuart Sexton

March 2015

FRDC Project No **2013/053**

ISBN 978-1-921563-73-7

Summer spawning patterns and preliminary Daily Egg Production Method survey of Jack Mackerel and Australian Sardine off the East Coast

2013/053

2015

This publication (and any information sourced from it) should be attributed to:

Ward, T.M., Burnell, O., Ivey, A. Carroll, J. Keane, J., Lyle, J., Sexton, S. South Australian Research and Development Institute (Aquatic Sciences) 2015, *Summer spawning patterns and preliminary Daily Egg Production Method survey of Jack Mackerel and Sardine off the East Coast*, March.

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
Web: www.frdc.com.au

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

Contents

Tables

Figures

Acknowledgments

The Fisheries Research and Development Corporation (FRDC) provided funding (Project No: 2013/053). Mr Crispian Ashby's assistance with organising the surveys is gratefully acknowledged. We thank the masters, crews, owners and staff of the *Dell Richey II* and *FV Western Alliance* for their professional and cheerful assistance. The efforts of Mr Stuart Richey and Mr David Guillot in ensuring the success of the surveys are recognised with gratitude. Mr Matt Lloyd, Mr Graham Hooper and Mr Lorenzo Andreacchio (SARDI) helped collect plankton samples from the *Dell Richey II*. Ms Kylie Tonon from AFMA assisted with collection of adult samples from the *Western Alliance*. Mr Lorenzo Andreacchio, Mr Josh Fredberg and Mr Damian Matthews (SARDI) helped to sort the plankton samples and identify eggs and larvae. Dr Chris Leigh (University of Adelaide) did the histology. Dr Richard McGarvey (SARDI) provided statistical advice on model selection and calculation of confidence intervals. Dr Adrian Linnane 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 Professor Gavin Begg (SARDI Aquatic Sciences).

Abbreviations

AFMA	Australian Fisheries Management Authority
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
NSW	New South Wales
NSW DPI	New South Wales Department of Primary Industries
PCR	Polymerase Chain Reaction
POF	Post-Ovulatory Follicles
RAG	Resource Assessment Group
RBC	Recommended Biological Catches
SARDI	South Australian Research and Development Institute
SNP	Single Nucleotide Polymorphisms
SPF	Small Pelagic Fishery
SPF RAG	Small Pelagic Fishery Resource Assessment Group
SST	Sea Surface Temperature
TAC	Total Allowable Catch
Tas DPI	Tasmanian Department of Primary Industries
Vic DPI	Victorian Department of Primary Industries

Executive Summary

Overview

This study was undertaken collaboratively by fisheries scientists from the South Australian Research and Development Institute (SARDI) and the University of Tasmania. It was the first dedicated application of the Daily Egg Production Method (DEPM) to Jack Mackerel, *Trachurus declivis*. It successfully collected large numbers of samples of eggs and adults concurrently from the key spawning area off eastern Australia during what has been previously identified as the main spawning period. The study established an effective method for sampling adult Jack Mackerel and provides the first estimates for this species of the adult reproductive parameters required for application of the DEPM. The spawning biomass of Jack Mackerel off eastern Australia during January 2014 was estimated to be approximately 157,805 t (95% CI = 59,570 – 358,731). Most of the estimates of spawning biomass obtained in sensitivity analyses were between approximately 95,000 t and 215,000 t. Plausible values for only two parameters provide estimates of spawning biomass that were outside that range; both of these parameters were estimated with a high degree of confidence in the present study.

This was also the first study to investigate the spawning habitat of Australian Sardine *Sardinops sagax* off eastern Australia during summer. It showed that during January 2014 spawning occurred between northern Tasmania and southern Victoria. The spawning biomass at this location during this period was approximately 10,962 t. This estimate should be treated with caution as adult samples were not collected during the study. It also is important to note that this not an estimate of the total adult biomass of Australian Sardine off eastern Australia. It is only an estimate of the portion of the population that was spawning in this southern part of the range during that period. The main spawning area of Australian Sardine off eastern Australia occurs off southern Queensland and northern NSW during late winter and early spring.

Background

The Small Pelagic Fishery (SPF) was established in 1992 and is managed by the Australian Fisheries Management Authority (AFMA). It extends from the Queensland/New South Wales border, typically outside three nautical miles from the coastline, around southern Australia, including Tasmania, and to Lancelin, north of Perth, Western Australia. The fishery is divided into two zones (East and West) by a line through Bass Strait. The target species are Jack Mackerel *Trachurus declivis*, Redbait *Emmelichthys nitidus*, Blue Mackerel *Scomber australasicus*, and Australian Sardine *Sardinops sagax*.

Jack Mackerel and Australian Sardine are the only two SPF species that have not been subject to dedicated DEPM surveys off eastern Australia. This project was conducted to acquire the knowledge needed to support ongoing ecologically sustainable management of these species. Knowledge of the summer spawning patterns of Jack Mackerel and Australian Sardine is needed to underpin future assessment of these stocks. The DEPM was used to estimate the population size of Jack Mackerel and Australian Sardine off eastern Australia because this is the preferred stock assessment technique specified in the harvest strategy for the SPF and considered to be the most appropriate for this species.

Objectives of the study

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

Methods

Surveys to estimate DEPM parameters were conducted concurrently from two vessels during January 2014. Plankton samples were collected from the *FV Dell Richie II* at 292 stations along 49 transects perpendicular to the coast between South East Cape, Tasmania and Port Stephens, New South Wales (NSW). Fish trawls for adult Jack Mackerel were undertaken from the *FV Western Alliance* at 20 locations between St Helens, Tasmania and Eden, NSW, which included the main spawning area. Standard laboratory procedures were used to identify and stage eggs of Jack Mackerel and Australian Sardine. Egg identifications of Jack Mackerel were confirmed using molecular techniques. Four models were used to estimate egg production (P_0). The value of egg production (P_0) used to estimate spawning biomass was the mean of the estimates obtained from non-linear least squares regression and two GLMs. Spawning area was estimated using the Voronoi nearest neighbour method. Standard statistical approaches were used to estimate adult parameters. Adult samples of Australian Sardine were not collected during the study; adult parameters were obtained from South Australian surveys conducted between 1998 and 2014. Sensitivity analyses were undertaken to determine the influence of uncertainty in individual parameters on estimates of spawning biomass.

Results, implications and recommendations

A total of 3,530 live Jack Mackerel eggs were collected from 117 of the 292 (40.1%) stations sampled. Jack Mackerel eggs were collected from waters between Eden and Triabunna where sea surface temperatures ranged between 15 and 22°C. The highest densities of Jack Mackerel eggs were recorded in waters off north-eastern Tasmania and in Bass Strait. The estimated spawning area was 23,960 km^2, comprising 37.8% of the total area sampled (63,355 km^2). Mean daily egg production (P_0) was 28.9 eggs.day^{-1}.m^{-2} (95% CI = 15.9 – 48.7). Mean sex ratio (R) was 0.47 (0.44 – 0.51). Mean female weight (W) was 208.8 g (187.2 – 230.7 g). Mean batch fecundity (F) was 34,068 eggs (21,584 – 82,010). Mean spawning fraction (S) was 0.056 (0.035 – 0.080). Five of the six DEPM parameters were estimated from a large number of samples and are considered robust. The only adult parameter that was not estimated from a large number of samples was batch fecundity. Sensitivity analyses showed that variation in this parameter has limited effects on the estimates of spawning biomass. The estimate of the spawning biomass of Jack Mackerel, i.e. 157,805 t (95% CI = 59,570 – 358,731), is considered robust and suitable for setting recommended biological catches as outlined under the harvest strategy for the SPF. Most of the estimates of spawning biomass obtained in sensitivity analyses were between approximately 95,000 t and 215,000 t. Plausible values for only two parameters provide estimates of spawning biomass that were outside that range; both of these parameters were estimated with a high degree of confidence in the present study. The estimate in the represent study is within the range of estimates of spawning biomass for Jack Mackerel off eastern Australia provided by Neira

(2011) of 114,900 to 169,000 t and within the range of plausible estimates for the ecosystem suggested by Fulton (2013) of 130,000 to 170,000 t.

This study showed that Australian Sardine spawned off northern Tasmania and in Bass Strait during January 2014. As 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 10,962 t should be treated with caution. It is also only an estimate of the portion of the population that was spawning off northern Tasmania and in Bass Strait during summer. Results of this survey support the hypothesis that the main spawning area occurs off southern Queensland and northern NSW during late winter and early spring, where the spawning biomass in July 2004 was ~25,000–35,000 t. A survey conducted off southern Queensland and northern NSW during August-September 2014 will provide a useful estimate of the spawning biomass of Australian Sardine off eastern Australia (FRDC Project 2014/033, *Egg distribution, reproductive parameters and spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the East Coast during late winter and early spring*). The present study provides insights into the catch levels that may be suitable for any developmental fishery that may be established in the region (i.e. off Tasmania and in Bass Strait). Egg samples collected in the present and related studies (e.g. FRDC Project 2014/033) could potentially be used to support a cost-effective study of the stock structure of Australian Sardine off eastern Australia.

This study made some crucial technical developments (e.g. established an adult sampling method for Jack Mackerel) and filled several key knowledge gaps (e.g. provided the first estimates of adult reproductive parameters for Jack Mackerel). However, a follow up study is required to fill both remaining gaps (e.g. the size-fecundity relationship for Jack Mackerel) and those identified during the course of the project (e.g. spatial, temporal and size-related variations in spawning fraction of Jack Mackerel) and to further improve the accuracy and precision of future estimates of spawning biomass of Jack Mackerel off eastern Australia.

Keywords: Jack Mackerel, *Trachurus declivis*, Australian Sardine, *Sardinops sagax,* Daily Egg Production Method, Spawning Biomass, Small Pelagic Fishery, eastern Australia, Tasmania, Bass Strait

1. Introduction

1.1 Small Pelagic Fishery

The Small Pelagic Fishery (SPF) was established in 1992 and is managed by the Australian Fisheries Management Authority (AFMA). It extends from the Queensland/New South Wales (NSW) border, typically outside 3 nautical miles from the coastline, around southern Australia, including Tasmania, to a line at latitude 31°S (near Lancelin, north of Perth, Western Australia). The fishery is divided into two zones (East and West) by a line through Bass Strait extending through southern Tasmania. The target species are Jack Mackerel *Trachurus declivis*, Redbait *Emmelichthys nitidus*, Blue Mackerel *Scomber australasicus*, and Australian Sardine *Sardinops sagax* (Ward *et al.* 2014a).

A harvest strategy for the SPF was established in 2009 (AFMA 2009) and last updated in April 2013. The harvest strategy is used to develop advice on the recommended biological catches (RBCs) for each quota (target) species. A three tiered approach is used to determine the RBCs for each quota species. Stocks are allocated to a tier based upon the level of knowledge about stock size, with Tier 1 representing the highest level of available information and Tier 3 the lowest (Moore and Skirtun 2012). Corresponding individual transferable quotas (ITQs) are established; Tier 1 stocks have the largest quota (by weight), and Tier 3 the smallest (Tracey *et al.* 2013). The tiered system was introduced to ensure that significant exploitation only occurs in stocks where there is a high level of confidence that such exploitation can be sustained (Moore and Skirtun 2012). The SPF Harvest strategy specifies that estimates of spawning biomass are to be obtained using the Daily Egg Production Method (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. Total allowable catches 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 Daily Egg Production Method (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; Neira and Lyle 2008; Dimmlich *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 calculated by dividing the mean number of pelagic eggs produced per day throughout the spawning area (i.e. total daily egg production) by the mean number of eggs produced per unit mass of adult fish (i.e. mean daily fecundity; 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 the product of mean sex ratio (by weight, R), mean batch fecundity (number of oocytes in a batch, F), mean spawning fraction (proportion of mature females spawning each day/night, S) and mean female weight (W).

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

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

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 vessel, 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).

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, b; Gaughan *et al.* 2004). For example, P_0 has been determined using a variety of statistical approaches. Ward *et al.* (2011) showed that these approaches provide different estimates of P_0 and suggested that the log-linear model of Piquelle and Stauffer (1985) should be used for Australian Sardine because it fits strongly over-dispersed egg density data better and provides more logically consistent and precautionary estimates of P_0 than the exponential mortality model and most generalised linear models. 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.

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 to 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 Commonwealth Small Pelagic Fishery (SPF), including Australian Sardines in the East Zone (Ward and Rogers 2007), Blue Mackerel, *Scomber australasicus*, in the East and West Zones (Ward and Rogers 2007; Ward et al. 2009a), Redbait, *Emmelichthys nitidus*, in the East Zone (Neira *et al.* 2008) and Yellowtail Scad, *T. novaezelandiae*, in the East Zone (Neira 2009). A preliminary investigation of the application of the DEPM to Jack Mackerel in the East Zone, based on surveys designed for other species, was conducted by Neira (2011).

1.3 Jack Mackerel

Jack Mackerel (*Trachurus declivis*, Jenyns, 1841) is a member of the family Carangidae. It is found over the continental shelf and outer shelf slope across southern Australia from Wide Bay, Queensland to Shark Bay, Western Australia (Gomon *et al*. 2008). It occurs in depths up to 500 m, but is most common over the continental shelf (Pullen 1994). Two closely related species also occur in the waters off south-eastern Australia; the common Yellowtail Scad, *T. novaezelandiae,* and the rarer Peruvian Jack Mackerel, *T. murphyi*. While Jack Mackerel and Yellowtail Scad share a similar geographical range, the former are more abundant off Tasmania and Victoria and in the Great Australian Bight, whereas the latter are most abundant off NSW (Kailola 1993). It has been suggested that resident populations of Jack Mackerel occur in Tasmanian waters (Wolfe 1971; Jordan 1995), while other fish move seasonally along the east coast of Australia, and show a preference for water temperatures near the 17°C isotherm (Maxwell 1979).

Modern fishing for small to medium-sized pelagic fishes began in Australia in 1950 when approximately 4 t of Jack Mackerel was taken during purse-seine trials off Hobart, Tasmania (Moore and Skirtun 2012). A commercial fishery for Jack Mackerel was established soon thereafter and catches increased rapidly during the 1960s. A fish-meal plant operated at Triabunna during 1973/74 to process the catch (Kailola *et al.* 1993; Pullen 1994). In 1985, fish-meal production recommenced at Triabunna. The annual catch reached 36,804 t in 1986/87 and 33,194 t in 1987/88, making it the largest fishery in Australia, but fell to 7,573 t in 1988/89 (Ward *et al.* 2014a). Catches increased to 15,910 t in 1990/91 and have not exceeded 10,000 t in any year since then and since 2000/01 have not exceeded 3,340 t (Ward *et al.* 2014a).

In 2001/02, a 6-month trial of mid-water pair-trawling was undertaken to target subsurface schools of Jack Mackerel. Nearly 90% of the catch was Redbait. A multi-purpose 50 m mid-water trawler commenced fishing in late 2002 and Redbait dominated the catches. Purse-seine catches of Jack Mackerel in Tasmanian State waters have not yielded more than 1,000 t since 2007/08 (Ward *et al.* 2014a).

Jack Mackerel is a serial spawner that reproduces during late spring to mid-summer off eastern Australia (Webb 1976; Maxwell 1979; Marshall *et al.* 1993; Jordan *et al.* 1995). Estimates of mean length at maturity off south-eastern Australia are 31.5 cm fork length (FL) by Marshall *et al.* (1993) and ~27 cm by Webb (1976). Spawning off the east coast appears to occur progressively southwards, starting in northern NSW in spring and continuing down to Tasmania throughout summer (Maxwell, 1979; Neira, 2011). The main spawning region off south-eastern Australian is located within Bass Strait, and waters surrounding Tasmania (Bulman *et al.* 2012), where spawning peaks during mid to late summer (Kailola *et al*. 1993). Jordan *et al.* (1995) recorded the highest egg densities off eastern Tasmania during early to late summer. Both Neira (2011) and Jordan *et al*. (1995) suggested that *T. declivis* favours deeper waters for spawning. Pullen (1994), Kailola *et al.* (1993) and others have noted that smaller fish occur near the surface in inshore waters and larger specimens occur further offshore.

Preliminary estimates of spawning biomass for Jack Mackerel off NSW were provided by Neira (2011) of ~115,000-169,000 t. Egg samples were collected from surveys timed and located to target peak spawning seasons and areas of another SPF species (Blue Mackerel). Adult samples were not collected during the surveys and reproductive parameters were sourced from small samples from commercial vessels or published accounts of other species in the genus (i.e. *Trachurus trachurus*, *T. murphyi* and *T. symmetricus*).

1.4 Australian Sardine

The Australian Sardine is found throughout southern Australia between Rockhampton (Queensland) and Shark Bay (Western Australia), including northern Tasmania (Gomon *et al.* 1994). Catches off the east coast peaked at 4,768 t in 2008/09 before declining to approximately 1,100 t in 2012/13 (Ward *et al.* 2014a). 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 11 vessels in 2012/13 (Ward *et al.* 2014a).

The gonadosomatic index (GSI) of Australian Sardine peaks from late spring to early summer off Victoria (Hoedt and Dimlitch 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 had been widely applied to clupeoids both within Australia and overseas. A preliminary DEPM spawning biomass estimate for Australian Sardine in the East Zone was undertaken in 2007 using ichthyoplankton samples collected in July 2004 (Rogers and Ward 2007). Egg data were obtained from an ichthyoplankton survey conducted between Bundaberg and Newcastle during July 2004 from a study that targeted Blue Mackerel, *S. australasicus*. Existing data and published parameter estimates were combined to provide best, minimum and maximum estimates of spawning biomass using the DEPM. The best estimate of spawning biomass of Australian Sardine off eastern Australia during July 2004 was ~29,000 t, with likely estimates ranging from ~25,000–35,000 t (Rogers and Ward 2007).

1.5 Need

This project was developed to support the ecologically sustainable management of the Small Pelagic Fishery (SPF) and address community concerns regarding ecological and social impacts of large scale harvesting of small pelagic fishes. 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 Jack Mackerel and Australian Sardine off the east coast as the highest priority for the fishery. Knowledge of the summer spawning patterns of Jack Mackerel and Australian Sardine is needed to underpin future assessment of these stocks and to underpin the ecologically sustainable development of pelagic fish resources off the east coast of Australia.

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

2. Objectives

The objectives of the project were to:

1. Establish methods for estimating adult reproductive parameters of Jack Mackerel and Australian Sardine off the east coast;
2. Determine distribution and abundance of eggs of Jack Mackerel and Australian Sardine off the east coast during summer;
3. Produce preliminary estimates of the spawning biomass of Jack Mackerel and Australian Sardine off the east coast during summer.

3. Methods

3.1 Study Area and Environmental Variables

3.1.1 Study area

Surveys were conducted concurrently aboard the *FV Dell Richie II* and *FV Western Alliance* during January 2014. Plankton samples were collected from the *FV Dell Richie II* at 292 stations along 49 transects running perpendicular to the coastline between South East Cape, Tasmania and Port Stephens, NSW (Figure 1). Fish trawls for adult samples were undertaken from the *FV Western Alliance* at 20 locations between St Helens, Tasmania and Eden, NSW (Figure 1).

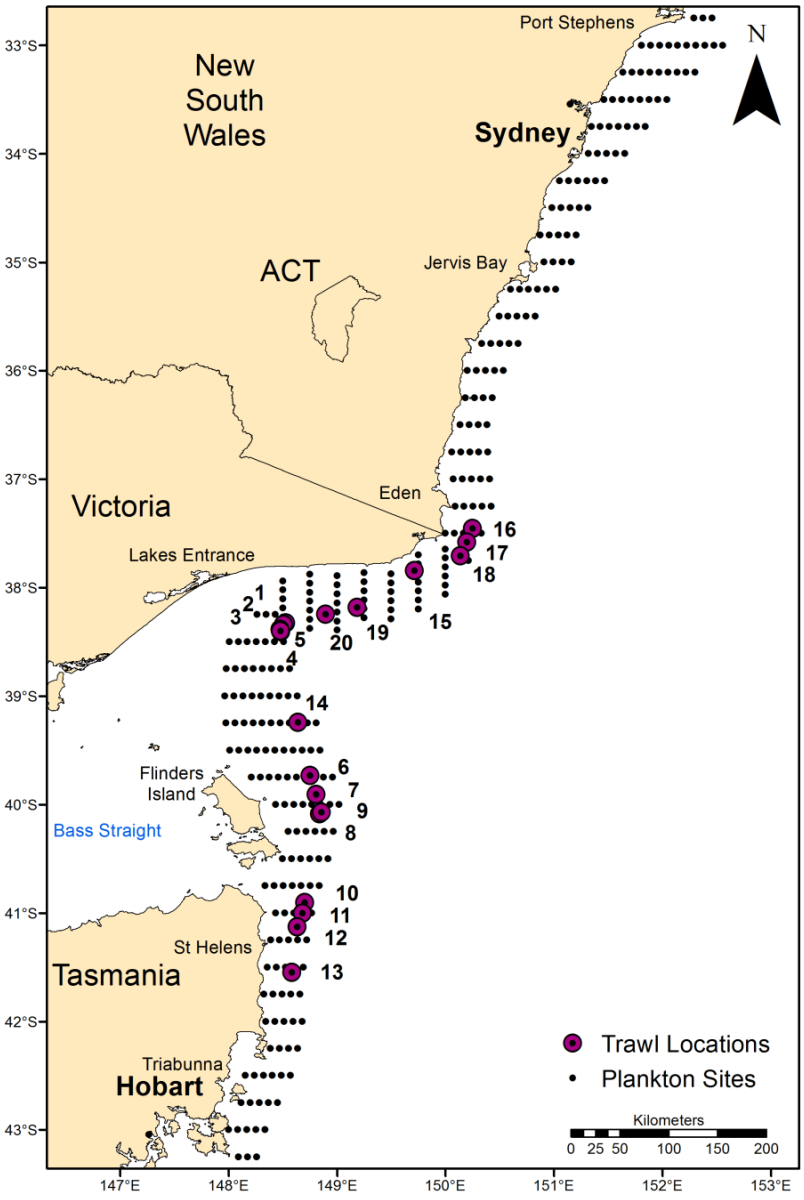


Figure 1. Egg and adult survey locations.

3.1.2 Water temperature

At each plankton sampling station (Figure 1), a *Sea-Bird* Conductivity-Temperature-Depth (CTD) recorder was lowered to a depth of 10 m from the seabed up to a maximum cast length of 200 m. Estimates of water temperature at a depth of 3 m were extracted from each profile. Spatial plots of Sea Surface Temperature (SST) were prepared using ArcGIS® (Version 10.1).

3.2 Daily Egg Production and Spawning Area

3.2.1 Plankton sampling

Plankton samples were collected from 8-22 January 2014 (Figure 1) in vertical tows of paired bongo plankton nets. Each bongo net had an internal diameter of 0.6 m, 500 µm mesh and plastic cod-ends. During each tow the bongo nets were lowered to a depth of 10 m from the seabed up to a maximum cast length of 200 m and retrieved vertically at a speed of ~1 $m.s^{-1}$. General Oceanics™ 2030 flow-meters 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-meters, the relationship between wire length released and flow-meter units was used to determine which was correct and that value was used for both nets. Upon retrieval of the nets, the samples from each of the two cod-ends were washed into a sample container. Plankton samples were fixed using 5% buffered formaldehyde and seawater. Replicate plankton tows were undertaken at 37 sites throughout the study region and preserved using 96% ethanol for genetic validation of Jack Mackerel eggs and other morphologically similar species (i.e. Yellowtail Scad, *Trachurus novaezelandiae* and Silver Trevally, *Pseudocaranx dentex*) (see Appendix 1).

3.2.2 Laboratory analysis

Jack Mackerel

Eggs of Jack Mackerel and similar species preserved in formalin and ethanol solutions were identified using morphological criteria and counted. Eggs were identified and staged using characteristics described for *Trachurus* eggs by Ahlstrom and Ball (1954), Crossland (1981) and Cunha *et al.* (2008). The main diagnostic features are: a) spherical with a diameter ranging from approximately 0.70 to 1.03 mm, b) smooth chorion, c) narrow perivitelline space, d) prominent segmented yolk sac (irregular and indistinct in early stages), e) single pigmented oil globule oriented posteriorly on yolk sac in later stages of development, and f) stout bodied embryo with prominent melanophores along the dorsal surface. Sub-samples of the ethanol preserved eggs were identified using the molecular techniques developed by Perry (2011) and refined by Neira *et al.* (2014). Results of the molecular studies were used to evaluate and develop morphological criteria for distinguishing Jack Mackerel eggs from those of similar species, especially Yellowtail Scad (see Appendix 1).

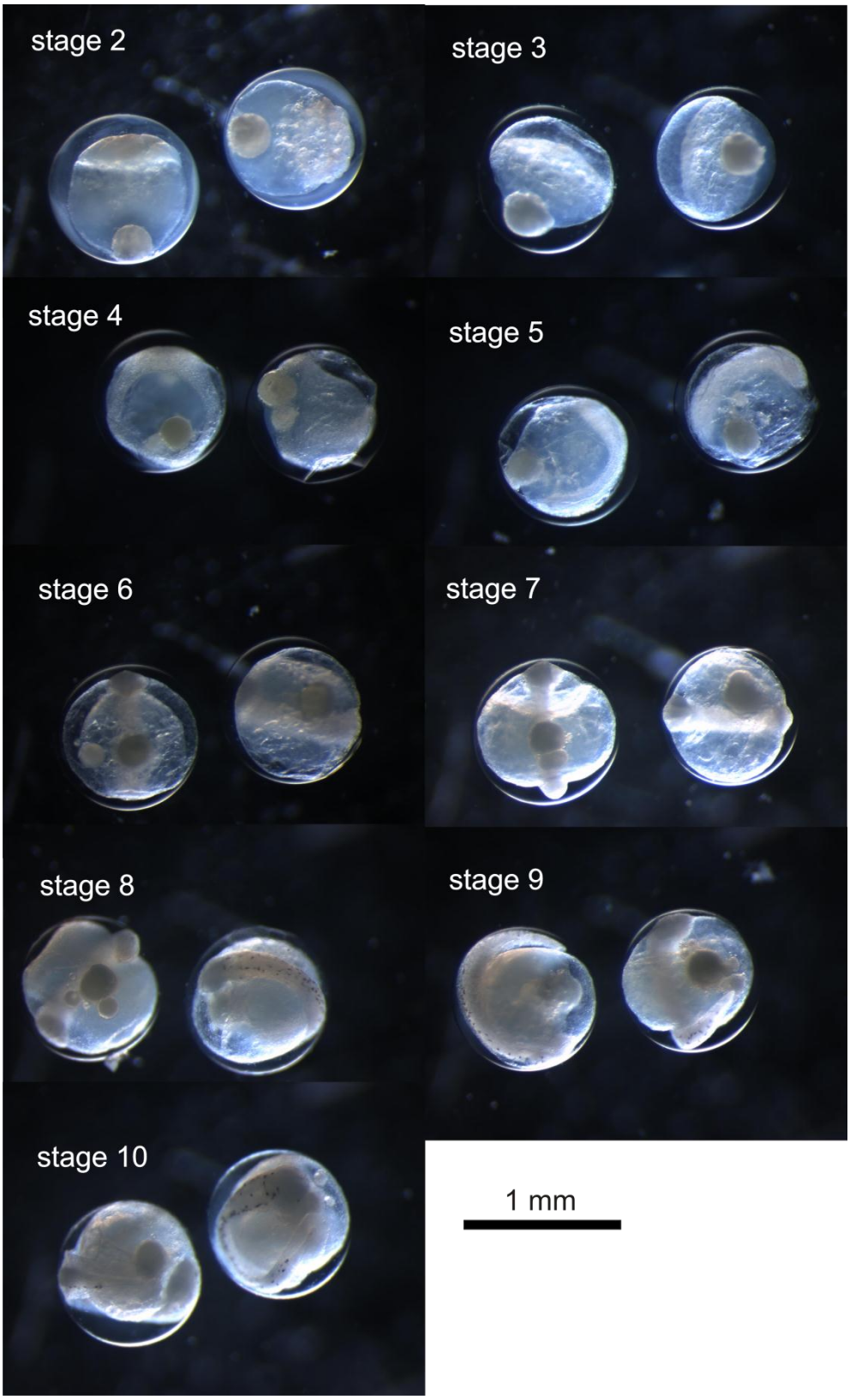


Figure 2. Jack Mackerel eggs staged after descriptions and key in Cunha *et al.* (2008).

Australian Sardine

Australian Sardine eggs were identified in each sample using published descriptions (White and Fletcher 1996; Neira *et al.* 1998). Eggs were staged and assigned approximate ages based on descriptions and temperature-development keys (White and Fletcher 1996).

3.2.3 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:

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

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

3.2.4 Spawning area

The Voronoi natural neighbour (VNN) method or Dirichlet tessellation (Watson 1981) was implemented using the Dirichlet function in the R package spatstat (R 3.1.0, Baddeley and Turner 2005) and used to generate a polygon around each sampling site with the boundary as the midpoint equidistant between each sampling site (Figure 3). The area represented by each station (km^2) was then determined. The spawning area (A) was defined as the total area of grids where live Jack Mackerel and Australian Sardine eggs were found.

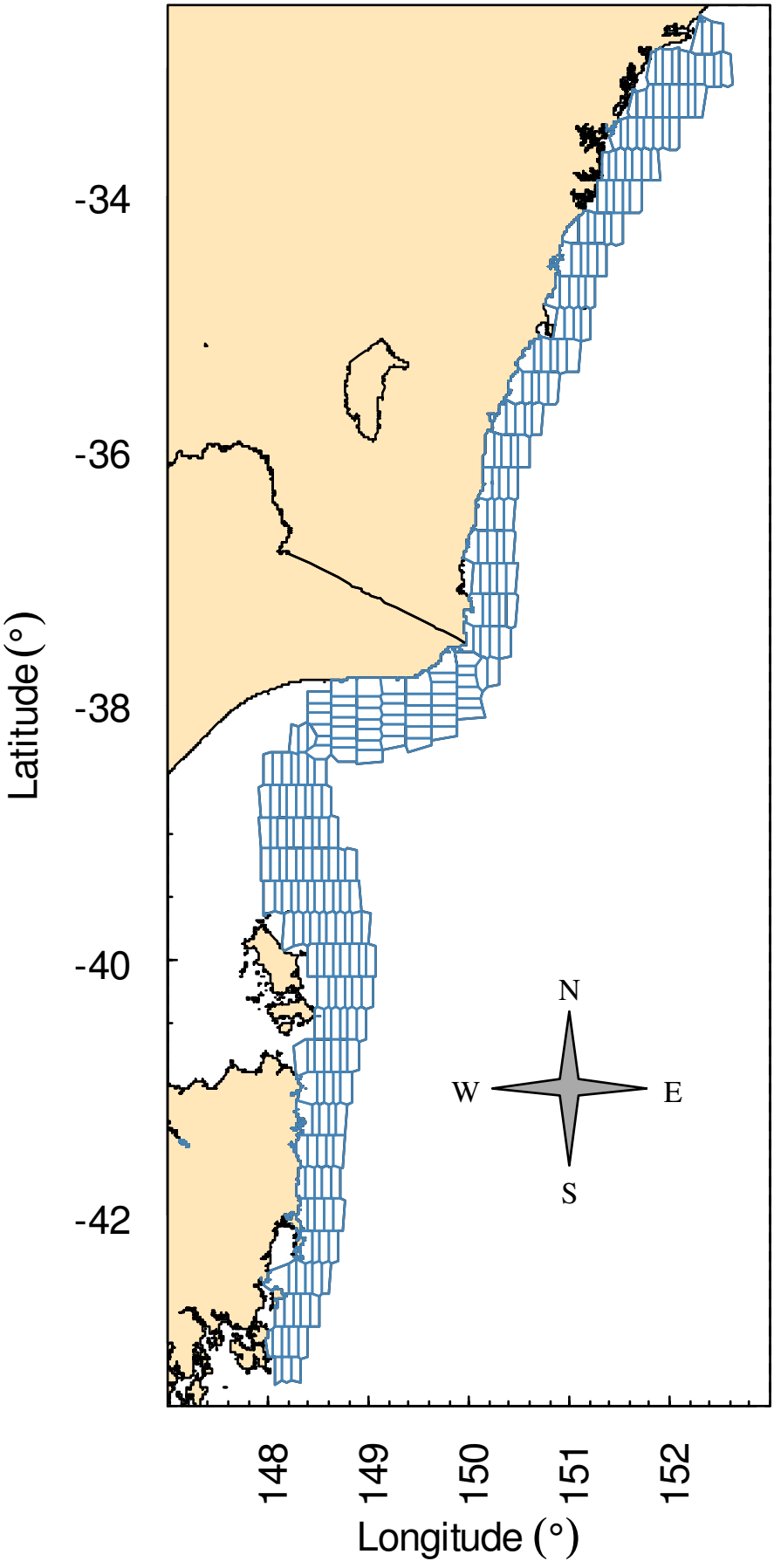


Figure 3. Voronoi natural neighbour polygons used to estimate spawning area.

3.2.5 Daily egg production (P_0) and egg mortality

Kernel density smoothing was applied to counts of each egg stage at time of sampling to identify the peak time of sampling for each stage. These plots were used to infer the peak spawning time (12:00 am). Egg stages were assigned to age day classes (day 1, 2 or 3) based on the temperature-development key of Cunha *et al.* (2008). The estimated age of each egg stage derived from our analysis was plotted against the estimates of the age of each stage at five different temperatures provided by Cunha *et al.* (2008). The age of each egg stage in each day class was determined by subtracting the time each sample was collected from the peak spawning time. P_0 and mean daily egg mortality (z) were calculated using egg stages 2-11 for Jack Mackerel and stages 2-12 for Sardine. P_0 and mean daily egg mortality (z) can be estimated using the exponential model (Picquelle and Stauffer 1985), a log-linear model (Picquelle and Stauffer 1985) and several generalised linear models (Ward *et al.* 2011).

Model options

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

$$P_t = P_0 e^{-z\,t} \qquad \text{Equation 3}$$

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):

$$\ln P_{i,t} = \ln P_b - z\,t \qquad \text{Equation 4}$$

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):

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

where σ^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.* 2011). For observed egg densities (P_i), the GLMs were of the form:

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

where g is the link function from an exponential family distribution, and X and b express the model.

The two GLMs used in this study assumed a Quasi distribution with a log link function with variance proportional (i) the mean and (ii) 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. The log-linear model, which has been used elsewhere for strongly over-dispersed data (e.g. Ward *et al*. 2011), was not used to estimate spawning biomass in this study because it provided an estimate of egg production (P_0) that was implausibly lower than the mean density of day-1 eggs (Appendix 1). Non-linear least squares regression and the two GLMs provided similar estimates of P_0. The mean value of egg production (P_0) obtained using these three methods was used to estimate the spawning biomass of both species.

3.3 Adult Reproductive Parameters

3.3.1 Adult Sampling

Jack Mackerel

Sampling for adult Jack Mackerel occurred from 12-18 January 2014 (i.e. during the egg survey) in shelf and slope waters between St Helens, Tasmania and Eden, NSW (see Figure 1). Sites likely to yield catches of Jack Mackerel were chosen based upon information provided from local commercial fishers. Samples were collected from the *FV Western Alliance* using a modified demersal trawl towed at a speed of 4.5 knots. The net was a wing trawl specifically designed to provide high lift and wide spread, with a maximum floatation of 225 kg. Ground line weight was 250 kg and there was 100 m of spread between the boards. After the net was retrieved, fish were removed and dissected immediately. Mature and immature males and females were counted. The gonads of mature females were removed and fixed in 5% buffered formaldehyde solution. Mature males and females were then frozen. Calculations of mean female weight, sex ratio, batch fecundity and spawning fraction were based on samples collected from 16 of the 20 trawl sites. Four trawl sites did not include sufficient numbers of Jack Mackerel for estimating these parameters.

Table 1. Date, time and locations of trawls for adult Jack Mackerel (* indicates no Jack Mackerel caught, # indicates insufficient numbers for sample).

Shot No.	Date	Start Time End Time	Start Latitude Longitude End Latitude Longitude	Description of Area	Depth (m)
1*	12 January	22:31 00:29	38°17.42'S 148°34.65'E 38°22.10'S 148°28.39'E	Off the Rigs	167-200
2	13 January	05:57 08:00	38°17.67'S 148°33.21'E 38°23.46'S 148°27.17'E	Off the Rigs	145-155
3	13 January	09:41 11:42	38°20.12'S 148°30.72'E 38°26.47'S 148°26.02'E	Off the Rigs	145-160
4	13 January	13:48 15:48	38°22.42'S 148°31.08'E 38°28.64'S 148°26.75'E	Off the Rigs	200-250
5	13 January	18:40 20:44	38°20.86'S 148°31.00'E 38°'27.44'S 148°26.37'E	Off the Rigs	170-200
6	14 January	07:28 09:58	39°40.21'S 148°43.52'E 39°47.81'S 148°46.38'E	Flinders Island	130-140
7	14 January	11:00 13:41	39°49.96'S 148°46.16'E 39°59.26'S 148°50.61'E	Flinders Island	130-140
8	14 January	14:10 16:45	40°01.14'S 148°51.22'E 40°09.55'S 148°49.14'E	Flinders Island	130-140
9*	14 January	19:36 21:34	40°00.91'S 148°51.15'E 40°08.04'S 148°51.61'E	Flinders Island	130-140
10*	15 January	06:31 08:31	40°50.89'S 148°42.51'E 40°58.01'S 148°41.65'E	St Helens	125-135
11	15 January	09:23 11:25	40°56.75'S 148°42.52'E 41°03.69'S 148°39.16'E	St Helens	130-140
12	15 January	12:14 14:13	41°04.22'S 148°38.93'E 41°11.34'S 148°36.85'E	St Helens	135-140
13	15 January	17:05 19:08	41°29.27'S 148°35.21'E 41°36.61'S 148°34.43'E	St Helens (South)	130-135
14#	16 January	13:58 15:51	39°17.93'S 148°38.00'E 39°11.34'S 148°38.62'E	The Hole	125-165
15	17 January	07:16 09:16	37°53.84'S 148°59.52'E 37°47.86'S 150°04.56'E	Eden (South)	130-155
16	17 January	13:49 15:49	37°23.85'S 150°16.50'E 37°31.20'S 150°13.27'E	Eden (North)	140-155
17	17 January	16:30 18:28	37°31.47'S 150°13.17'E 37°38.68'S 150°10.29'E	Eden	140-155
18	17 January	19:06 21:06	37°39.08'S 150°10.09'E 37°45.95'S 150°05.90'E	Eden	140-155
19	18 January	06:39 08:42	38°09.02'S 149°14.83'E 38°13.05'S 149°07.13'E	South of Eden	155-165
20	18 January	10:11 11:58	38°13.60'S 148°57.21'E 38°16.12'S 148°49.98'E	South of Eden	150-190

Australian Sardine

No samples of adult Australian Sardine were collected during the study.

3.3.2 Adult Parameters

The following sections describe the methods used to estimate adult reproductive parameters for Jack Mackerel. These are also the methods usually used to estimate these parameters for Australian Sardine. However, as no adult samples of Australian Sardine were collected during the present study adult parameters were obtained from studies conducted in South Australia (see Section 3.5).

Female weight (W)

Mature females from each sample were thawed and weighed (± 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 mean weight of mature females in the population was calculated from the average of sample means weighted by proportional sample size:

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

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

Male weight

Mature males in each sample were thawed and weighed (± 0.01 g). The mean weight of mature males in the population was calculated from the average of sample means weighted by proportional sample size as described above for females.

Sex ratio

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

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

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:

$$\overline{R_i} = \frac{F_i}{F_i + M_i}$$ 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)

Batch fecundity 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 and weighed. The total batch fecundity for each female was calculated by multiplying the mean number of oocytes per gram of ovary segment by the total weight of the ovaries. The relationship between female weight (ovaries removed) and batch fecundity was determined by linear regression analysis and used to estimate the batch fecundities of mature females in all samples.

Spawning fraction (S)

Ovaries of mature females were sectioned and stained with haematoxylin and eosin. Several sections from each ovary were examined to determine the presence/absence of post-ovulatory follicles (POFs). POFs were aged according to the criteria outlined by Ganias (2012). The spawning fraction of each sample was estimated as the mean proportion of females with hydrated oocytes plus day-0 POFs (*d*0) (assumed to be spawning or have spawned on the night of capture), day-1 POFs (*d*1) (assumed to have spawned the previous night) and day-2 POFs (*d*2) (assumed to have spawned two nights prior). The mean spawning fraction of the population was then calculated from the average of sample means weighted by proportional sample size.

$$S = \overline{\left[\frac{\overline{S_i}\, n_i}{N}\right]}$$ 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:

$$\overline{S_i} = \frac{d0 + d1 + d2}{3\, n_i}$$ 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.

Australian Sardine

As no adult samples were collected during this study, adult parameters used in these analyses were from Australian Sardine obtained from South Australian surveys conducted between 1998 and 2014.

3.4 Spawning Biomass and Bootstrapping Procedures

3.4.1 Spawning biomass

Jack Mackerel

Spawning biomass for Jack Mackerel was calculated according to Equation 1 using the mean value of egg production (P_0) obtained from three models (i.e. non-linear least squares regression and the two GLMs, Section 3.2.5) and the estimates of adult reproductive parameters obtained from the 2014 survey.

Australian Sardine

Spawning biomass for Australian Sardine was calculated using the same approach, but as no adult samples of Australian Sardine were collected during the present study, estimates of adult parameters were obtained from South Australian surveys conducted between 1998 and 2014 (see Section 3.5).

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 10,000 iterated bootstrap values. For each iteration, the average of the estimates from three models (i.e. non-linear least squares regression and the two GLMs, Section 3.2.5) was used to estimate P_0. Resampling for all four adult parameters used a two-stage bootstrap with 10,000 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 value $W / R.F.S$ was used in the calculation of bootstrapped confidence intervals for spawning biomass. The 95% confidence intervals of spawning biomass were estimated by calculating the spawning biomass 10,000 times from A and the 10,000 bootstrapped estimates of P_0 and $W/(R\ F\ S)$ using the percentile method. Parameter estimates were calculated independently in Excel 2007 and using R 3.1.0. Confidence intervals were estimated using R 3.1.0.

3.5 Sensitivity Analysis

Sensitivity analyses were conducted to determine which parameters exert 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 manipulation.

Jack Mackerel

Values used in the sensitivity analysis are considered plausible as they were obtained from literature values for Jack Mackerel (*T. declivis*) and other *Trachurus* species worldwide (Table 2) or from the range of values obtained in the present study.

The estimate of egg production (P_0) used in this study was relatively low compared to other studies. The lowest value used in the sensitivity analysis was the value obtained in the present study using the linear version of the exponential model (Ward *et al.* 2011). The highest value was that obtained from Neira (2011).

The estimate of spawning area obtained in this study was similar to that provided by Neira (2011). The upper and lower values of spawning area (*A*) used in the sensitivity analysis were ± 20% of the estimate used to calculate spawning biomass. The presence of high densities of eggs at stations on the western end of transects in Bass Strait (Figure 4) suggests that the spawning area may have been underestimated in the present study. Hence, the effect of the increase in spawning area on the estimate of spawning biomass is of the most relevance to interpretation of our results.

Other relevant studies of Jack Mackerel (e.g. Neira 2011) have used adult samples collected from commercial fishing operations, which are inherently biased because they target large fish, to estimate mean female weight. The values used in the sensitivity analysis were the minimum and maximum mean values for individual samples collected in the present study.

The minimum and maximum values for sensitivity analysis of batch fecundity (*F*) were calculated using the relationships with gonad-free female weight reported for *T. trachurus* and *T. murphyi*, which returned the highest and lowest batch fecundity when applied to the mean gonad-free female weight estimated in this study (Karlou-Riga and Economidis 1997; Ruiz *et al.* 2008; Table 2).

The sex ratio (*R*) of 0.47 was within the range of values reported for Jack Mackerel in south-eastern Australia (Webb and Grant 1979; Neira 2011). The maximum value for *R* of 0.53 was based upon samples taken from commercial and research vessel catches taken off the east coast between 1973 and 1976 and using length-weight relationships provided by Webb and Grant (1979). The minimum value of *R* of 0.35 was based upon samples taken off the east coast in 2002 (Neira 2011).

The measured spawning fraction (*S*) of 0.056 was lower than any previous reports for *Trachurus* spp. and used as the minimum estimate for this parameter in this study. The maximum value of *S* was based on averages reported for *T. trachurus* of ~0.2 (Karlou-Riga and Economidis1997; Goncalves *et al.* 2009).

Australian Sardine

In the absence of adult parameters, the sensitivity analyses for Australian Sardine were conducted using the minimum, mean and maximum estimated in South Australian DEPM surveys conducted between 1998 and 2014 (Table 3).

Table 2. Range of adult parameters in available literature for *Trachurus* spp. used to inform sensitivity analysis for spawning fraction, *S* and batch fecundity, *F*. All data are presented in the rawest available form. Values based on annual averages ($^{+}$). Values based on authors calculations from literature (*). Values based on total weight ($^{\#}$). g_{gf}: grams of gonad free weight.

Source	Species / Location	Female Weight (W, g_{gf})	Spawning Fraction (S)	Batch Fecundity (F, eggs. female^{-1})	eggs.g_{gf}^{-1}
Karlou-Riga and Economidis (1997)	*T. trachurus* Europe	63 – 223	0.171 – 0.209	4,551 – 54,355	205
Goncalves *et al.* (2009)	*T. trachurus* Europe		0.076 – 0.339		124 – 175
Abaunza *et al.* (2003)[a]	*T. trachurus* Europe		0.083		172
Ruiz *et al.* (2008)$^{+}$	*T. murphyi* Chile	192 – 625	0.07 – 0.194	26,069 – 55,419	89 – 124*
Macewicz and Hunter (1993)	*T. symmetricus* California	586 – 1262	0.202	31,752 – 171,466	112

[a]Primary references for Abaunza *et al.* (2003) are Eltink (1991), Borges *et al.* (1993) and Priede (1994).

Table 3. Mean, minimum and maximum adult Australian Sardine parameters determined in DEPM surveys in South Australia between 1998 and 2014 (Ward *et al.* 2014b).

Parameter	Mean 1998-2014 (min-max)
Sex Ratio (R)	0.53 (0.36 – 0.68)
Fecundity (F, eggs.female^{-1})	17,250 (10,904 – 24,790)
Spawning Fraction (S)	0.122 (0.040 – 0.179)
Female Weight (W, g)	58.3 (45.2 – 78.7)

4. Results

4.1 Environmental Variables

4.1.1 Sea surface temperature

Sea surface temperatures (SSTs) ranged from 14.3 to 25.8°C (Figure 4) during January 2014. The highest temperatures were recorded in the north and the lowest in the south.

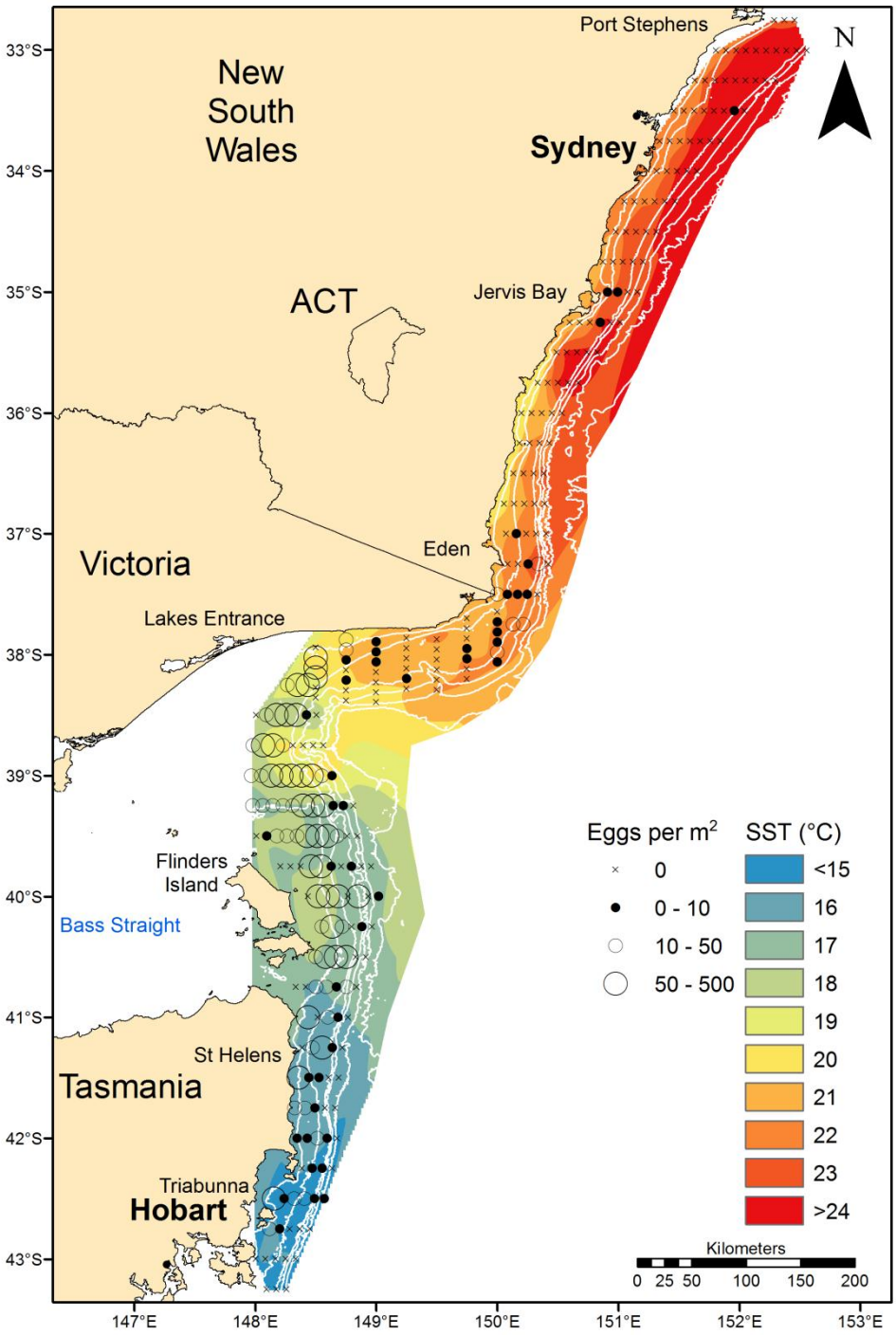


Figure 4. Sea surface temperature (SST) with egg densities of Jack Mackerel and 50 m depth contours.

4.2 Jack Mackerel

4.2.1 Distribution and Abundance of Eggs

Plankton samples were successfully collected from 292 sites located between South East Cape and just south of Port Stephens. Approximately 15 sites located on two transects off Port Stephens could not be sampled due to bad weather.

A total of 3,530 live Jack Mackerel eggs were collected from 117 of the 292 (40.1%) stations sampled. Jack Mackerel eggs were collected from waters between Eden and Triabunna where SSTs ranged between 15 and 22°C (Figure 4). The highest densities of Jack Mackerel eggs were recorded in waters off north-eastern Tasmania and in Bass Strait. Most Jack Mackerel eggs were collected from sites with SSTs in the range of 16.5 to 20°C, however, a small peak in egg abundance occurred off Triabunna where the SST was ~15°C.

The distribution of eggs was influenced by depth, with most eggs collected from <130 m. Approximately 70% of eggs were sampled at stations in depths of <100 m, with the greatest densities between 40 and 80 m.

4.2.2 Egg Identification and Ageing

Kernel density smoothing plots of the modal time of capture of each egg stage suggested that spawning occurred at approximately 12:00 am (Figure 5).

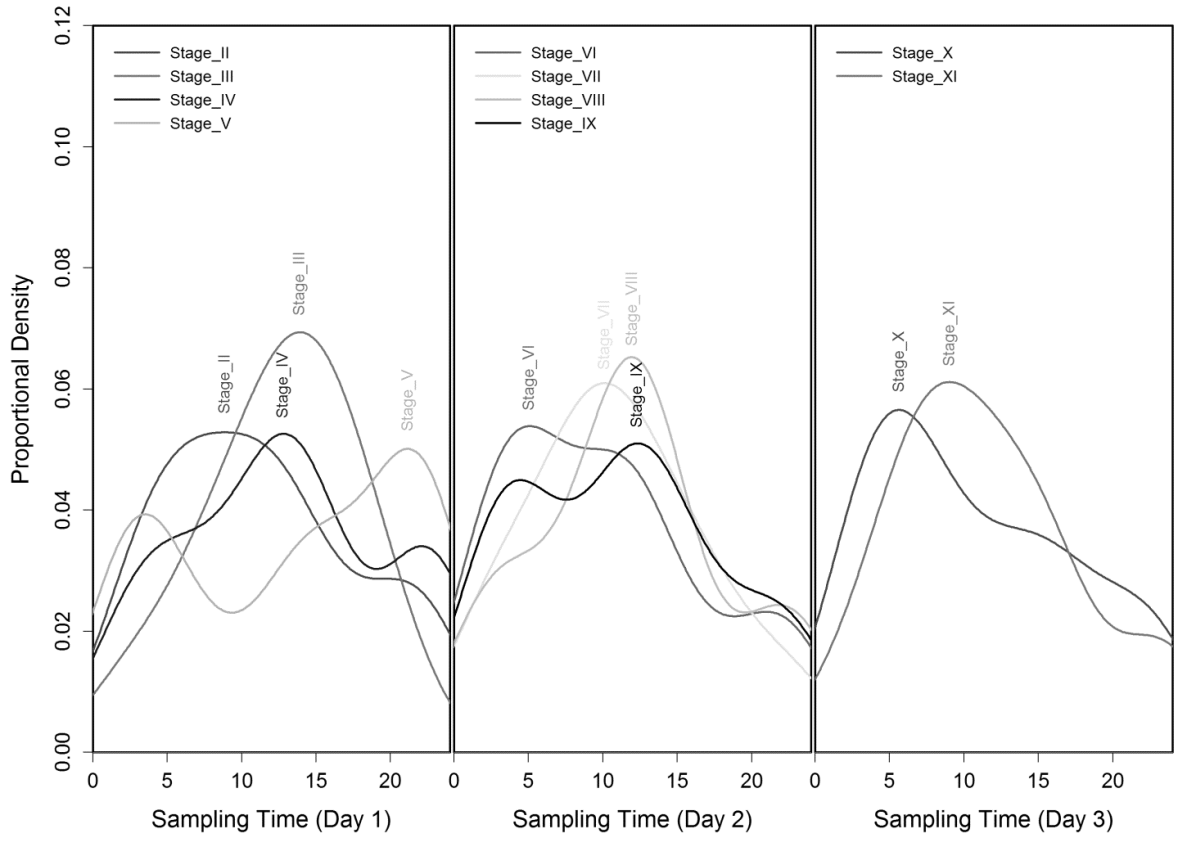


Figure 5. Kernel density smoothing plots of counts of each egg stage by sampling time.

The plots of the age of each egg stage estimated by Kernel density smoothing against the development curves derived from Cunha *et al.* (2008) confirmed that the developmental rates of Jack Mackerel eggs are similar to those of *Trachurus trachurus* (Figure 6). The peak time of collection of egg stages taken at temperatures < 20°C and ≥ 20°C were similar (e.g. Stage 11 eggs were estimated to be approximately 57 hours old for both temperature ranges).

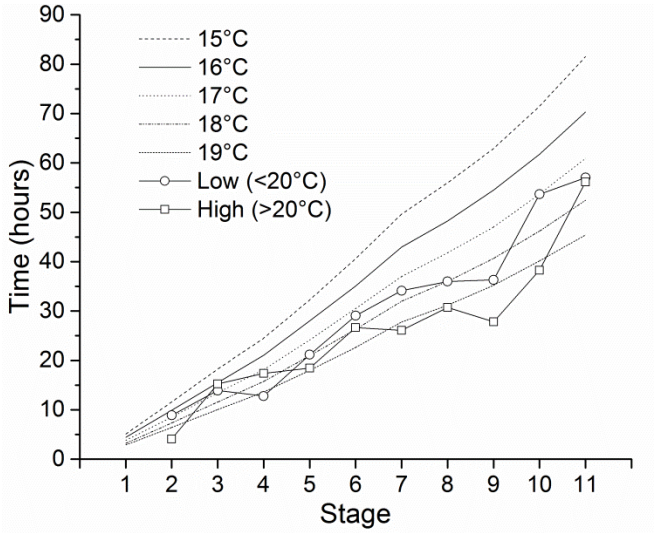


Figure 6. Mean age of each egg stage of *T.* declivis derived from kernel density smoothing (symbols) and plotted against development curves for *T. trachurus* eggs derived experimentally by Cunha *et al.* (2008).

4.2.3 Spawning Area

The estimated spawning area for Jack Mackerel was 23,960 km^2, comprising 37.8% of the total area sampled (63,355 km^2, Table 4).

Table 4. Survey area, spawning area (*A)*, percentage of area containing eggs and spawning biomass of Jack Mackerel.

Area sampled (km ^{2})	Spawning area *A* (km ^{2})	Percentage of area sampled	Spawning biomass (t)
63,355	23,553	37.2	157,805

4.2.4 Daily Egg Production (P_0)

The estimate of mean daily egg production, P_0 obtained by averaging the estimates obtained using the exponential model and the two GLMs was 28.9 eggs.day^{-1}.m^{-2} (95% CI = 15.9 – 48.7, Table 5, 8). The

estimate obtained using the linear version of the exponential model was 17.9 eggs.day^{-1}.m^{-2} (95% CI = 10.9 – 28.4, Table 5, 8)

Table 5. Mean daily egg production of Jack Mackerel estimated using four alternative models. The value used for biomass estimation is highlighted in bold. Ranges are 95% confidence intervals.

Model	P_0 (eggs.day^{-1}.m^{-2})
Exponential model, ρ ~ exp (-Z age), NLS	27.9 (15.4 – 52.8)
Linear version of exponential model, ln(ρ) ~ age, corrected, NLS	17.9 (10.9 – 28.4)
GLM, ρ ~ age, Quasi family, log link, var(y)=μ(y)	28.7 (15.6 – 48.3)
GLM, ρ ~ age, Quasi family, log link, var(y)=μ(y)^2	30.2 (16.1 – 49.1)
Mean of models (excluding linear)	**28.9 (15.9 – 48.7)**

4.2.5 Adult Samples

Sixteen of the 20 trawls undertaken from the *FV Western Alliance* caught sufficient numbers Jack Mackerel to be used for estimation of adult reproductive parameters (i.e. $n > 30$). In these 16 trawls a total of 2,704 adult Jack Mackerel were collected, including 1,285 females and 1,419 males (Table 6), with an average fork length (FL) of 266.6 mm (range: 204 – 398 mm). Most Jack Mackerel were caught during the day. Catch rates during the night were low. Samples collected between 125 and 250 m depths included significant numbers of spawning adults. Estimates of the adult female reproductive parameters used in calculations of spawning biomass are provided in Tables 6 and 7. Bootstrapped parameter estimates that provided 95% confidence intervals are shown in Table 8.

4.2.6 Mean Female Weight

The mean weight of mature females in samples ranged from 133.9 to 250.9 g (Table 6). The weighted mean weight of mature females was 208.8 g (95% CI = 187.2 – 230.7, Tables 6, 8).

4.2.7 Sex ratio

The sex ratio calculated from the survey was 0.47 (95% CI = 0.44 – 0.51, Tables 6, 8).

Table 6. Number of Jack Mackerel in samples by sex and estimates of female weight, *W* and sex ratio, *R* (proportion of females by weight) for samples collected in 2014. Values in bottom row are sums (*) and weighted means (#).

Trawl #	Location	Date	Male	Female	Mean Male Weight (g)	Mean Female Weight (*W, g*)	Sex Ratio (*R*)
2	Off The Rigs	13/01/2014	80	100	187.5	188.5	0.557
3	Off The Rigs	13/01/2014	86	100	159.4	171.6	0.556
4	Off The Rigs	13/01/2014	133	102	278.0	290.1	0.445
5	Off The Rigs	13/01/2014	125	100	254.7	250.2	0.440
6	Flinders Island	14/01/2014	76	98	250.3	231.8	0.544
7	Flinders Island	14/01/2014	71	77	222.2	196.5	0.489
8	Flinders Island	14/01/2014	134	99	240.2	236.6	0.421
11	St Helens	15/01/2014	65	49	228.9	230.0	0.431
12	St Helens	15/01/2014	106	101	232.8	238.4	0.494
13	St Helens (South)	15/01/2014	49	49	258.5	250.9	0.492
15	Eden (South)	17/01/2014	117	100	151.6	147.5	0.454
16	Eden (North)	17/01/2014	161	104	203.2	193.7	0.381
17	Eden	17/01/2014	100	100	169.0	163.8	0.492
18	Eden	17/01/2014	29	33	157.4	133.9	0.492
19	South of Eden	18/01/2014	50	50	182.3	168.9	0.481
20	South of Eden	18/01/2014	37	23	207.3	200.8	0.376
	Grand Total		**1419***	**1285***	**215.2#**	**208.8#**	**0.470#**

4.2.8 Batch fecundity

Batch fecundity ranged from 16,599 to 94,743 hydrated oocytes for the 14 hydrated female Jack Mackerel examined. Three of these fish were excluded from further analyses because of the apparently anomalous egg counts (two low and one high). The batch fecundity of the remaining 11 hydrated females ranged from 22,084 to 49,279 (mean = 33,749) hydrated oocytes.

The relationship between Batch Fecundity and Gonad-free Female Weight was poor (Batch Fecundity = 6.091 x Gonad-free Female Weight + 32,826, R^2 = 0.0001, Figure 7). For mean gonad free female weight (204.0 g) for all samples collected mean batch fecundity was estimated to be 34,068 hydrated oocytes per batch (95% CI = 21,584 – 82,009, Table 8). If the three outliers were included, the mean batch fecundity was 38,414 hydrated oocytes per batch (95% CI = 21,644 – 82,604).

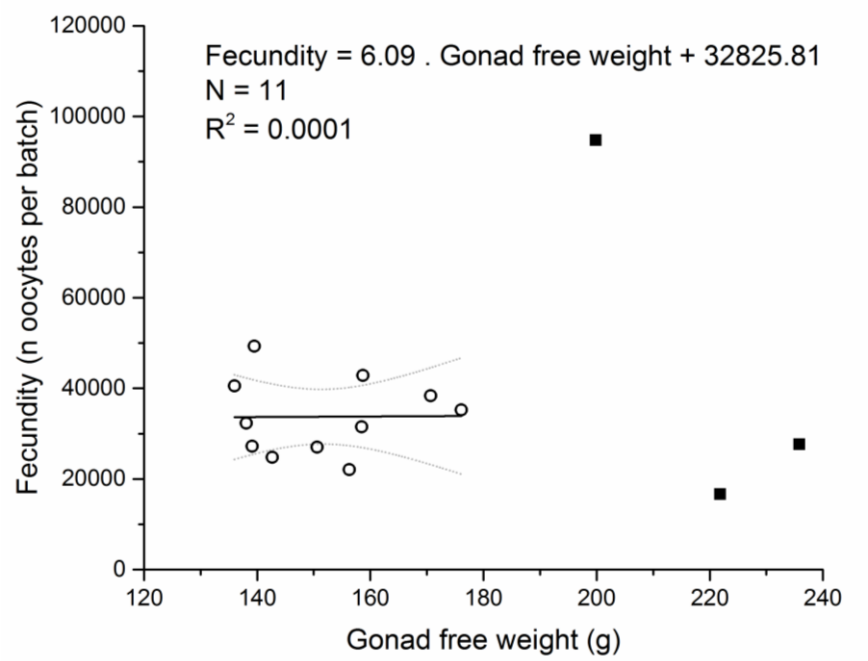


Figure 7. Relationship between gonad-free weight and batch fecundity in 2014 (dotted line = 95% CI). Excluded values are indicated by shaded squares.

4.2.9 Spawning fraction

Of the 1285 ovaries examined, 71 had hydrated oocytes and/or day-0 POFs, 55 had day-1 POFs and 91 day-2 POFs (Table 7). The spawning fraction of females in samples ranged from 0.000 to 0.134. The weighted mean spawning fraction for all 2014 data was 0.056 (95% CI = 0.035 – 0.080).

Table 7. Number of female Jack Mackerel in samples and estimates of spawning fraction, *S*, for samples collected in 2014. [+] Includes hydrated females. Values in the bottom row are sums* and weighted means[#].

Shot #	Location	Date	POF 0[+]	POF 1	POF 2	Total	Spawning Fraction (S)
2	Off The Rigs	13/01/2014	0	0	6	100	0.020
3	Off The Rigs	13/01/2014	7	5	7	100	0.063
4	Off The Rigs	13/01/2014	0	0	0	102	0.000
5	Off The Rigs	13/01/2014	1	1	0	100	0.007
6	Flinders Island	14/01/2014	8	6	19	98	0.112
7	Flinders Island	14/01/2014	12	5	14	77	0.134
8	Flinders Island	14/01/2014	8	8	10	99	0.088
11	St Helens	15/01/2014	0	10	6	49	0.109
12	St Helens	15/01/2014	15	2	5	101	0.073
13	St Helens (South)	15/01/2014	1	6	5	49	0.082
15	Eden (South)	17/01/2014	14	7	6	100	0.090
16	Eden (North)	17/01/2014	0	1	3	104	0.013
17	Eden	17/01/2014	0	3	6	100	0.030
18	Eden	17/01/2014	3	1	1	33	0.051
19	South of Eden	18/01/2014	2	0	3	50	0.033
20	South of Eden	18/01/2014	0	0	0	23	0.000
			71*	55*	91*	1285*	0.056[#]

Table 8. Parameters used in the calculations of spawning biomass of Jack Mackerel in 2014.

Parameter	Number (95% CI)
Egg Production (P_o, eggs.day^{-1}.m^{-2})	30.2 (16.1 – 49.1)
Sex Ratio (R)	0.47 (0.44 – 0.51)
Fecundity (F, eggs.female^{-1})	34,068 (21,584 – 82,010)
Spawning Fraction (S)	0.056 (0.035 – 0.080)
Female Weight (W, g)	208.8 (187.2 – 230.7)
Spawning Area (A, km^2)	23,553

4.2.10 Spawning Biomass

The estimate of Jack Mackerel spawning biomass calculated using data from the 2014 survey was 157,805 t (95% CI = 59,570 – 358,731; Table 4).

4.2.11 Sensitivity Analysis

The sensitivity of estimates of spawning biomass to plausible variations in each parameter is shown in Figure 8. Most of these estimates of spawning biomass range from ~95,000 t to 215,000 t. Plausible values for only two parameters, i.e. egg production (P_0) and spawning fraction (S), provide results that are outside that range (i.e. ~436,000 t for P_0 and ~44,000 t for S). The mean estimate of egg production (28.9 eggs.day^{-1}.m^{-2}) and spawning fraction (0.056) obtained in this study are both low compared to the estimates of these parameters obtained in the other studies that were used in the sensitivity analysis (i.e. 78.4 eggs.day^{-1}.m^{-2} and 0.2, respectively). However, in the present study both parameters were estimated with a high degree of confidence. For example, estimates of egg production were based on a relatively large number of samples (117) and there was good agreement among the three models used to estimate egg production (range 27.9 – 30.2 eggs.day^{-1}.m^{-2}). In addition, estimates of spawning fraction were based on a large number of samples obtained using a method that may sample the adult population without bias. Most importantly, the low values for these two parameters provide consistent insights into the spawning patterns of the population during the study period (i.e. both suggest that spawning rates were low).

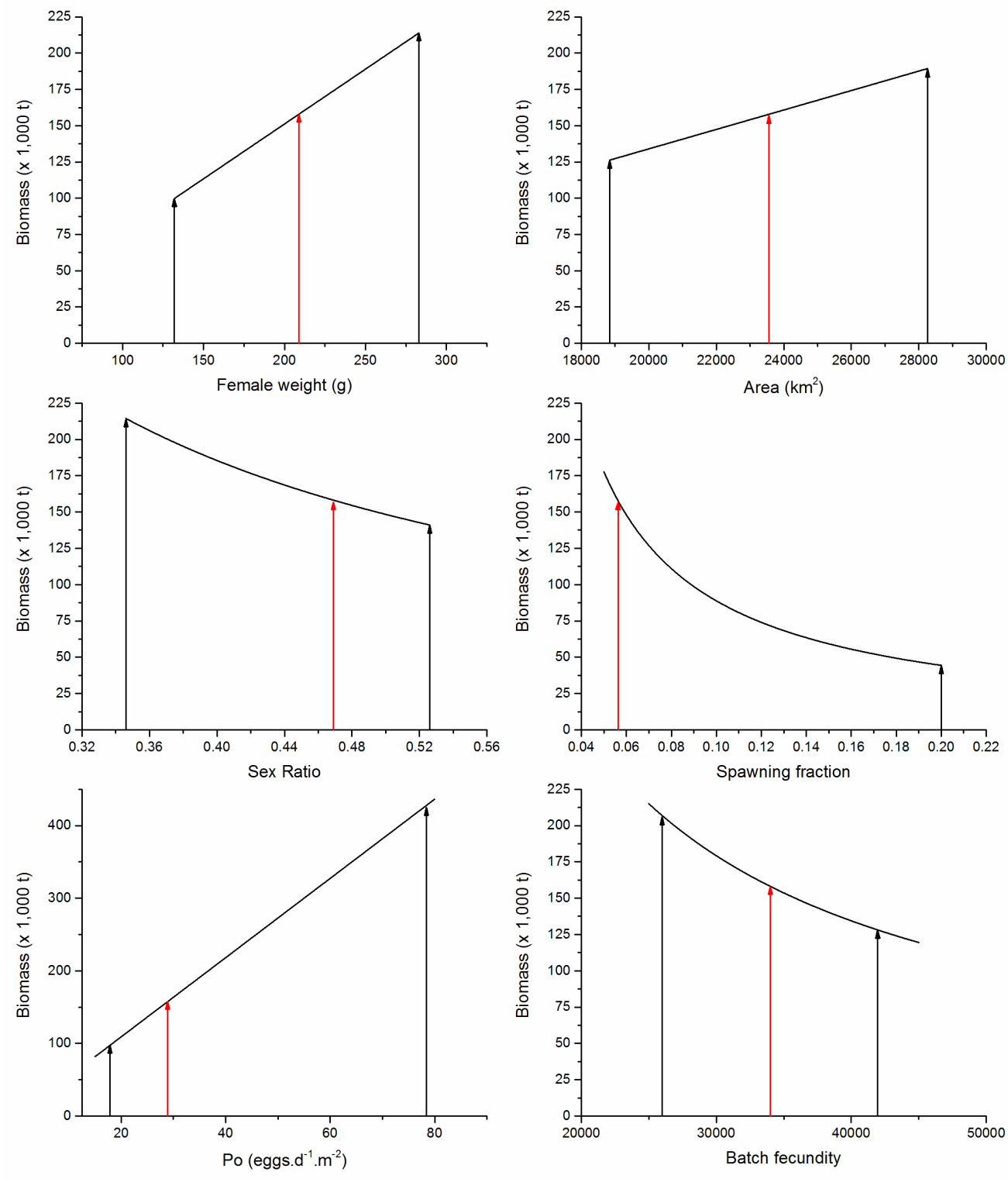


Figure 8. Sensitivity analysis of the effects of individual parameters on estimates of spawning biomass of Jack Mackerel. Red and black arrows are mean, minimum and maximum values as described in Table 2.

4.3 Australian Sardine

4.3.1 Distribution and Abundance of Eggs

A total of 1,429 live Australian Sardine eggs were collected from 59 of the 292 (20.2%) stations sampled. Most Australian Sardine eggs were collected from waters off north-eastern Tasmania and in Bass Strait where sea surface temperatures were between 16 and 21°C (Figure 9).

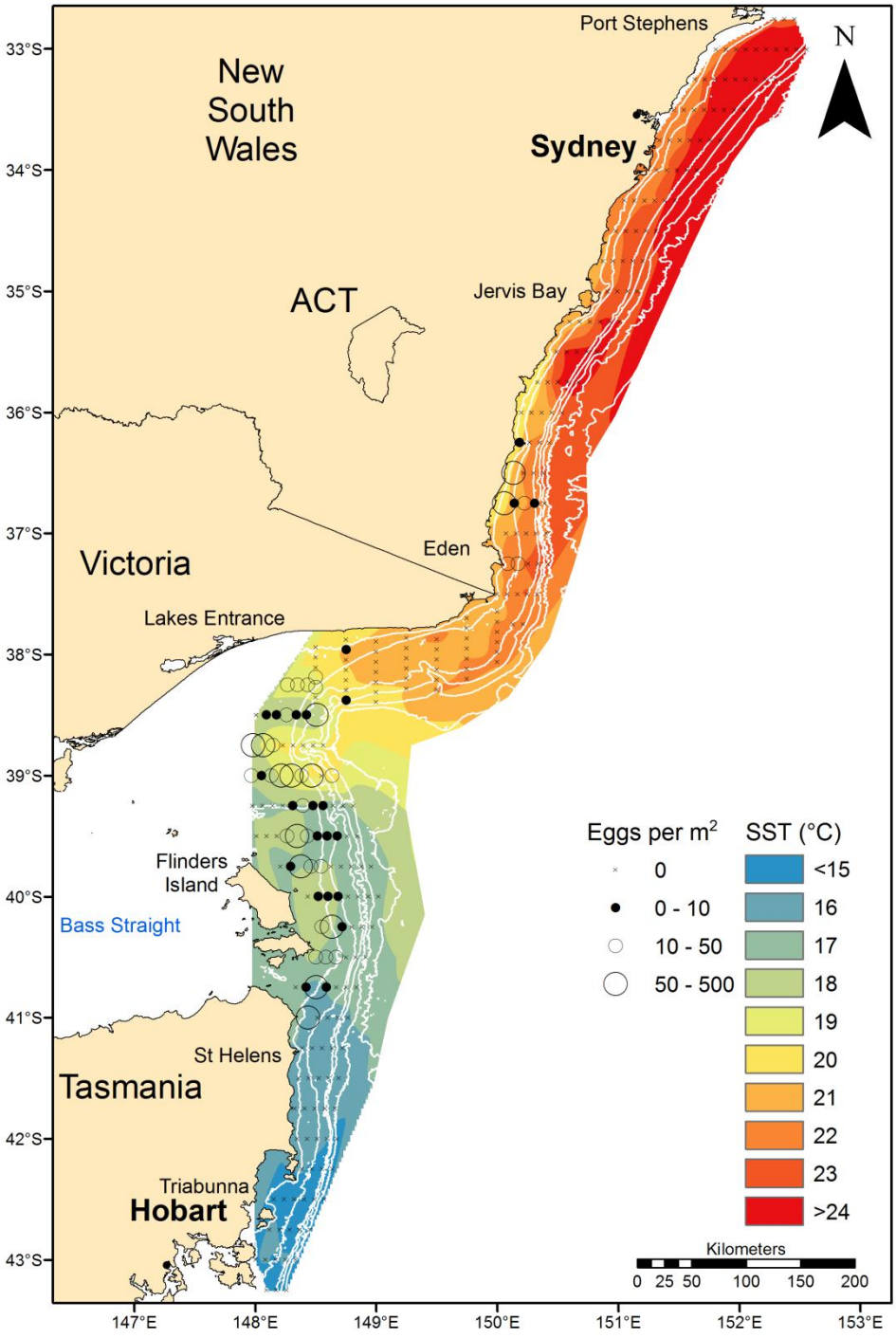


Figure 9. Sea Surface Temperature (SST) with Australian Sardine egg densities and depth contours.

4.3.2 Spawning Area

The estimated spawning area for the entire survey area was 11,906 km^2, comprising 18.8% of the total area sampled (63,355 km^2, Table 9).

Table 9. Survey area, spawning area (*A)*, percentage of area containing eggs and spawning biomass of Australian Sardine.

Area sampled (km^2)	Spawning area *A* (km^2)	Percentage of area sampled	Spawning biomass (t)
63,355	11,906	18.8	10,962

4.3.3 Daily Egg Production (P_0)

The estimate of mean daily egg production, P_0 obtained by averaging the estimates obtained using the exponential model and the two GLMs was 17.6 eggs.day^{-1}.m^{-2} (95% CI = 6.0 – 40.3, Table 10).

Table 10. Mean daily egg production of Australian Sardine estimated using four alternate models. The value used for biomass estimation is highlighted in bold. Ranges are 95% confidence intervals.

Model	P_0 (eggs.day^{-1}.m^{-2})
Exponential model, ρ ~ exp(age), NLS	17.8 (4.8 – 35.0)
Linear version of exponential model, ln(ρ) ~ age, corrected, NLS	15.6 (6.6 – 34.9)
GLM, ρ ~ age, Quasi family, log link, var(y)=μ(y)	17.6 (5.7 – 36.8)
GLM, ρ ~ age, Quasi family, log link, var(y)=$μ(y)^2$	17.3 (6.2 – 43.7)
Mean of models (excluding linear)	**17.6 (6.0 – 40.3)**

4.3.4 Adult Reproductive Parameters

Data for estimating reproductive parameters of adult Australian Sardines were sourced from South Australian DEPM surveys between 1998 and 2014 (Table 3).

Mean female weight

The estimate of mean female weight (*W* = 52.8 g) for NSW waters between 2003 and 2009 was calculated from data provided by Stewart *et al.* (2010). The mean weight of mature females obtained from South Australian surveys between 1998 and 2014 was 58.3 g and the minimum and maximum values were 45.2 g in 1998 and 78.7 g in 2004 (Table 3).

Sex ratio

Estimates of mean sex ratio (*R* = 0.64) from NSW waters between 2003 and 2009 were calculated from Stewart *et al.* (2010). Although these data are the most spatially relevant to the survey region, commercial

purse seining vessels are often biased towards females (Ward and McLeay 1998) and the value of 0.64 is unlikely to be representative of the population. The mean sex ratio obtained from fishery-independent samples from South Australia was 0.53 and the minimum and maximum values were 0.36 in 2009 and 0.68 in 2013 (Table 3).

Batch fecundity

Females with hydrated oocytes suitable for estimating batch fecundity have not been collected from south-eastern Australian waters. The mean batch fecundity (*F*) obtained for South Australia was 17,250 eggs and ranged from 10,904 eggs in 2003 to 24,790 eggs in 2004 (Table 3).

Spawning fraction

No estimates of spawning fraction (*S*) are available from south-eastern Australian waters. The mean estimate of *S* for South Australia was 0.12, with values ranging from 0.04 in 2014 to 0.18 in 2001 (Table 3).

4.3.5 Spawning Biomass

The estimate of spawning biomass, calculated using estimates of egg production and spawning area obtained from the 2014 survey and mean estimates of adult parameters from South Australia was 10,962 t (Table 9, Figure 10).

4.3.6 Sensitivity Analysis

The relative sensitivity of estimates of spawning biomass to variation in each parameter is shown in Figure 10. These show that estimates of spawning biomass are between ~ 8,000 t and 15,000 t for plausible values of all parameters except spawning fraction. A spawning fraction value of 0.04 produces a spawning biomass estimate of approximately 33,000 t.

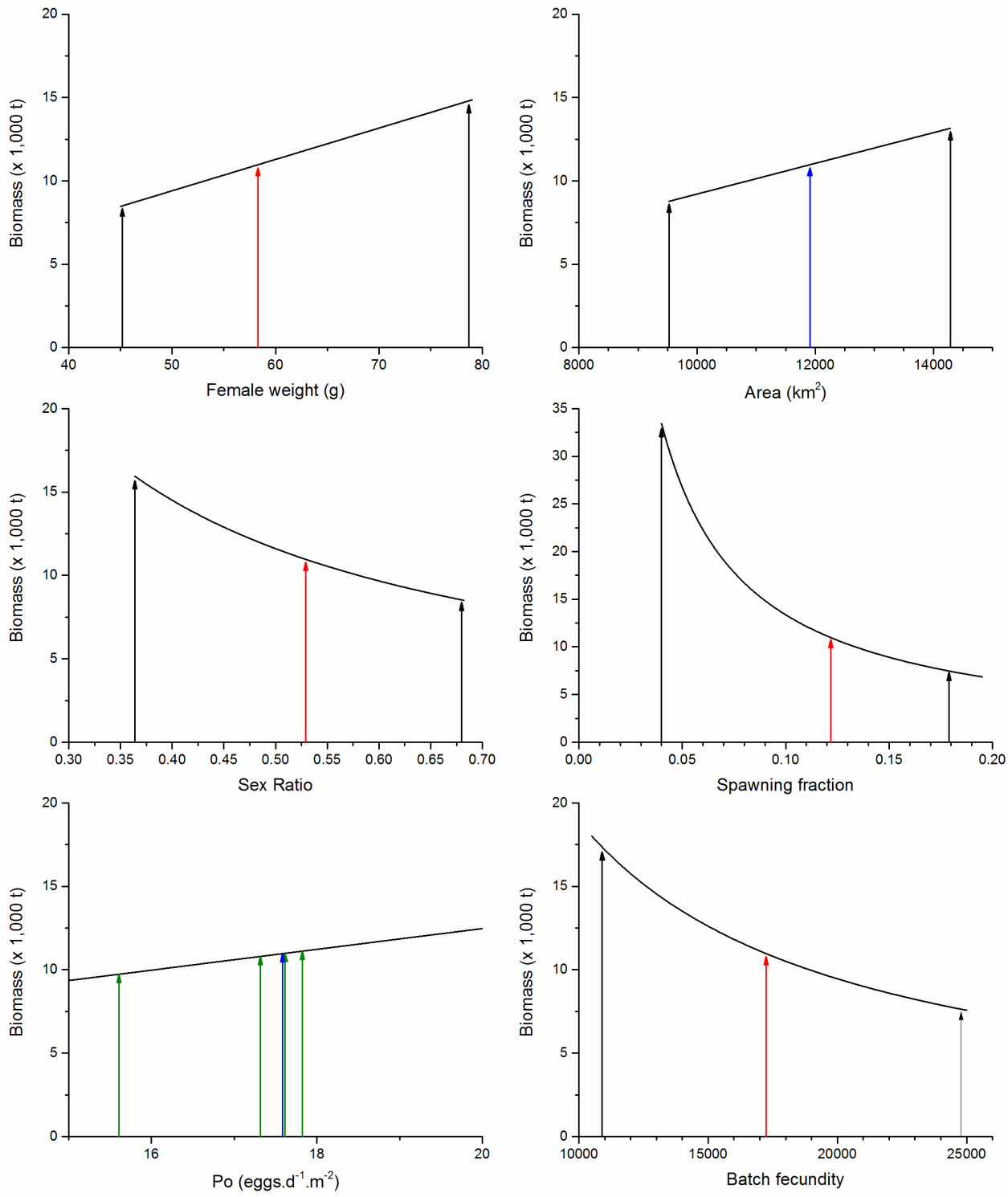


Figure 10. Sensitivity analysis of the effects of individual parameters on estimates of spawning biomass of Australian Sardine. Blue arrows are values estimated in this study, green arrows are alternate models used to estimate P_0 and red and black arrows are mean, minimum and maximum values as described in Table 3.

5. Discussion

5.1 Jack Mackerel

Spawning patterns

Our results support the conclusions of Kailola *et al.* (1993), Bulman *et al.* (2012) and others who have suggested that the main spawning region off south-eastern Australian during summer is located in Bass Strait and waters off Tasmania. In the present study, most Jack Mackerel eggs were collected from sites with SSTs in the range of 16.5 to 20°C, which is consistent with the suggestion that spawning occurs seasonally along the east coast in water temperatures around 17°C (Maxwell 1979). High egg abundances were recorded at similar SSTs in surveys off NSW during October 2002 and 2003 and Victoria and Tasmania during February 2003 and 2004 (Neira 2011). However, the present study also identified a small peak in egg abundance at a SST of ~15°C off Triabunna, Tasmania which is consistent with the findings of Jordan *et al.* (1995) who also found high densities of Jack Mackerel eggs off eastern Tasmania in SSTs in the range of 15 to 17.5°C (Jordan *et al.* 1995).

The present study provides strong evidence that during January 2014 spawning occurred in relatively shallow shelf waters, mainly between 40 and 80 m. The high densities of eggs in shallow waters contrasts the findings of most previous surveys that found greater densities of eggs at the shelf break (e.g. Jordan 1995; Neira 2011). For example, spawning near the shelf break was inferred from surveys off eastern Tasmania during 1990 and 1991 where ~87% and 95% of Jack Mackerel eggs, respectively, were found at the outermost of stations located at the shelf break (Jordan *et al.* 1995). However, during 1989 eggs were more evenly distributed among inshore, mid-shelf and shelf-break stations, which the authors suggested may have been due to rapid onshore advection of eggs during a La Nina event (Jordan *et al.* 1995).

Adult data collected during the present study suggest that the high numbers of eggs collected from inshore stations reflect the high spawning rates of Jack Mackerel in shallow waters (Sexton 2014). The high spawning rates recorded in inshore waters are unexpected because our results suggest, as others such as Shuntov (1969), Stevens *et al.* (1984); Kailola *et al.* (1993), Pullen (1994), Furlani *et al.* (2000) and Bulman *et al.* (2012) have noted, that Jack Mackerel in shallower waters were generally smaller than those found further offshore. A latitudinal pattern in fish size and spawning activity was also discernible, with larger fish with higher spawning rates occurring in the south (Sexton 2014). Future studies of the spawning patterns of Jack Mackerel off eastern Australia should be designed to assess temporal variations in the relationship between depth, latitude, fish size and spawning rates of Jack Mackerel throughout the summer on the spawning grounds off eastern Tasmania and in Bass Strait.

DEPM Parameters

The present study provides the first estimate of the spawning area of Jack Mackerel off eastern Australia (23,960 km^2) based on a targeted large-scale survey. The previous estimate of spawning area (i.e. 21,327 km^2) provided by Neira (2011) was constructed from several smaller surveys that were conducted over multiple years and targeted the eggs of other species. The estimate of spawning area provided in the

present study is likely to be negatively biased as high densities of eggs were found at stations on the western end of transects in Bass Strait (Figure 6), suggesting that additional spawning may have occurred west of the survey area.

The estimate of egg production provided in this study (28.9 eggs.day^{-1}.m^{-2}) is considered robust. Three of the four models used provided estimates of egg production were similar. The estimate of egg production provided by the fourth (linear) model is considered implausible because it was lower than the mean density of day- 1 eggs (Appendix 1).

This is the first study to establish a fishery-independent sampling technique for collecting samples of adult Jack Mackerel for estimation of reproductive parameters required for application of the DEPM. The collection of adult parameters from the main spawning area during the egg survey is a significant improvement on the previous study of the spawning biomass of Jack Mackerel off eastern Australia which was based on relatively small adult samples collected from commercial fishing activities conducted in locations and during time periods that did not coincide with the egg surveys (Neira 2011).

There is no evidence to suggest that samples of adult Jack Mackerel obtained using the modified demersal trawling method developed were not representative of the population. The estimate of mean sex ratio (0.47) is biologically plausible and estimates of this parameter obtained for individual sites were relatively consistent. In short, there was no evidence of sex-related net avoidance. The mean weight of females (209 g) collected in trawl samples during this study is low compared with historical estimates which ranged from approximately 287 to 373 g (Webb and Grant 1979; Blaber and Bulman 1987; Pullen and Lyle 1994; Neira 2011). However, previous studies were generally obtained from commercial vessels which may have selectively targeted large fish. It is possible that the trawling technique used in this study may be biased towards small fish, due to larger fish having stronger swimming ability and greater capacity to avoid the net. However, the mean size of females in samples varied considerably (i.e. 134 to 290 g) which shows that our technique successfully sampled fish of a broad range of sizes.

The trawling technique developed in this study may provide unbiased estimates of spawning rates. Samples were collected during the day when the fish appeared to be relatively dispersed, rather than during the night when many pelagic species are known to separate into spawning and non-spawning schools (Ganias *et al.* 2003; Stratoudakis *et al.* 2006). The development of a sampling technique that may estimate spawning fraction without bias is a major step forward in the application of the DEPM to Jack Mackerel. Difficulties obtaining reliable estimates of spawning fraction (*S*) are often the greatest impediment to the application of the DEPM (e.g. Hunter and Lo 1997; Stratoudakis *et al.* 2006; Ward *et al.* 2009; Neira 2011). The low value of spawning fraction obtained in the current survey (0.056) suggests that the survey may not have been conducted during the peak spawning season (Karlou-Riga and Economidis 1997). However, spawning fraction also varies among years and it is possible that 2014 was a year with low spawning rates (see Abaunza *et al.* 2003). As noted above, it would be useful to have a better understanding of the spatial and temporal variations in the spawning rates of Jack Mackerel off eastern Tasmania and in Bass Strait during summer.

The only adult parameter that was not estimated from a large number of samples during the present study was batch fecundity. It has previously been recommended that fecundity estimates for multiple spawning fishes, e.g. Northern Anchovy *Engraulis mordax*, should be estimated from ≥50 individuals (Hunter *et al.*

1985). Our estimate of batch fecundity was based on only 14 females with hydrated oocytes and three of these were excluded from the analysis as they had apparently anomalous fecundities (both high and low egg numbers). The females used in the analysis were also very similar in size (i.e. 136 to 176 g) which resulted in a poor relationship between mean female gonad free weight and fecundity (i.e. $R^2 = 0.001$). However, the relationship (i.e. eggs/g) obtained in this study is similar to those established for other *Trachurus* species (Karlou-Riga and Economidis 1997; Ruiz *et al.* 2008). Future applications of the DEPM to Jack Mackerel should have a strong focus on sampling hydrated females needed for estimation of batch fecundity (e.g. by jigging at night) or using alternative methods to estimate this parameter (e.g. Ganias *et al.* 2010).

Spawning biomass and sensitivity analyses

This is the first study to estimate the spawning biomass of Jack Mackerel off eastern Australia based on concurrent, targeted surveys of both eggs and adults. The estimate of spawning biomass of Jack Mackerel obtained for January 2014, i.e. 157,805 t (95% CI = 59,570 – 358,731) appears to be robust. It is within the range of estimates of spawning biomass for Jack Mackerel off eastern Australia provided by Neira (2011) of 114,900 to 169,000 t and within the range of plausible estimates of biomass suggested for the ecosystem by Fulton (2013) of 130,000 to 170,000 t.

This estimate of spawning biomass was based on large numbers of samples of both eggs and adults. Most of the estimates of spawning biomass obtained in the sensitivity analyses were between ~95,000 t and ~215,000 t and within the 95% confidence intervals for the primary estimate (i.e. 40,218 to 347,213 t). Estimates of spawning biomass outside those ranges were obtained for potentially plausible values for only two parameters, i.e. ~428,000 t for the estimate of egg production obtained from Neira (2011) and ~46,000 t for the estimate of the average spawning fraction for *T. trachurus.* The estimates of egg production (28.9 eggs.day^{-1}.m^{-2}) and spawning fraction (0.56) obtained in this study are both low compared to the estimates of these parameters obtained in the other studies (e.g. 78 eggs.day^{-1}.m^{-2} from Neira *et al.* (2011) and 0.2 for *T. trachurus*). However, there is strong evidence that estimates of both parameters obtained in the present study are reliable. For example, estimates of egg production were based on a relatively large number of samples (117) and estimates provided by three of the four models used were similar (range 27.9 – 30.2 eggs.day^{-1}.m^{-2}). Estimates of spawning fraction were also based on a relatively large number of samples (16) that were obtained using a method that may sample the adult population without bias. Perhaps most importantly, the low values for two parameters provide two independent lines of evidence that suggest the spawning rates of Jack Mackerel off eastern Australia during January 2014 were low.

5.2 Australian Sardine

This is the first large scale study of the spawning patterns of Australian Sardine off eastern Australia to be conducted during summer. It was the first to identify that a significant spawning area exists off northern Tasmania and in Bass Strait during this period, although Neira (2005) collected two Australian Sardine eggs from stations located near the oil rigs in Bass Strait during February 1999. As no adult samples were collected during this study and data from South Australia were used to estimate adult parameters, the

estimate of spawning biomass for Australian Sardine off eastern Australia during summer (i.e. 10,962 t), should be treated with caution. This estimate may also be negatively biased as the entire spawning area may not have been sampled. It is important to note that this not an estimate of the total adult biomass of Australian Sardine off eastern Australia, it is only an estimate of the portion of the population that was spawning in this part of the range during that period.

This study provides unequivocal evidence that Australian Sardine occurs in this area at least in some years and provides insights into the quantum of catches that may be suitable for any developmental fishery that may be established in the region. The small number of Australian Sardine eggs collected off the mainland coast during the survey suggested that limited spawning was occurring in that region during this period. The main spawning area for Australian Sardine off eastern Australia occurs off northern NSW and southern Queensland during late winter and early spring (Ward and Staunton-Smith 2002). During July 2004, the spawning biomass of Australian Sardine off eastern Australia was estimated to be ~29,000 t, with likely estimates ranging from ~25,000–35,000 t (Rogers and Ward 2007). Results from a DEPM survey conducted off northern NSW and southern Queensland during September 2015 (e.g. FRDC 2014-033, Egg distribution, reproductive parameters and spawning biomass of Blue Mackerel, Australian Sardine and Tailor off the East Coast during late winter and early spring) will provide an estimate of the current abundance of adult Australian Sardine off eastern Australia. Samples of Australian Sardine eggs obtained in the present and related studies (e.g. FRDC 2014-033) and stored in ethanol provide a cost-effective opportunity for using molecular techniques to investigate the stock structure of this species off eastern Australia. Future application of the DEPM to Australian Sardine off eastern Australia should have a strong focus on collecting representative samples of the adult population.

6. Conclusions

This study established an effective fishery-independent method (i.e. modified demersal trawling) for sampling adult Jack Mackerel. As a result of this development, it is the first study to provide estimates of adult reproductive parameters for this species for application of the DEPM. Samples collected during these surveys enabled reliable estimates of the spawning biomass of Jack Mackerel off eastern Australia to be calculated for the first time and provided important new insights into the spawning patterns of this species in this region during summer. For example, during this study most spawning occurred in much shallower depths than was observed in previous more restricted studies and overall spawning fractions were much lower than those that have been estimated for similar species. These findings have important implications for the design of adult surveys conducted as part of future applications of the DEPM to Jack Mackerel off eastern Australia. For example, future surveys should be stratified by depth and latitude to ensure that fish of different sizes with potentially different spawning rates are sampled representatively.

In contrast to Jack Mackerel, adult samples of Australian Sardine were not obtained during this study, mainly because of logistical constraints (i.e. time required to collect the plankton samples). However, the fishery-independent method (gill-netting) that has been used successfully to sample Australian Sardine off South Australia since 1998 (e.g. Ward *et al.* 2011) has been shown to work off the east coast (e.g. Staunton-Smith and Ward 2000). Future applications of the DEPM to Australian Sardine off eastern Australia should focus on

using gill-nets and/or demersal trawling to obtain samples required for estimation of adult reproductive parameters.

The plankton surveys conducted in this study successfully identified the distribution and abundance of the eggs and larvae of Jack Mackerel and Australian Sardine off the east coast during the summer of 2014. As expected, most Jack Mackerel eggs found were off Tasmania and in Bass Strait. Most Australian Sardine eggs were also collected mainly from this region with relatively few obtained from waters off the east coast of the mainland. Our results suggest that future DEPM surveys of Jack Mackerel and Australian Sardine off eastern Australia during summer may not need to extend as far north as the survey that was conducted in January 2014.

The estimate of the spawning biomass of Jack Mackerel off eastern Australia during January 2014 is based on robust estimates of all DEPM parameters, except batch fecundity. This parameter is closely linked to female weight and has relatively limited influence of estimates of spawning biomass. Hence, the estimate of the spawning biomass of Jack Mackerel off eastern Australia during January 2014 provides a suitable basis for setting future catch limits for this species. The estimate of spawning biomass for Australian Sardine off eastern Australia during January 2014 should be treated with caution but provides insights into the catch limits that may be set for any developmental fishery that may be established in the region.

7. Implications and recommendations

This study provides the first estimates of the spawning biomass of Jack Mackerel and Australian Sardine off eastern Australia during summer. These results are suitable for use in the future management of the fisheries for this species.

The estimate of the spawning biomass of Jack Mackerel (i.e. 157,805; 95% CI = 59,570 – 358,731), is considered robust and suitable for setting recommended biological catches as outlined under the Harvest strategy for the SPF.

The estimate of the spawning biomass of Australian sardine (i.e. 10,962 t) has a greater level of uncertainty than the estimate for Jack Mackerel, but is considered useful for informing future management of any fishery for this species that may be developed off Tasmania in the next few years. The localised area from which the eggs of Australian Sardine were collected during this study, suggests that the hypothesis that separate sub-populations (stocks) of this species may occur off eastern Australia may warrant investigation.

8. Further development

As the first dedicated application of the DEPM to Jack Mackerel, this study made some crucial technical developments (e.g. establishing a fishery-independent sampling method) and filled several critical knowledge gaps (e.g. provided the first robust estimates for five of the six DEPM parameters). However, the study also identified some additional knowledge gaps that should be filled to further improve the accuracy and precision of future estimates of spawning biomass. As discussed above, there is a need to assess the temporal

variations in the relationship between depth, latitude, fish size and spawning rates of Jack Mackerel during the summer on the spawning grounds off eastern Tasmania and in Bass Strait.

The information required to address this issue would ideally be collected as part of a second targeted application of the DEPM to this stock. That study should also involve regular (e.g. fortnightly) concurrent sampling of eggs and adults throughout the entire summer (i.e. December to February) from one or more cross-shelf transects located in the main spawning area. This information on the variations in spawning rates of Jack Mackerel would be used to optimise the design of future applications of the DEPM. The adult survey that would be conducted as part of the DEPM survey should also be stratified by depth and latitude (to examine spatial patterns in spawning rates) and extended beyond the known spawning area to provide insights into the relative abundance of non-spawning adult Jack Mackerel off the east coast during the summer spawning period.

The plankton survey conducted as part of a second targeted application of the DEPM to Jack Mackerel would again provide valuable information on the spawning area and egg production of Australian Sardine. If this study is conducted it is important that adult samples of Australian Sardine are collected from Tasmanian waters and in Bass Strait over the same period that the plankton survey is conducted.

Samples of Australian Sardine eggs obtained in this and related studies (e.g. FRDC 2014-033) and stored in ethanol provide a cost-effective opportunity for investigating the stock structure of this species off eastern Australia.

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 the RAG to identify recommended biological catches for Jack Mackerel and Australian Sardine.

Results of this project will also be presented at stakeholder fora to be held in Canberra and Hobart and at other fora as requested by AFMA and/or FRDC.

Planned Outcomes and Benefits

1. Estimates of spawning biomass will be used to set sustainable TACs for Jack Mackerel in the SPF and inform sustainable management of Australian Sardine fisheries along the east coast. Methods developed will improve reliability of future estimates of spawning biomass. Beneficiaries include: AFMA, NSW DPI, Vic DPI, Tas DPI, commercial and recreational fishers in all jurisdictions along the East Coast, conservation groups and the broader community.
2. Potential increases in the TACs for Jack Mackerel in the SPF through updating of DEPM estimates of spawning biomass. Under the current harvest strategy indicative exploitation rates are halved when biomass estimates are more than 5 years old.
3. Enhanced sustainability of the Australian Sardine fisheries off the east coast. Reduced risk to licence holders through enhanced social licence to operate due to increased public confidence in fisheries' sustainability.

10. Project materials developed

Some of the data obtained during this study was used in an Honours project undertaken during 2015.

Sexton, S (2014). Characterizing the spawning habitat of Jack Mackerel off eastern Australia to optimise future survey design. Honours Thesis, Flinders University. 40 p.

A manuscript based on the thesis is currently being prepared for submission to Fisheries Research.

A manuscript that covers aspects of the study included in the present report will also be submitted to Fisheries Research later this year.

Outputs and outcomes

The primary outputs and outcomes of the project are:

1. A new fishery-independent method for sampling adult Jack Mackerel.
2. The first estimates of key reproductive parameters (spawning fraction, batch fecundity) of Jack Mackerel.
3. Estimate of the spawning area and total mean daily egg production of Jack Mackerel and Australian Sardine off eastern Australia during summer.
4. Identification of environmental factors influencing the distribution abundance of eggs and larvae and spawning patterns of adult Jack Mackerel off eastern Australia during summer.
5. Preliminary estimates of spawning biomass of Jack Mackerel and Australian Sardine off the east coast during summer.
6. Improved assessment and management of the SPF.

11. References

Abaunza, P., Gordo, L., Karlou-Riga, C., Murta, A., Eltink, A.T.G.W., Santamaria, M. T. G., Zimmermann, C., Hammer, C., Lucio, P., Iversen, S. A., Molloy, J. and Gallo, E. (2003). Growth and reproduction of horse mackerel, *Trachurus trachurus* (Carangidae). *Reviews in Fish Biology and Fisheries* **13**: 27-61.

AFMA (Australian Fisheries Management Authority) (2009). Small Pelagic Fishery management plan 2009. AFMA, Canberra, Federal Register of Legislative Instruments F2010L00081.

Ahlstrom E.H. and Ball. O.P. (1954). Description of eggs and larvae of jack mackerel (*Trachurus symmetricus*) and distribution and abundance of larvae in 1950 and 1951. *Fishery Bulletin United States* **56**: 209-245.

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**(2): 750-767.

Baddeley, A. and Turner, R. (2005). spatstat: An R Package for Analyzing Spatial Point Patterns. Journal of Statistical Software 12(6), 1-42. URL http://www.jstatsoft.org/v12/i06/.

Bernal, M., Stratoudakis, Y., Wood, S., Ibaibarriaga, L., Valde´s, L., and Borchers, D. (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* **68**: 528–536.

Blaber, S.J.M. and Bulman, C.M. (1987). Diets of fishes of the upper continental slope of eastern Tasmania: Content, calorific values, dietary overlap and trophic relationships. *Marine Biology***. 95:** 345-56.

Bulman, C., Condie, S., Findlay, J., Hender, J., Ward, B. and Young, J. (2008). Management zones from small pelagic fish species stock structure in southern Australian waters. FRDC project no. 2006/076. Report to the Fisheries Research and Development Corporation and Australian Fisheries Management Authority. 126 pp

Crossland, J. (1981). Fish eggs and larvae of the Hauraki Gulf, New Zealand. *Fisheries Research Bulletin of the New Zealand Ministry of Agriculture and Fisheries* **23**: 1-61

Cunha, M.E., Vendrell, C. and Goncalves, P. (2008). Experimental study of the dependence of embryonic development of *Trachurus trachurus* eggs on temperature. *Ices Journal of Marine Science* **65**: 17-24.

Dimmlich, W.F., Ward, T.M. and Breed 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 Tibshirani, R.J. (1993). An Introduction to the Bootstrap. New York, Chapman and Hall, Inc.

Fletcher, W. J., Lo, N.C.H., Hayes, E.A., Tregonning, R.J. and Blight, S.J. (1996). Use of the daily egg production method to estimate the stock size of western Australian sardines (*Sardinops sagax*). *Marine and Freshwater Research* **47**(6): 819-825.

Furlani, D., Williams, A. and Bax, N. (2000). Fish biology (length and age). In: *Habitat and fisheries production in the South East Fishery ecosystem.* Final report to the Fisheries Research and Development Corporation, Project No. 94/040, (eds N.J. Bax and A. Williams). Division of Marine Research, CSIRO Marine Laboratories, Hobart.

Ganias,K, Rakka, M., Vavalidis, T., and Nunes, T. (2010). Measuring batch fecundity using automated particle counting. Fisheries Research 106 (3), 570-

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

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

Gomon, M., Bray, D. and Kuiter, R. (2008). *Fishes of the Australia's South Coast*. Museum of Victoria. New Holland Publishers (Australia). 928 pp.

Goncalves, P., Costa, A.M. and Murta, A.G. (2009). Estimates of batch fecundity and spawning fraction for the southern stock of horse mackerel (Trachurus trachurus) in ICES Division IXa. *Ices Journal of Marine Science* **66**: 617-622.

Gray, C.A. and Miskiewicz, A.G. (2000). Larval Fish Assemblages in South-east Australian Coastal Waters: Seasonal and Spatial Structure. *Estuarine, Coastal and Shelf Science* **50**: 549–570

Hoedt, F.E. and Dimmlich, W.F. (1995). Egg and Larval Abundance and Spawning Localities of the Anchovy (*Engraulis australis*) and Pilchard (*Sardinops neopilchardus*) near Phillip Island, Victoria. *Marine and Freshwater Research* **46**: 735-743.

Hunter, J.R., Lo, N.C.H. and Leong, R.J.H. (1985). Batch fecundity in multiple spawning fishes. *NOAA Technical Report NMFS* **36**: 67-77.

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

Jordan, A., Pullen, G., Marshall, J. and Williams, H. (1995). Temporal and spatial patterns of spawning in jack mackerel, *Trachurus declivis* (Pisces, Carangidae), during 1988-91 in eastern Tasmanian waters. *Marine and Freshwater Research* **46**: 831-842.

Kailola, P., Williams, M.J., Stewart, P.C., Reichelt, R.E., McNee, A. and Grieve, C. (eds). (1993). *Australian Fisheries Resources*. Bureau of Resource Sciences, Department of Primary Industries and Energy and the Fisheries Research and Development Corporation, Canberra.

Karlou-Riga, C. and Economidis, P.S. (1997). Spawning frequency and batch fecundity of horse mackerel, *Trachurus trachurus* (L.), in the Saronikos Gulf (Greece). *Journal of Applied Ichthyology-Zeitschrift Fur Angewandte Ichthyologie* **13**: 97-104.

Lasker, R. (1985). An egg production method for estimating spawning biomass of pelagic fish: application to northern anchovy, *Engraulis mordax*. *NOAA Tech. Rep. NMFS*. **36**: 1-99.

Macewicz, B.J. and Hunter, J.R. (1993). Spawning frequency and batch fecundity of jack mackerel, *Trachurus symmetricus*, off California During 1991. *California Cooperative Oceanic Fisheries Investigations Reports* **34**: 112-121.

Marshall, J., Pullen, G. and Jordan, A. (1993). Reproductive-biology and sexual maturity of female jack mackerel, Trachurus declivis (Jenyns), in eastern Tasmanian waters. *Australian Journal of Marine and Freshwater Research* **44**: 799-809.

Maxwell, J.G.H. (1979). Jack mackerel. CSIRO Division of Fisheries and Oceanography Fishery Situation Report No. 2. 18 pp.

May, J.L., and Blaber, S.J.M. (1989). Benthic and pelagic fish biomass of the upper continental slope off eastern Tasmania. *Marine Biology* **101**: 11-25.

McGarvey, R. and Kinloch, M.A. (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.

Moore, A. and Skirtun, M. (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., Miskiewicz, A.G., and Trnski, T. (1998). Larvae of temperate Australian fishes: Laboratory guide for larval fish identification. University of Western Australia Press: Western Australia. 474 pp.

Neira, F.J., Sporcic, I. and Longmore, A.R. (1999). Biology and fishery of pilchards, *Sardinops sagax* (Clupeidae), within a large south-eastern Australian bay. *Marine and Freshwater Research* **50**: 43-55.

Neira F.J. (2005). Summer and winter plankton fish assemblages around offshore oil and gas platforms in south-eastern Australia. *Estuarine Coastal and Shelf Science* **63**: 589-604.

Neira, F.J. and Lyle, J.M. (2008). 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. (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. (2011). Application of daily egg production to estimate biomass of jack mackerel, *Trachurus declivis* – a key fish species in the pelagic ecosystem of south-eastern Australia. Final Report to the Winifred Violet Scott Charitable Trust. Fisheries, Aquaculture and Coasts Centre, Institute for Marine and Antarctic Studies (IMAS), University of Tasmania. 42pp.

Neira, F.J., Perry, R., Burridge, C., Lyle, J. and Keane, J. (2014). Molecular discrimination of shelf-spawned eggs of two co-occurring *Trachurus* spp. (Carangidae) in southeastern Australia: a key step to future egg-based biomass estimates. *ICES Journal of Marine Science* doi:10.1093/icesjms/fsu151.

Perry, R.A. (2011). Spawning habitat of two commercial mackerel species (*Trachurus spp.*) in south-eastern Australia based on distribution of eggs identified using molecular techniques. Honours Thesis, University of Tasmania. 111pp

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

Picquelle, S. and Stauffer, G. (1985). Parameter estimation for an egg production method of anchovy biomass assessment. In: Lasker, R. "An egg production method for estimating spawning biomass of pelagic fish: application to northern anchovy, *Engraulis mordax*." *NOAA Tech. Rep. NMFS*. **36**: 7-17.

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

Pullen, G. and Lyle, J. (1994). Jack mackerel research review. The Department of Primary Industry and Fisheries, Marine Resources Division.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/)

Rogers, P. J. and Ward, T. M. (2007). Application of a 'case-building approach' to investigate the age distributions and growth dynamics of Australian sardine (*Sardinops sagax*) off South Australia. *Marine and Freshwater Research.* **58**:5, 461-474.

Ruiz, P., Sepulveda, A., Cubillos, L., Oyarzun, C. and Chong, J. (2008). Reproductive parameters and spawning biomass of Chiean Jack Mackerel (*Trachurus murphyi*) in 1999-2008), determined by the Daily Egg Production Method. Eighth International Meeting: SWG: Jack Mackerel Sub-Group - SP-08-SWG-JM-02.

Sexton, S (2014). Characterizing the spawning habitat of Jack Mackerel off eastern Australia to optimise future survey design. Honours Thesis, Flinders University. 40 p

Shuntov, V.P. (1969). Some features of the ecology of pelagic fishes in the Great Australian Bight. *Problems of Ichthyology* **9**: 801–809.

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

Staunton-Smith, J. and T. M. Ward (2000). Stock assessment of pelagic bait fishes in southern Queensland with special reference to pilchards (*Sardinops sagax*). Brisbane, Department of Primary Industries, Queensland. FRDC Project 95/043. 126pp.

Stevens, J.D., Hausfeld, H.F. and Davenport, S.R. (1984). Observations on the biology, distribution and abundance of *Trachurus declivis, Sardinops neopilchardus* and *Scomber australasicus* in the Great Australian Bight. CSIRO Marine Laboratories. 29pp.

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

Stewart, J., Ballinger, G. and Ferrell (2010). Review of the biology and fishery for Australian sardines (Sardinops sagax) in New South Wales – *2010.* Industry and Investment NSW – Fisheries Research Report No. 26. ISSN 1837-2120. 59pp.

Taylor, L.R. (1961) Aggregation, variance and the mean. *Nature* **189:** 732–735.

Tracey, S., Buxton, C., Gardner, C., Green, B., Hartmann, K., Haward, M., Jabour, J., Lyle, J. and McDonald, J. (2013). Super trawler scuppered in Australian fisheries management reform. *Fisheries* **38**: 345–350.

Ward, T.M., Kinloch, M., G.K., J. and Neira, F.J. (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. 324pp.

Ward, T.M. and McLeay, L.J. (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. 41pp.

Ward, T.M., Hoedt, F., McLeay, L., Dimmlich, W.F., Jackson, G., Rogers, P.J. and Jones, K. (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? *Mar. Ecol. Prog. Ser.* **220**: 241-251.

Ward, T.M., Hoedt, F., McLeay, L.J., Dimmlich, W.F., Jackson, G., Kinloch, G., McGarvey, R., Rogers, P. J. and Jones, K. (2001b). Effects of the 1995 and 1998 mass mortalities on the spawning biomass of *Sardinops sagax* in South Australia. *ICES J. Mar. Sci.* **58**: 865-875.

Ward, T.M. and Staunton-Smith, J. (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. and Rogers, P.J., Eds. (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. Adelaide, SARDI Aquatic Science. 468pp.

Ward, T.M., Rogers P.J. and L.J. McLeay (2009) Evaluating use of the Daily Egg Production Method for stock assessment of blue mackerel, *Scomber australasicus*. *Marine and Freshwater Research* **60**: 112–128.

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

Ward, T.M., Ivey, A.R. and Earl, J. (2014a). 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. 105pp.

Ward, T.M., Ivey, A.R. and Carroll, J.D. (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. 33pp.

Watson, D.F. (1981). Computing the n-dimensional Delaunay tessellation with application to Voronoi polytopes. *Computer Journal* **24**: 167-172.

Webb, B.F. (1976). Aspects of the biology of Jack Mackerel. *Trachurus declivis* (Jenyns) from south-east Australian waters. *Tasmanian Fisheries Research* **10**: 1-14.

Webb, B.F. and Grant, C.J. (1979). Age and growth of jack mackerel, *Trachurus declivis* (Jenyns), from southeastern Australian waters. *Australian Journal of Marine and Freshwater Research* **30**: 1-9.

White, K.V. and Fletcher, W.J. (1996). Identifying the developmental stages for eggs of the Australian sardine, *Sardinops sagax.* Fisheries Western Australia. Fisheries Research Report No. 103pp.

Wolfe, D. C. (1971). Pelagic fish survey. 1. Seasonal availability of fish schools in inshore waters. *Tasmanian Fisheries Research* **5**: 2-11.

12. Appendices

Appendix 1. Genetic identification of Jack Mackerel eggs

Keane, J.P. and Lyle, J.M.

A1.1 Introduction

Eggs and larvae of Jack Mackerel *(Trachurus declivis*) and Yellowtail Scad (*T. novaezelandiae*) occur simultaneously in shelf waters of south eastern Australia. Morphologically the eggs of these species possess almost identical distinguishing characteristics apart from slightly differing but overlapping egg diameters (Neira *et al.* 2014). In addition, eggs of other carangid species, such as Silver Trevally (*Pseudocaranx dentex*) also possess near-identical morphological features and are known to occur off south eastern Australia at similar times. Furthermore, it is not known if eggs of other species present in the area possess similar morphological characters to Jack Mackerel as the eggs of just a few fish species have been described in Australia. As a consequence morphological identification of *Trachurus* eggs to species level remains challenging.

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 differentiate ethanol preserved eggs of Jack Mackerel from Yellowtail Scad and Silver Trevally, and investigate the effect of preservation method on egg diameters; egg diameter has been suggested as a diagnostic characteristic in separating Jack Mackerel from the other co-occurring Carangids (Crossland 1981; Neira *et al.* 2014). Furthermore, since fish eggs can undergo large morphological changes during development, a sample of eggs possessing similar but not identical morphological characteristics to *Trachurus* were also tested molecularly.

A1.2 Methods

A1.2.1 Samples

Replicate plankton tows to the main DEPM survey (see section *3.2.1 Plankton Sampling*) were completed at 37 stations (Figure 1) and captured plankton was immediately drained of excess seawater and preserved in 96% ethanol. Replicate tows were conducted at stations 2 (shelf break) and 4 (10 nm shoreward of the shelf break) on every second transect in the mid survey region and every forth transect at the survey extremities. The sampling regime was implemented to cover the temperature and depth characteristics of spawning habitat of Jack Mackerel described from previous studies (Jordan *et al.* 1995; Perry, 2011).

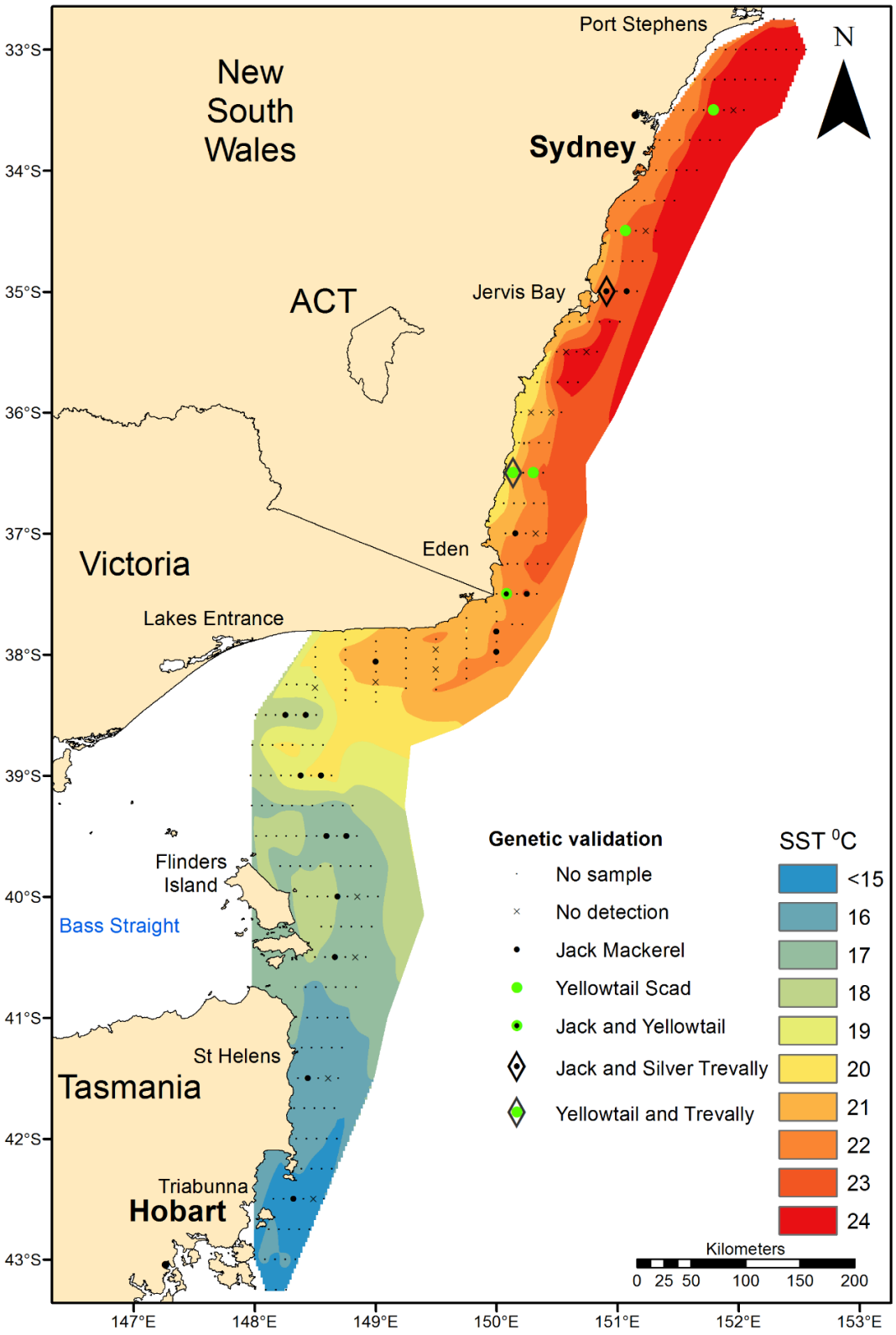


Figure 1. Map of south eastern Australia showing location of replicate plankton hauls for molecular analyses and location of molecular confirmed eggs of Jack Mackerel, Yellowtail Scad and Silver Trevally.

Ichthyoplankton samples were collected using a bongo sampler equipped with 500 um mesh, two 3 m long plankton nets enclosed in a purpose built, weighted stainless steel frame to facilitate vertical drops. The mouth of each net (0.6 m diameter) was fitted with a General Oceanics flowmeter to estimate total volume of water filtered during each vertical haul. The net was lowered to within 10 m of the seabed or to a maximum of 200 m.

A.1.2.2 Morphometric identification

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

Preserved eggs were rehydrated in distilled water (immersion time of approximately 5 min) to better reflect diameters of fresh eggs, and measured digitally to 0.01 mm under a stereomicroscope. Diameters for rehydrated eggs identified genetically as Jack Mackerel*,* Yellowtail Scad or Silver Trevally were plotted using standard box and whisker plots. Formalin preserved eggs were measured in the same manner for comparison. Since eggs of Yellowtail Scad and Silver Trevally could not be distinguished morphologically they were grouped as 'Other Carangidae'. Variances of mean egg diameters were tested using a standard F-Test.

A.1.2.3 Molecular identification

A molecular approach of Mitochondrial DNA (mtDNA) extraction, amplification, and sequencing for *Trachurus* spp. developed by Perry (2011) and refined by Neira *et al.* (2014) was employed to discriminate between eggs of Jack Mackerel, Yellowtail Scad and Silver Trevally*.* DNA extractions from eggs identified based on morphological characters were carried out using the QIAamp DNA Micro Kit (QIAGEN, USA) following the manufacturer's protocol for tissue extraction. The concentration and quality of DNA extracted was tested using the NanoDrop 8000 Spectrophotometer, with samples having less than the detection limit of 2.5ng/ul considered to have insufficient DNA for identification.

Amplification by polymerase chain reactions (PCRs) were performed using MyTaq HSTM DNA Polymerase (Bioline) with PCR product purification and bi-directional sequencing performed by Macrogen Inc. (Seoul, Republic of Korea) (see Neira *et al.* 2014 for full methods). The primers F3 and R3 (F3: 5'-GTA GGA AAY ACC CTC GTC CA-3', R3: 5'-ATT GATCGG AGA ATG GCG TA-3') were used in the PCRs to amplify a 385 bp fragment of cytochrome B (*Cyt b*) containing species-diagnostic Single Nucleotide Polymorphisms (SNPs) in positions 558, 588, and 825 (Table 1; Rozen and Skaletsky, 2000; Perry, 2011; Neira *et al.* 2014). Sequences were aligned to reference data in the Fish Barcode of Life Database (BOLD) using BioEdit biological sequence alignment editor.

Table 1. Species-diagnostic Single Nucleotide Polymorphisms (SNPs) in positions 558, 588, and 825 for Jack Mackerel, Yellowtail Scad and Silver Trevally (Rozen and Skaletsky, 2000; Perry, 2011; Neira et al., 2014).

	Base		
Base position	558	588	825
Jack Mackerel	G	C	A
Yellowtail Scad	A	T	G
Silver Trevally	A	C	G

The number of eggs subjected to mtDNA testing varied between stations according to raw abundances of eggs morphologically identified as *Trachurus*. All such eggs were tested in stations with 20 or fewer eggs, while for stations with >20 eggs, a minimum of 20 eggs were randomly selected for testing. Eggs from all developmental stages (Cunha *et al.* 2008) were tested. In addition, up to three eggs possessing similar characteristics to *Trachurus* (e.g. diameter, pigmentation) were selected per station if present across the entire survey area for mtDNA testing.

A1.3 Results

A total of 267 eggs identified morphologically as *Trachurus* spp. as well as 27 eggs possessing similar characteristics to *Trachurus* were subjected to mtDNA analysis. Of the suspected *Trachurus* eggs, a total of 242 (89%) yielded quality DNA to enable identification to species level (Table 2). Alignment of the *cyt b* sequences revealed a total of 194 Jack Mackerel, *23* Yellowtail Scad and five Silver Trevally. A further 20 eggs were regarded as Trachurus "variant haplotypes" as they exhibited variation in the diagnostic nucleotide base pairs (See Neira *et al*. 2014; Table 3). From the 27 suspected non-*Trachurus* eggs subjected to molecular analyses, none amplified using Primers F3 and R3 indicating they are eggs of species from families other than Carangidae.

Table 2. *Cyt b*-based specific identifications of eggs collected during the January 2014 survey along southeastern Australia, and initially identified as *Trachurus* using morphological characters.

Species	**n**	**%**
Jack Mackerel	194	80.2
Yellowtail Scad	23	9.5
Silver Trevally	5	2.1
Trachurus variant	20	8.3
Total eggs sequenced	242	

Eggs of Jack Mackerel were confirmed to be present in samples via mtDNA analyses between Triabunna, Southern Tasmania (42°30' S) and Eden, southern NSW (37°0' S) as well as being present off Jervis Bay (35°0' S; Figure 1). Eggs of Yellowtail Scad and Silver Trevally were verified at stations along the NSW coast (north of 37°30' S), with Silver Trevally only identified at two stations (Figure 1).

Table 3. Summary of variant haplotypes obtained in eggs of Jack Mackerel and Yellowtail Scad found during this study. Station, latitude and egg diameter (mm) is also provided.

Transect-Station	Latitude (S)	Diameter (mm)	Haplotype	Inferred species
4-2	42.5	0.87	ACA	Jack Mackerel
8-4	41.5	0.89	GCG	Jack Mackerel
8-4	41.5	0.86	GCG	Jack Mackerel
16-4	39.5	1.03	GCG	Jack Mackerel
18-4	39.0	0.94	GCG	Jack Mackerel
20-2	38.5	0.96	ACA	Jack Mackerel
20-2	38.5	0.96	ACA	Jack Mackerel
22-3	38.3	0.93	GGA	Jack Mackerel
24-4	38.1	0.94	ACA	Jack Mackerel
24-4	38.1	0.94	ACA	Jack Mackerel
28-4	37.8	0.94	GCC	Jack Mackerel
30-2	37.5	0.93	TCG	Jack Mackerel
30-4	37.5	0.83	GCG	Jack Mackerel
30-4	37.5	0.67	GTG	Yellowtail Scad
34-2	36.5	0.78	GTG	Yellowtail Scad
38-2	35.5	0.91	GTG	Yellowtail Scad
38-4	35.5	0.93	GTG	Yellowtail Scad
40-2	35.0	0.81	GTG	Yellowtail Scad
42-4	34.5	0.8	ACG	Yellowtail Scad
42-4	34.5	0.81	GTT	Yellowtail Scad

The mean diameter of molecular verified and rehydrated Jack Mackerel eggs (0.947 mm) was significantly larger than those of Yellowtail Scad (0.804 mm) and Silver Trevally (0.775 mm; Table 4, Figure 2). There was no significant difference in the mean diameter of rehydrated ethanol preserved Jack Mackerel eggs compared to formalin preserved Jack Mackerel eggs (0.954 mm). Ethanol preserved Jack Mackerel eggs, however, had a broader range (Figure 2) and significantly higher variance ($P<0.05$) than formalin preserved

eggs. This resulted in an overlap in the diameters of ethanol preserved Jack Mackerel and Yellowtail Scad eggs whereas there was minimal overlap in the diameters of formalin preserved Jack Mackerel eggs and those of other Carangids (Figures 2, 3).

Table 4. Mean diameter, variance, minimum, maximum and confidence intervals of ethanol and formalin preserved Jack Mackerel, Yellowtail Scad and Silver Trevally eggs.

	Ethanol preserved			Formalin preserved	
	Jack Mackerel	Yellowtail Scad	Silver Trevally	Jack Mackerel	Other Carangidae
Mean	0.947	0.804	0.775	0.954	0.787
Var.	0.00195	0.00345	0.00004	0.00134	0.00090
Min.	0.806	0.675	0.768	0.853	0.674
Max.	1.103	0.944	0.782	1.046	0.858
95% CI	0.006	0.022	0.007	0.004	0.005
n	207	30	5	373	159

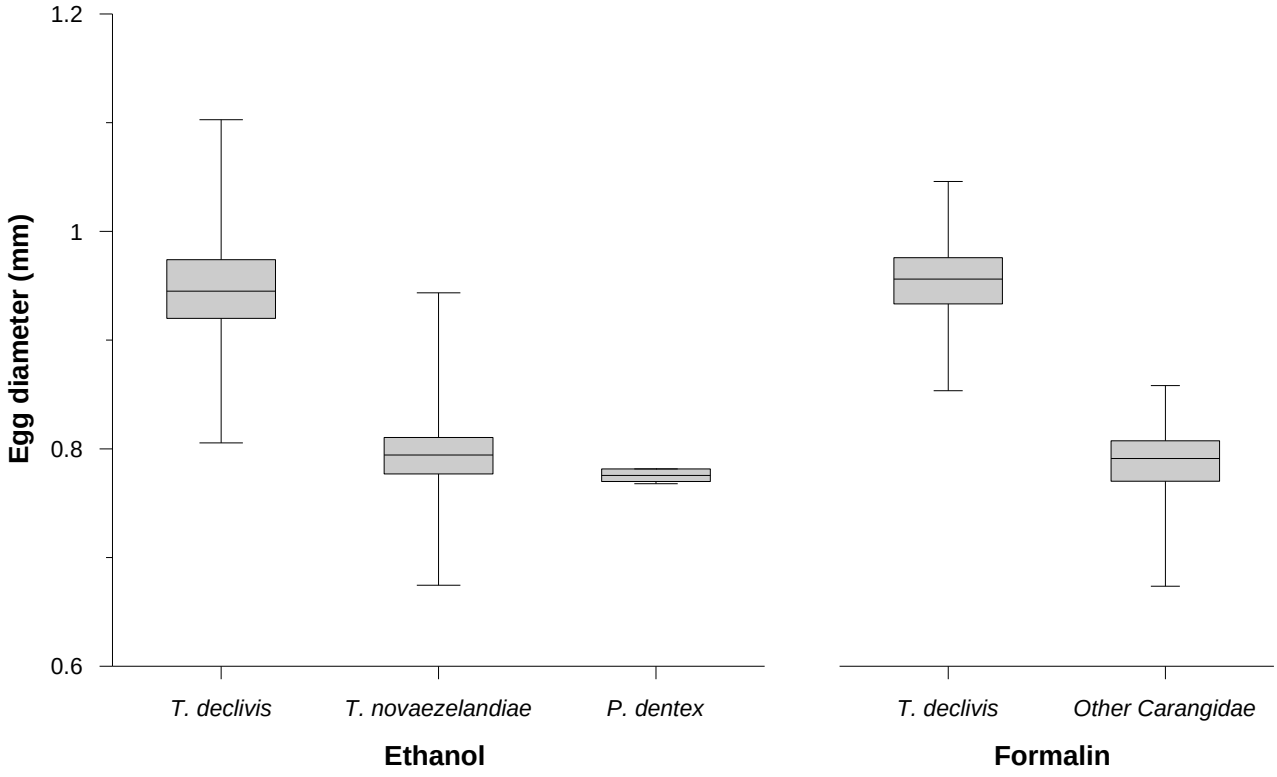


Figure 2. Diameters (mm) of mtDNA verified rehydrated ethanol preserved eggs of Jack Mackerel (n = 207), Yellowtail Scad (n = 30) and Silver Trevally (n = 5) (left) and morphologically identified formalin preserved eggs of Jack Mackerel (n=373) and other Carangids (Yellowtail Scad, Silver Trevally; n=159; right). Data on each box–whisker plot correspond to the median, 1st and 3rd quartiles, and range (minimum and maximum).

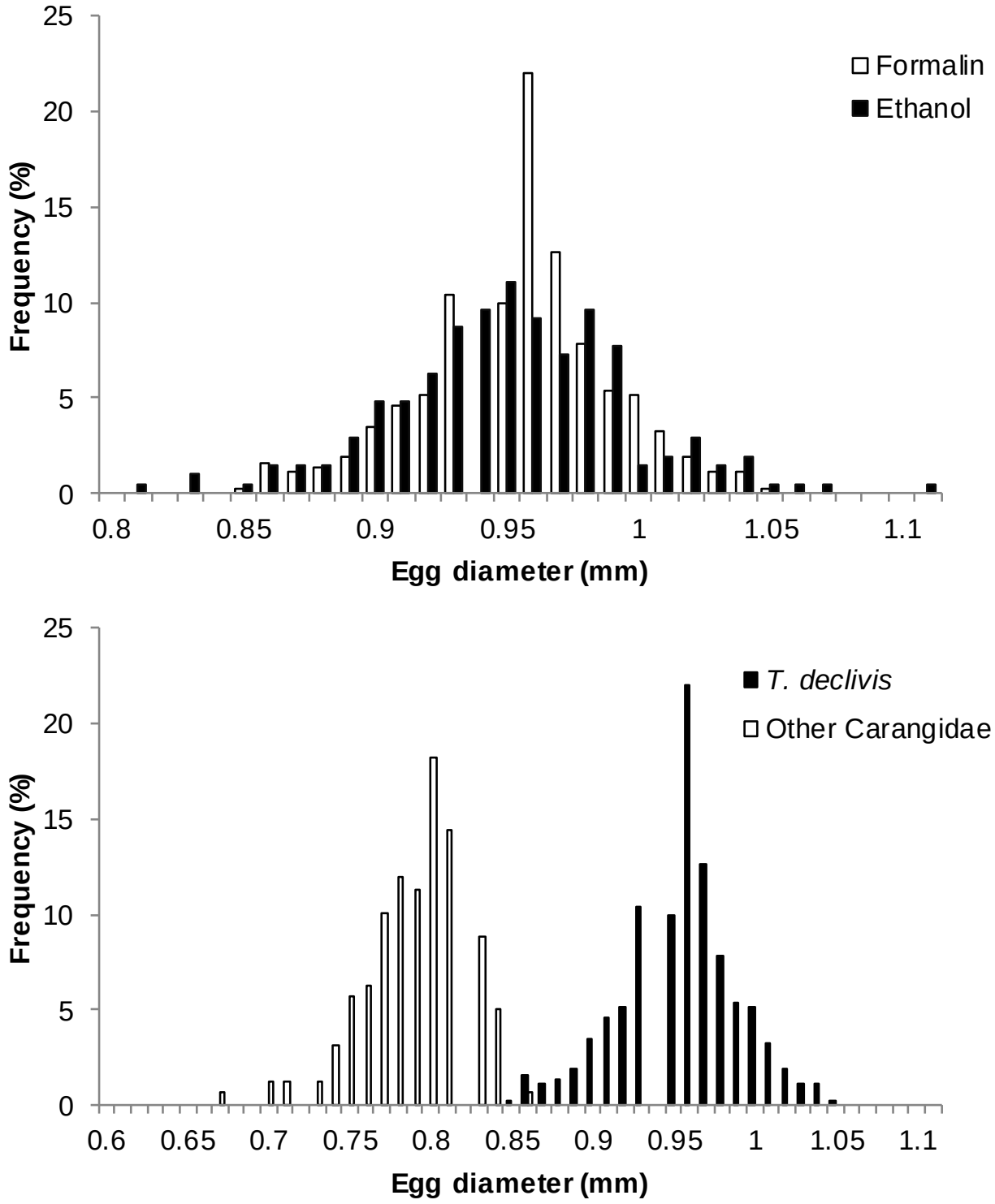


Figure 3. Length-frequency histograms of formalin preserved eggs of Jack Mackerel (n=373) compared to ethanol preserved eggs of Jack Mackerel (n=207) (top) and formalin preserved eggs of other Carangids (Yellowtail Scad, Silver Trevally; n=159) (bottom).

A1.4 Discussion

Molecular analyses successfully validated eggs identified as *Trachurus* using species-specific morphological characters and allowed Jack Mackerel eggs to be categorically separated from those of the morphologically similar Yellowtail Scad and Silver Trevally based on egg diameters. Despite interspecies mtDNA sequence divergence in *Trachurus* being among the lowest recorded in any marine fish genus (Cardenas *et al*. 2005), primers F3 and R3 successfully amplified a 385 bp fragment of Cyt b containing the diagnostic SNPs at positions 558, 588, and 825 allowing for robust approach to species identification.

Previous studies have shown that ethanol preserved eggs of *T. declivis* are significantly larger than those of Yellowtail Scad (and Silver Trevally) but with some overlap in diameters (Perry, 2011; Neria *et al.* 2014). The overlap in egg diameters not only makes morphometric identification problematic in some cases but also introduces uncertainty into species spawning area and habitat characterisation, as well as estimating egg abundances for use in DEPM assessments.

Through the comparison of replicate samples preserved in ethanol or formalin and the use of molecular methods to confirm the identity of eggs this study established that preservation of eggs in formalin significantly reduced the variation in egg diameters compared with ethanol preservation, resulting in minimal overlap in the egg diameter range of Jack Mackerel with that of Yellowtail Scad and Silver Trevally. This finding means that egg diameter, coupled with other morphological characters, can be used with a high level of confidence to distinguish Jack Mackerel from Yellowtail Scad and Silver Trevally in formalin preserved samples, but not the latter two species from each other. Higher variation in the diameters of ethanol preserved eggs is likely attributable to damage caused during egg dehydration and rehydration processes when interchanging between alcohol and water media.

Diameters of molecular validated eggs were marginally smaller but not dissimilar to those found by Neira (2014) which were 0.78 – 1.10 mm for Jack Mackerel (0.97 mm ± 0.01), 0.70 – 0.98 mm for Yellowtail Scad (0.82 ± 0.01) and 0.75 – 0.99 mm for Silver Trevally (0.82 ± 0.02). Minor discrepancies in diameter may reflect varied environmental conditions, the structure of the adult spawning populations or variations in preservation (Karaiskou *et al*. 2007). The latter is noteworthy given samples used in the Neira study were approximately 10 years old.

Overall results show that morphological characters are able to be used to accurately identify Jack Mackerel eggs and separate them from those of Yellowtail Scad and Silver Trevally.

A1.4 References

Ahlstrom and Ball (1954) Description of eggs and larvae of jack mackerel (*Trachurus symmetricus*) and distribution and abundance of larvae in 1950 and 1951. Fishery Bulletin United States 56: 209-245

Ahlstrom, E.H., Moser, H.G. (1980) Characters Useful in Identification of Pelagic Marine Fish Eggs. *CA COOP. Oceanic Fisheries Investment Reports* 21:131pp.

Crossland, J. (1981) Fish eggs and larvae of the Hauraki Gulf, New Zealand. *Fisheries Research Bulletin of the New Zealand Ministry of Agriculture and Fisheries* 23:1-61

Cunha, M.E., Vendrell, C., Goncalves, P. (2008) Experimental study of the dependence of embryonic development of Trachurus trachurus eggs on temperature. *ICES Journal of Marine Science* 65:17-24

Goodsir, F., Armstrong, M.J., Witthames, P.R., Maxwell, D.L., and Fox, C.J. (2008) The use of species-specific TaqMan probes for identifying early stage gadoid eggs following formaldehyde fixation. – *ICES Journal of Marine Science*, 65: 1573–1577.

Fulton, E.A. (2013). Simulation analysis of jack mackerel stock sizes; ecosystem model based plausibility study, CSIRO, Australia. 24 p.

Jordan, A., Pullen, G., Marshall, J., Williams, H. (1995) Temporal and spacial patterns of spawning in jack mackerel, Trachurus declivis (Pisces : Carangidae), during 1988-91 in eastern Tasmanian waters. *Marine and Freshwater Research* 46:831-842

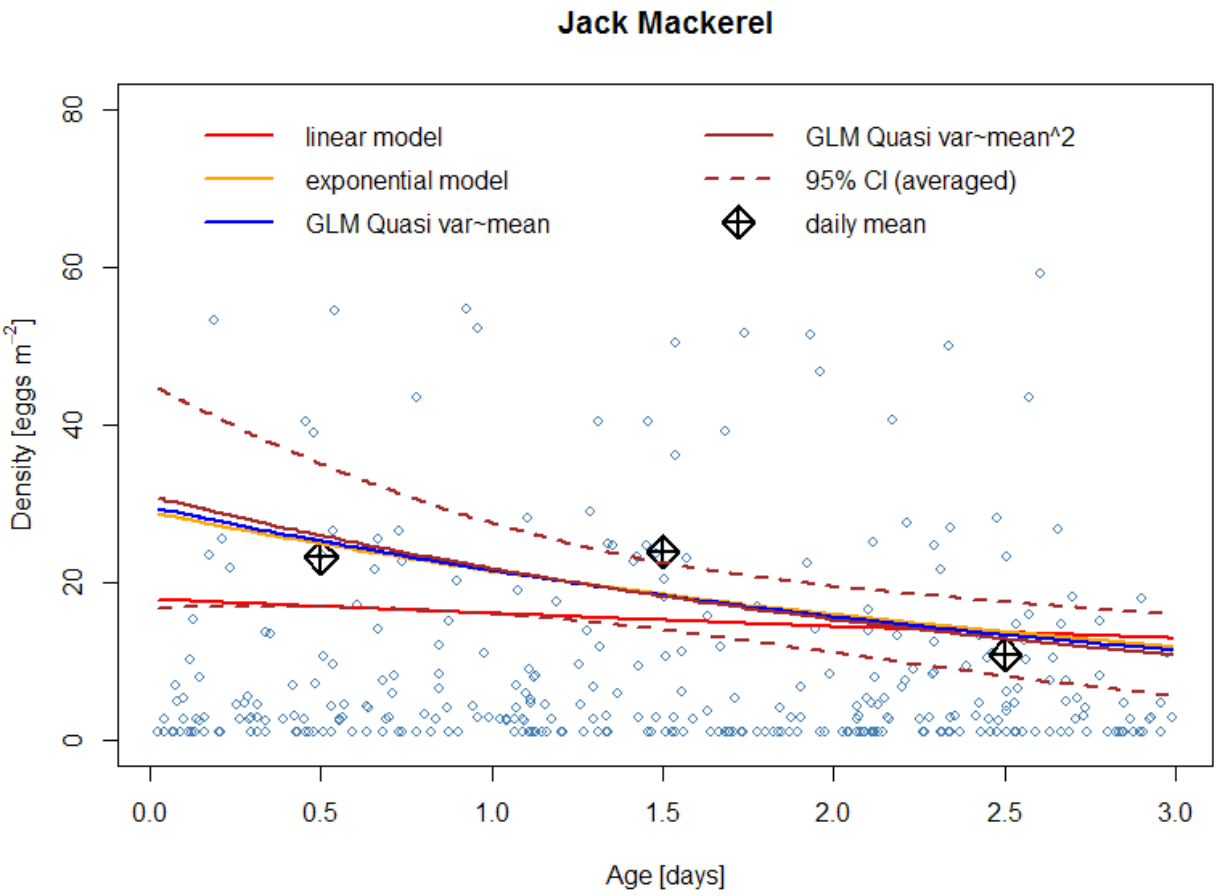
Karaiskou, N., Triantafyllidis, A., Alvarez, P., Lopes, P., Garcia-Vazquez, E., Triantaphyllidis, C. (2007) Horse mackerel egg identification using DNA methodology. *Marine Ecology-an Evolutionary Perspective* 28:429-434

Neira, F.J., Perry, R.A., Burridge, C.P., Lyle, J.M. and Keane, J.P. (2014) Molecular discrimination of shelf-spawned eggs of two co-occurring Trachurus spp. (Carangidae) in southeastern Australia: a key step to future egg-based biomass estimates. ICES Journal of Marine Science; doi: 10.1093/icesjms/fsu151

Perry, R.A. (2011) *Spawning habitat of two commercial mackerel species (Trachurus spp.) in south-eastern Australia based on distribution of eggs identified using molecular techniques.* Honours Thesis, University of Tasmania. 111pp.

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.

Appendix 2: Egg density versus egg age for Jack Mackerel. Plots are of the four models used to estimate P_0. Mean densities of day-1, day-2 and day-3 eggs are also shown.



Appendix 3: Project Staff

Authors

Associate Professor Tim Ward (SARDI)

Dr Owen Burnell (SARDI)

Mr Alex Ivey (SARDI)

Dr Jonathan Carroll (SARDI)

Mr Stuart Sexton (Flinders University of South Australia)

Dr John Keane (Institute for Marine and Antarctic Studies, University of Tasmania)

Dr Jeremy Lyle (Institute for Marine and Antarctic Studies, University of Tasmania)

Other Technical staff

Mr Lorenzo Andreacchio

Mr Matt Lloyd (SARDI)

Mr Graham Hooper (SARDI)

Ms Kylie Tonon (AFMA)

Owners of FV Dell Richie II and FV Western Alliance

Mr Stuart Richie and Mr David Guillot

Skippers and crews

Mr John Richey *FV Dell Richey II*

Mr Ian Rule

Mr Jerrod McDermott

Mr William Rouwhoust

Mr Dion Lodum

Mr Brendan Ashley *FV Western Alliance*

Mr Ashley McNamara

Mr Michael McNamara

Mr Michael Joseph