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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

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Final Report

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

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Executive summary

Spawning stock biomass of jack mackerel (*Trachurus declivis*) in south-eastern Australia is currently unknown. Accordingly, Commonwealth harvest strategies in place for this commercially-fished mackerel, along with that for the closely-related yellowtail scad (*T. novaezelandiae*), are likely to remain at Tier 2 until biomass estimates are obtained. The lack of stock information on jack mackerel prompted the author to seek external funding to attempt determine spawning biomass levels of this species applying the daily egg production method (DEPM). This method constitutes a well-known fishery-independent approach presently applied to estimate biomass of clupeoid and a few non-clupeiod species worldwide. The study presented a number of challenges due to the lack of key adult parameters required by the DEPM, including spawning fraction and batch fecundity. Another major hurdle constituted the lack of a species-specific temperature-dependent incubation model to assign ages to eggs according to developmental stage, as required when estimating daily egg production by area. Best estimates obtained during this study were thus based on the availability of pelagic eggs of jack mackerel collected during egg surveys carried out off south-eastern Australia in October 2002 to examine spawning dynamics of blue mackerel (*Scomber australasicus*), and adult parameters derived from jack mackerel specimens caught in eastern Tasmania and literature information on *Trachurus* spp. from the Northern Hemisphere.

Identifications of eggs suspected as *Trachurus* spp. from the October 2002 survey were verified using molecular techniques that included the extraction, PCR amplifications and sequencing of two gene regions of the mtDNA (*cytochrome oxidase* and 16*s* rRNA). However, test results proved highly ambiguous to separate eggs at the species level, with sequences returning positive matches (83-100%) for jack mackerel and yellowtail scad, as well as *T. japonicus* and *T. trachurus* which do not occur in Australasian waters. Such results led to relying on morphological descriptions based on New Zealand populations to identify jack mackerel eggs and distinguish these from those of the co-occurring yellowtail scad. Jack mackerel eggs were thus separated from those of yellowtail scad by their larger diameter, i.e. 0.93-1.04 c.f. 0.75-0.80 mm.

Considering the whole shelf region sampled in October 2002, jack mackerel eggs occurred along the NSW coast between Sugarloaf Point (32.5^{o}S) and Cape Howe (37.0^{o}S), corresponding to an area estimated to be 21,327 km^2. Mean weighted daily egg production at that time (P_0) was estimated in 3.80-3.92 eggs/0.05m^2/day based on an exponential decay model using egg abundance-at-age data fitted with a GLM assuming a negative binomial distribution. Abundance data were obtained

after each jack mackerel egg (n = 2,627) was staged (I-XI) and aged (days) using incubation models developed for *T. trachurus* from the north-east Atlantic and *T. murphyi* from the eastern south Pacific. Weighted P_0s were also computed from identical abundance data sets using the traditional least squares non-linear regression method (NLS). Two adult parameters, namely female weight (W = 311.4 g) and sex ratio (R = 0.346), originated from mid-water trawl samples collected along eastern Bass Strait and Tasmania in 2002. Batch fecundity (F = 62,947 eggs) was derived from a relationship combining ovary-free weight of female jack mackerel weight from eastern Tasmania and average values of oocytes per gram from *T. trachurus*, while spawning fraction (S = 0.2 females/day) was based on data published for *T. trachurus* and *T. symmetricus*. Using all parameters, and the two statistical methods to estimate P_0s (GLM and NLS), spawning biomass of jack mackerel in October 2002 was estimated to be ~114,900-169,000 t. Variances could not be calculated given the lack of appropriate data. Proxies for spawning biomass limits provided herein (46,000-398,000 t) were calculated employing extreme values of each adult parameter, which combined actual jack mackerel data from eastern Tasmania and literature information.

Results of this study indicate that DEPM is an appropriate technique to estimate spawning biomass of jack mackerel. However, the estimates reported here are considered negatively biased and thus largely imprecise, and hence need to be treated with due caution. Key problems leading to this uncertainty are: (1) lack of reproductive data for jack mackerel in south-eastern Australia; (2) absence of a species-specific temperature-dependent incubation model to age eggs; (3) the October 2002 egg survey was timed to study spawning dynamics of blue mackerel along shelf waters of Qld and NSW, and did not correspond to the peak spawning period of jack mackerel; and (4) sampling design during that survey was not optimal to apply DEPM for jack mackerel. Given the biology distribution of jack mackerel in south-eastern Australia, three key aspects will need to be considered in future DEPM applications to provide biomass estimates for this species: (1) spawning of the south-eastern Australia stock may take place progressively south towards eastern Tasmania and possibly peak in summer; (2) the region defined as spawning area in October 2002 comprises a fraction of the entire area at peak spawning, which is likely to include eastern Bass Strait and Tasmania; and (3) daily egg production estimated at the start of the spawning season may not be as high as when the bulk of spawning takes place. Main issues concerning each parameter employed to estimate spawning biomass of jack mackerel are discussed, and several recommendations provided in relation to future work, including the need to collect adult reproductive data as well as additional molecular tests to verify with certainty the identity of jack mackerel eggs.

1. Introduction

1.1 Background

Estimating abundance of fishes constitutes a key undertaking of fisheries science, particularly in the pursuit to implement scientifically-based harvest strategies aimed at protecting stocks and preserving an adequate ecosystem balance. Biomass information is also fundamental to predict recruitment success and overall stock health, and is becoming increasingly essential in the evaluation of spatio-temporal changes in distribution and abundance of stocks under the looming global climate change scenario (IPCC; *www.ipcc.ch*). In the case of pelagic fishes, changes in ecosystem functioning directly affect selection of spawning area which in turn impacts on the survival of spawning products, i.e. eggs and larvae. In this context, early life history stages of fishes are highly sensitive to environmental fluctuations, and can be used as causal indicators of ecosystem perturbation (e.g. Neira and Sporcic, 2002) and, ultimately climate change.

Much of the research carried out during the last 25 years to estimate biomass of pelagic fishes worldwide has concentrated on the application of fishery-independent techniques such as the daily egg production method (DEPM). Rather than employing the standard approach of analysing catch and effort data, this technique combines egg production derived from intensive egg surveys over the spawning area with information on reproductive biology of the target species. Since its development around the mid 80s (Lasker, 1985), the DEPM has successfully been employed to estimate spawning biomass of pelagic fishes such as anchovies, sardines and mackerels (Stratoudakis *et al*., 2006; Cubillos *et al*., 2007), as well as mid-water fishes like redbait, *Emmelichthys nitidus* (Neira *et al*., 2008a; Neira and Lyle, 2011). A key pre-requisite before embarking on DEPM egg surveys is to define the spatio-temporal spawning habitat of the intended species, which derives from examining abundance and distribution of eggs and larvae together with high-resolution oceanographic data, particularly water column temperature and shelf bathymetry (Ibaibarriaga *et al*., 2007; Neira and Keane, 2008).

Pelagic schooling mackerels of the genus *Trachurus* support large commercial and recreational fisheries worldwide, though catches have declined markedly since the early 1990s. Two main species occur in south-eastern Australia, namely jack mackerel, *T. declivis* and yellowtail scad, *T. novaezelandiae*. Jack mackerel are distributed across temperate Australia from Wide Bay (Qld) to Shark Bay (WA), and are particularly abundant in Tasmanian waters (Gomon *et al*., 2008.

Commercial mackerel fishing off south-eastern Australia occurs between southern New South Wales (NSW) and south-eastern Tasmania, with catches mainly processed to feed farmed tuna and bait for higher-value species. Along with yellowtail scad, jack mackerel play a key role in the pelagic ecosystem of temperate Australia, both as predator and prey of piscivorous fishes including tuna and other game fish species, sea birds and diverse marine mammals (Gales and Pemberton, 1994; Young *et al*., 1997; Hedd and Gales, 2001).

Current information regarding the status of jack mackerel stocks in south-eastern Australia is limited. This particularly relevant to Tasmania, where the disappearance of large surface/sub-surface schools in the early 90s ended a productive purse-seine fishery with reported peak catches over 40,000 t in 86/87. With catches not exceeding ~3,000 t since 1999/2000, the sudden decline was described as climate induced, with the assumed shrinkage and dispersal of jack mackerel schools implying a possible shift in spawning pattern due to changes in key physical and biological drivers (Harris *et al*., 1987, 1992). Despite its importance as a commercial species and key component of the pelagic seascape of south-eastern Australia, no information is currently available on the biomass of jack mackerel across its distributional range (Ward *et al*., 2011). Earlier attempts to assess stocks of this species using hydroacoustic and aerial surveys of schools were unsuccessful, leading to the recognition that developing methods to estimate biomass of the species were of high priority.

1.2 Need and funding

The lack of information on biomass levels of jack mackerel in Australian waters has significant implications concerning the management both of the fishery and pelagic shelf ecosystem. Importantly, it also implies that harvest strategies currently in place by the Commonwealth Small Pelagic Fishery (SPF) to manage the mackerel fishery are likely to remain at Tier 2, with associated uncertainties and potential loss of profitability to industry until stock estimates are obtained. Consequently, this project was developed to evaluate spawning biomass levels of jack mackerel in south-eastern Australia based on the DEPM, in what constitutes the first attempt to provide a quantitative assessment of this pelagic species in Australia. As such, the use of DEPM is aligned to research priorities established by the Commonwealth SPF in 2009/10 on the pressing need for scientific advice on developing a cost-effective method to estimate exploitable biomass of SPF species. In this context, it is important to highlight that mackerels (family Carangidae) constitute the only

remaining species group included in the Commonwealth-managed SPF that have not been to date quantitatively assessed in the manner proposed in this project.

The study was funded by the Winifred Violet Scott Charitable Trust. To a large extent funding was facilitated by the availability of many samples containing jack mackerel eggs collected by the author (FJN) during five plankton surveys along shelf waters of south-eastern Australia between 2002 and 2004 (NSW to mid-eastern Tasmania). Furthermore, the study made use of the extensive dataset of biological and reproductive information on jack mackerel housed at Institute for Marine and Antarctic Studies (IMAS) of the University of Tasmania.

1.3 Intended outcomes and specific objectives

The main outcome of this project constitutes the provision of indicative spawning biomass estimates for the eastern zone jack mackerel. The estimates are aimed to address a major gap in understanding population characteristics of jack mackerel in south-eastern Australia, and are intended to underpin the decision-making process for the allocation of sustainable harvest levels of this pelagic resource. In addition, estimates are likely to assist the SPF Management and Resource Advisory groups in developing more scientifically-defensible catch limits for mackerels. This is particularly relevant to address serious concerns posed by recreational and conservation groups in relation to current harvest levels implemented for SPF species, particularly those assigned to jack mackerel (5,000 t eastern zone TAC) which are not scientifically based. Results of this study are also relevant to the impending expansion of the SPF fishery in south-eastern Australia, especially in terms of likely effects on other marine species and overall pelagic ecosystem.

Specific objectives of this project are:

1. To describe the developmental stages of eggs of jack mackerel.
2. To provide estimates of spawning biomass of jack mackerel in south-eastern Australia.
3. To characterise spawning habitat and boundaries of jack mackerel in south-eastern Australia using environmental descriptors.

2. Materials and methods

2.1 Background

Results of this study are in part based on mackerel (*Trachurus* spp.) eggs collected by the author during ichthyoplankton surveys carried out between southern Queensland (Qld) and southern New South Wales (NSW) in October 2002, October 2003, and July 2004, and between southern NSW and mid-eastern Tasmania in February 2003 and February 2004 (Fig. 1). The surveys were primarily conducted to obtain data on egg and larval distribution of *Scomber australasicus* (Scombridae), with the purpose of using this information to design adequate sampling strategies to estimate spawning biomass of this species using DEPM (Neira and Keane, 2008). Collection procedures of plankton samples and environmental data for these surveys are detailed in Keane and Neira (2008) and Neira and Keane (2008).

2.2 Identification of mackerel eggs

Eggs collected in 2002 were identified as *Trachurus* spp based on morphological descriptions provided by Ahlstrom and Ball (1954), Robertson (1975), Ahlstrom and Moser (1980), Crossland (1981) and Cunha *et al*. (2008). Crossland (1981) provided specific descriptions of jack mackerel and yellowtail scad eggs collected in shelf waters around New Zealand based on Robertson (1975), noting that jack mackerel eggs were slightly larger in diameter than those of yellowtail, i.e. 0.97-1.03 cf. 0.78-0.88 mm. These ranges were used to separate yellowtail scad eggs from those of jack mackerel, with egg measuring carried out southward from the northern-most station until no eggs of yellowtail scad were found (see Results).

Available mitochondrial DNA (mtDNA) methodology was employed to confirm species identity of field-caught mackerel eggs. The methodology has been successfully employed to separate both eggs (Karaiskou *et al*., 2007) and adults (Karaiskou *et al*., 2003; Cardenas *et al*., 2005) of various *Trachurus* species, and is being currently employed in the DNA barcoding world project (Herbert *et al*., 2003; Ward *et al*., 2005). Mackerel eggs tested for these analyses were randomly selected depending on total numbers available per sample, starting with the northern-most stations off northern NSW. Since DNA extraction requires that eggs be destroyed, each egg tested was measured to the nearest 0.01 mm with a stereo-microscope fitted with an eyepiece micrometer, and the developmental stage (I to XI) noted following the key developed by Cunha *et al*. (2008) for eggs of *T. trachurus*. In addition, each egg was photographed with a Leica DC300F camera attached to the

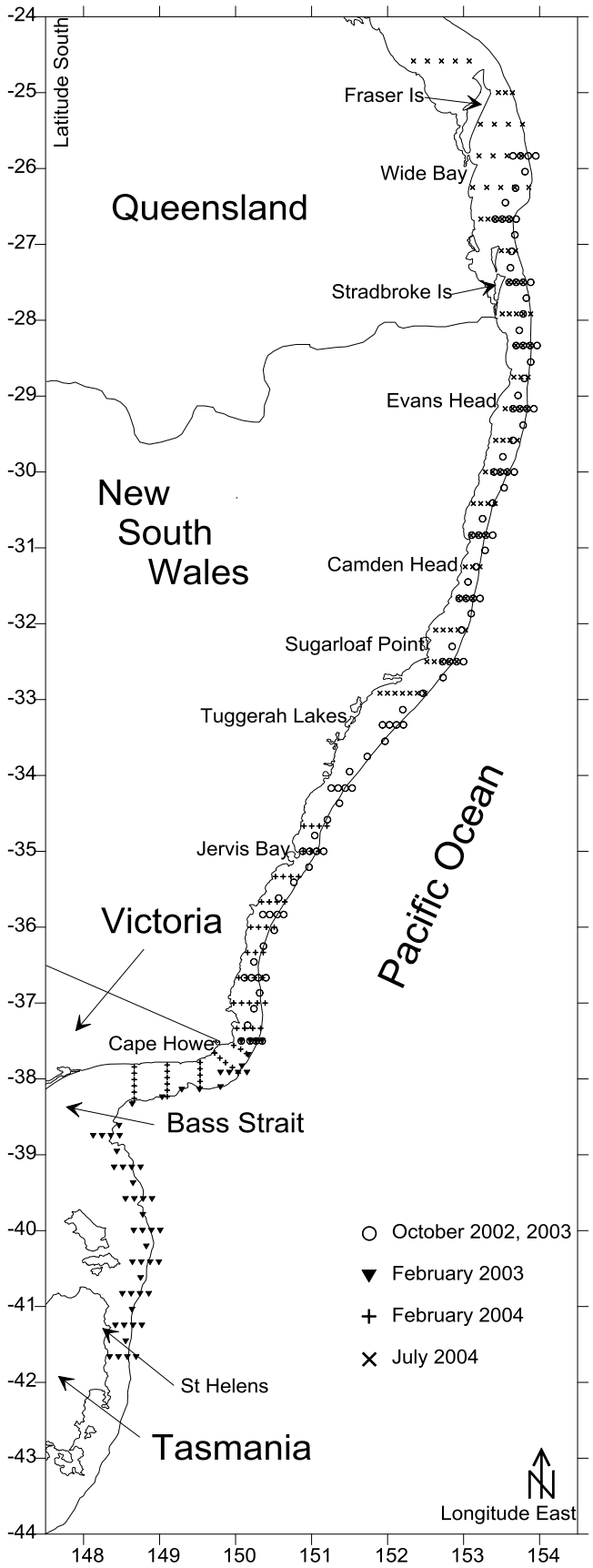

Figure 1. Stations sampled for mackerel eggs along shelf waters between southern Queensland and mid-eastern Tasmania between October 2002 and July 2004.

stereomicroscope, and the image recorded using Leica IM50 imaging software together with information on cruise, station and collection date.

The technique employed herein consisted of (a) extraction of mtDNA from individual eggs; (b) amplification using a Polymerase Chain Reaction (PCR); (c) sequencing of specific gene regions; and (d) comparison of sequences against those of adults of the target species archived in the public databases, namely GenBank (National Center for Biotechnology Information – NCBI) and Barcode of Life Data (BOLD) Systems. Extraction and amplification of mtDNA were conducted at the Central Science Laboratories (SCL) of the University of Tasmania.

Genomic DNA was extracted from eggs using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's protocol for tissue extractions. The optional addition of carrier RNA provided in the kit was used to increase DNA yield. Amplification of mtDNA used specific primers which targeted the 655 base-pair region of the protein-coding *cytochrome oxidase* gene (*COI*), as well as the 16*s* ribosomal DNA (Karaiskou *et al*., 2003; Garcia-Vazquez *et al*., 2006) (Table 2.1). The *COI* gene constitutes the marker currently used in the Fish BOLD being developed to barcode all fish species worldwide (Ward *et al*., 2005). Each PCR reaction mixture (25 µl of total volume) contained 2µl of template DNA, 12.5µl of ImmoMix (Bioline), 0.4µM of each primer, 8.5µl Milli-Q water. The PCR reactions were performed under the following conditions: initial denaturation of 10 min at 95°C, followed by 30 cycles of 30s denaturation at 95°C, 35s at the specific annealing temperature, and 50s extension at 72°C, then a final extension step of 72°C for 5 min. Resultant PCR products were visualised using gel electrophoresis (1% agarose at 85 volts for 30 minutes), and then sequenced using Big Dye chemistry and an ABI 3730xl sequencer by a commercial sequencing service (Macrogen Inc, Korea). Sequences were entered online into GenBank (*COI*, 16*s*) and BOLD (*COI*) databases for identification.

Table 2.1. Mitochondrial DNA regions targeted on field-caught mackerel eggs during this study, and relevant primer information. T_A = annealing temperature.

mtDNA region	*Primer*	*Primer sequence 5' – 3'*	*T_A (°C)*
COI	FishF1	TCAACCAACCACAAAGACATTGGCAC	54
	FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	
16*s*	LRN	CGCCTGTTTATCAAAAAC	52
	LRJ	CCGGTCTGAACTCAGATCACG	
D-loop	16498	CCTGAAGTAGGAACCAGATG	
	L15995	AACTCTCACCCCTARCTCCCAAAG	

2.3 Available egg data

Stations with jack mackerel eggs were identified southwards from 32°S, i.e. from just north of Sugarloaf Point in central NSW to just north of St Helens in eastern Tasmania (Fig. 1). However, spatial distribution of eggs varied significantly across years due to intrinsic variable spawning intensity and sampling effort. For example, eggs were reasonably abundant along southern NSW in October 2002 but were rather scarce along the same shelf region in October 2003, while noting that fewer stations were also sampled that year (Table 2.2). Thus, given the low numbers of jack mackerel eggs collected in October 2003 (n = 448) compared to October 2002 (n = 2,627), it was decided only to employ the egg data collected in 2002 to provide an overall early spring estimate of spawning biomass for southern NSW (32.0-37.5°S; Fig. 4). Likewise, both the overall low abundances and highly patchy spatial distribution of jack mackerel eggs observed during the February 2003 and February 2004 surveys (Table 2.2; Fig. 5) precluded the use of these data to estimate spawning biomass via the DEPM.

Table 2.2. Details of four ichthyoplankton surveys conducted along shelf waters of south-eastern Australia between southern New South Wales and central-eastern Tasmania in 2002-2004, with details of number of transects, stations sampled and number of jack mackerel eggs collected; positive stations refer to those where eggs of the target species were found.

Survey date	Shelf region sampled (States)	Range latitude °S	Transects	Stations sampled	Positive stations	No. eggs
12-22 October 2002	Sugarloaf Pt – Cape Howe (NSW)	32.5 - 37.5	13	43	36	2,627
5-11 February 2003	Cape Howe – Four Mile Creek (NSW-Tas)	37.5 - 41.7	12	55	15	109
1-8 October 2003	Sugarloaf Pt – Cape Howe (NSW)	32.5 - 37.5	7	28	8	448
5-12 February 2004	Kiama – Cape Conran (NSW-Vic)	34.7 - 38.2	14	60	12	341

2.4 Spawning biomass estimates

This objective followed a similar approach to that employed to estimate spawning biomass of redbait off eastern Tasmania (Neira *et al*., 2008; Neira and Lyle, 2011) and southern NSW (Neira and Lyle, 2008). Existing data for two of the four adult reproductive parameters needed for the DEPM model, namely average weight of females (W) and sex ratio (R), were obtained from data kept at the Fisheries, Aquaculture and Coast (FAC) Centre, of the Institute for Marine and Antarctic Studies (IMAS), University of Tasmania. Data of other two parameters, i.e.

spawning fraction (*S*) and batch fecundity (*F*), were obtained from available scientific literature for various species of *Trachurus* (see below).

2.4.1 Spawning biomass model

Spawning biomass (*B*, tonnes) was estimated separately for 2005 and 2006 using the equation of Parker (1985):

$$B = P_0 A \, k \, / \, (R \, F \, S \, / \, W_f) \qquad (2)$$

where P_0 is egg production per unit of area per day (eggs/0.05m^2/day); A spawning area (km^2); k factor to convert grams to metric tonnes; R fraction of mature females by weight (sex ratio); F batch fecundity (number of oocytes released per mature female per batch); S spawning fraction (proportion of mature females spawning each day); and W_f mean weight of mature females in the population (g). Variance of spawning biomass estimates could not be computed due to the lack of key reproductive data (see section 2.5).

2.4.2 Mean daily egg production – P_0

Parameter estimation employs egg abundance-at-age data, i.e. egg counts grouped by daily cohorts per station and standardized to area (numbers per m^2), with egg ages (days) assigned with a temperature-dependent egg incubation model following egg staging. However, such model has not yet been developed for *T. declivis* in temperate Australia. Therefore field-caught mackerel eggs examined for this study (n = 2,627) were each assigned one of 11 stages described for eggs of *T. trachurus* from the north-east Atlantic (Cunha *et al*., 2008), and subsequently assigned an age (days) based on a temperature-dependent incubation model developed for the same species by these authors based on a GLM with Poisson distribution and log-link function. In addition, and for comparative purposes, eggs were also assigned ages employing a described by a deterministic stage-to-age model obtained for Chilean mackerel (*T. murphyi*) by Sepulveda *et al*. (2009); i.e. $y = 4.1544 \; e^{-(0.038t + 0.0715i)} \; i^{(1.6558)}$, where y corresponds to the mean age (hours) of stage i at mean incubation temperature t°C. Runs employed collection hour of sample (EST; hour, min) and mean water temperature of each station to a depth of 50 m. Peak spawning hour was assumed to be 23:30 hrs (Sepulveda *et al*., 2009).

Mean daily egg production of jack mackerel at spawning time (P_0) and daily instantaneous mortality rate (Z; day^{-1}) of eggs were estimated using an exponential decay model based on egg abundance-at-age data (Picquelle and Stauffer, 1985).

The model assumes all eggs to be spawned and instantaneously fertilized at a specific time, and affected by a constant exponential mortality rate, with daily egg abundance-at-age data in each sample to be independent observations from a population with a common P_0 and Z (Stratoudakis *et al*., 2006). Two functions were fitted to the daily egg abundance-at-age data, namely the traditional least squares non-linear regression (NLS) model (Lo *et al*., 1996), and a generalized linear model (GLM) using a negative binomial error distribution (Cubillos *et al*., 2007; Neira and Lyle, 2011). The NLS, which assumes a constant mortality rate, is expressed as:

$$P_t = P_0 e^{-Zt}$$

where P_t corresponds to the number of eggs produced by unit of area at age t, P_0 the daily egg production at age 0, and Z the daily instantaneous mortality rate. Data employed in model runs included all positive stations as well as negative stations embedded within spawning area, and considered all egg stages (I to XI) even if catches did not include particular stages at a given station (G. Claramunt, Universidad Arturo Prat, Chile, *pers. comm.*). Details of how weighted means of P_0 estimates and corresponding CVs are estimated for the two models, as well as CVs for mortality (Z), are provided in Neira and Lyle (2011). When available, daily egg production levels were compared to those of *Trachurus* spp. from other locations. Functions for the two techniques are available in the library packages STATS and MASS available in R (Ihaka and Gentleman, 1996; www.r-project.org). Plots of daily egg cohort abundances per station ($\log_{10}$-transformed data; eggs/m^2 +1) against mean ages (days) provided for the GLM fit correspond to *R* outputs.

2.4.3 Spawning area

Spawning areas (i.e. positive area in km^2) for October 2002 was estimated using the same approach employed by Neira and Lyle (2011) for redbait off eastern Tasmania. For each survey the area regarded as the main spawning area encompassed all positive stations (i.e. with jack mackerel eggs) as well as negative stations embedded between positive stations. All negative stations outside the positive area were omitted from the area estimate.

2.4.4 Adult parameters

Data available for this study comprised average weight of mature females (W, g) and fraction of females by weight (sex ratio, R) from purse-seine and mid-water trawl samples of jack mackerel obtained annually between 2001 and 2004 along eastern Tasmania and eastern Bass Strait (Table 2.3). Equations used to compute

average female weight and sex ratio are provided in Neira *et al*. (2008a). For the purposes of biomass estimates it was assumed that all females counted and weighted were mature and/or in reproductive mode, i.e. with gonads macroscopically identified as being in stages III to V, with V corresponding to running ripe (= spawning).

Average batch fecundity of jack mackerel (*F*; eggs/batch) was estimated using the relationship $F = 205 \times W_{of}$ derived for *T. trachurus* (Karlou-Riga and Economidis, 1997), where W_{of} corresponds to the average ovary-free weight of females caught off eastern Tasmania between 2001 and 2004 (Table 2.3). Since the average 205 oocytes/g per female ovary-free weight falls within the 200-209 oocytes g^{-1} range reported for the same species by Abaunza *et al*. (2003) and Goncalves *et al*. (2009), average *F* estimates were calculated for the entire range. However, it should be noted that the whole range provided in the literature for *Trachurus* spp. is 90-725 oocytes/g (Abaunza *et al*., 2003; van Damme *et al*., 2005; Sepulveda *et al*., 2009). Average spawning fraction (*S*) was assumed to be 0.10 – 0.30/day based on values published for *T. trachurus* (Karlou-Riga and Economidis, 1997; Goncalves *et al*., 2009) and *T. symmetricus* (Macewicz and Hunter, 1993) (Table 2.4).

2.4 Spawning habitat characterisation

Abundance data of jack mackerel eggs were examined in terms of oceanographic conditions during that survey to describe selection of spawning habitat. These data include temperature and salinity by depth obtained simultaneously with each vertical plankton sample using a Seabird Electronics SBE19 Conductivity-Temperature-Depth (CTD) profiler attached to a 3m-long bongo sampler (Neira and Keane, 2008). Data were subjected to standard quotient analysis (Ibaibarriaga *et al*., 2007; Neira and Keane, 2008) using average temperatures of each site to a depth of 50 m. For this analysis, egg abundances (eggs per m^2) within each 0.5°C temperature interval were expressed as a percentage of total abundance, divided by the percentage frequency of occurrence under each temperature class and plotted. Quotients >1 indicate positive spawning location, i.e. favoured temperature range. Water masses off south-eastern Australia during all five surveys were identified using classification and NMDS ordination of temperature frequencies (Keane and Neira, 2008).

Table 2.3. Parameters for mature (gonadal stages III to V) jack mackerel (*Trachurus declivis*) in south-eastern Australia. Data were obtained from the database archived at FAC (IMAS). Average batch fecundity (*F*; oocytes^{-1}) was estimated using values of 200-209 oocytes in the relationship F = oocytes × W_{of} (ovary-free weight; g).

Year	**Weighted individuals**		**Total weight sample** (g)		**Sex ratio**	**Average female weight** (g)	**Average batch fecundity** (*n*)
	F	*M*	*F*	*M*			
2001	42	49	12,188	14,658	0.45	290.19	58,545 (38)
2002	14	21	4,359	8,249	0.35	311.36	62,947 (11)
2003	20	5	5,733	2,206	0.72	286.68	57,068 (20)
2004	24	4	7,990	935	0.90	332.92	73,555 (18)

Table 2.4. Literature-based reproductive parameters of mackerel (*Trachurus* spp.) employed for this study; * values based on total weight; ** number of eggs per gram of gutted female weight. Abbreviations: mnso = migratory nucleus-stage oocytes; ho = hydrated oocytes; POFs = postovulatory follicles; $POF_{1, 2}$ = days 1, 2; N/A = not available.

Species	**Locality**	**Average spawning fraction (*S*)** (females/day)	**Criteria to determine spawning fraction**	**Average batch fecundity (*F*)** (oocytes per g ovary-free weight)	**Female weight range** (ovary-free; g)	**Reference (s)**
T. murphyi	Chile	0.06627	POF_1	86 - 94*	381 – 1,012*	Sepulveda *et al.* (2009)
		0.07290	POF_2			
		0.06958	POF_{1+2}			
T. symmetricus	California	0.202	late mnso, ho and POFs to 54 h old	112	586 - 1,262	Macewicz and Hunter (1993)
T. trachurus	Saronikos Gulf, Aegen Sea - Greece	0.172 – 0.209	mnso and POFs	205	63 - 223	Karlou-Riga and Economidis (1997)
T. trachurus	E Atlantic (western stock)	0.083	mnso	208 - 209	N/A	Eltink (1991) and Priede (1994), in Abaunza *et al.* (2003)
T. trachurus	Portugal coast – E Atlantic (southern stock)	N/A	N/A	172	N/A	Borges *et al.* (1993) in Abaunza *et al.* (2003)
T. trachurus	Various locations NE Atlantic and Mediterranean	N/A	N/A	139 – 245**	N/A	Abaunza *et al.* (2008)
T. trachurus	Northeast Atlantic	0.10 – 0.30	mnso, ho and POFs	124 - 175	N/A	Goncalves *et al.* (2009)

3. Results

3.1 Identification of jack mackerel eggs

3.1.1 Standard techniques

Eggs were identified as *Trachurus* using diameter range (0.7-1.3 mm) together with morphological features typical of eggs of the genus, including smooth chorion, narrow perivitelline space, largely segmented yolk, and a single, pigmented oil globule which is positioned anteriorly in the yolk in late-stage eggs (Ahlstrom and Ball, 1954) (Fig. 2). Eggs between 0.97 and 1.03 mm in diameter were classified as *T. declivis* following Crossland (1981).

Diameters obtained for 14 ethanol-preserved eggs of the closely-related *T. novaezelandiae* collected at a station just north of NW Solitary Islands (29.80°S; 153.52°E) ranged between 0.75 and 0.80 mm (mean = 0.77; SD = 0.02). By contrast, diameters from a similar number of eggs collected off Jervis Bay (35.00°S; 151.07°E) ranged between 0.93 and 1.04 mm (mean = 0.99; SD = 0.04) and were therefore identified as jack mackerel.

3.1.2 Molecular techniques

Of the 50 field-caught *Trachurus* spp eggs subjected to genetic tests, PCR-based amplification and positive sequencing of mtDNA material was achieved for 33 eggs (66%). Tests for the remaining eggs were unsuccessful due to failed PCRs and poor sequencing. Regardless of which online database was used to compare sequences, runs returned 83-100% matches with four mackerel species, namely *T. declivis*, *T. novaezelandiae*, *T. japonicus* and *T. trachurus* (see Appendix). For *COI*, sequences stored in GenBank (NCBI) and BOLD matched more frequently *T. declivis* than *T. novaezelandiae* or *T. japonicus* for the same egg, though they provided higher frequencies of 99-100% matches for the two Australian species (Fig. 3). In addition, for a number of eggs the *COI* sequence matched 100% both with *T. declivis* and *T. novaezelandiae* using either database (Appendix). Targeting the *16s* rRNA gene region resulted in higher frequencies of 99-100% matches for *T. declivis* but still resulted in mostly a 96% match for *T. novaezelandiae*.

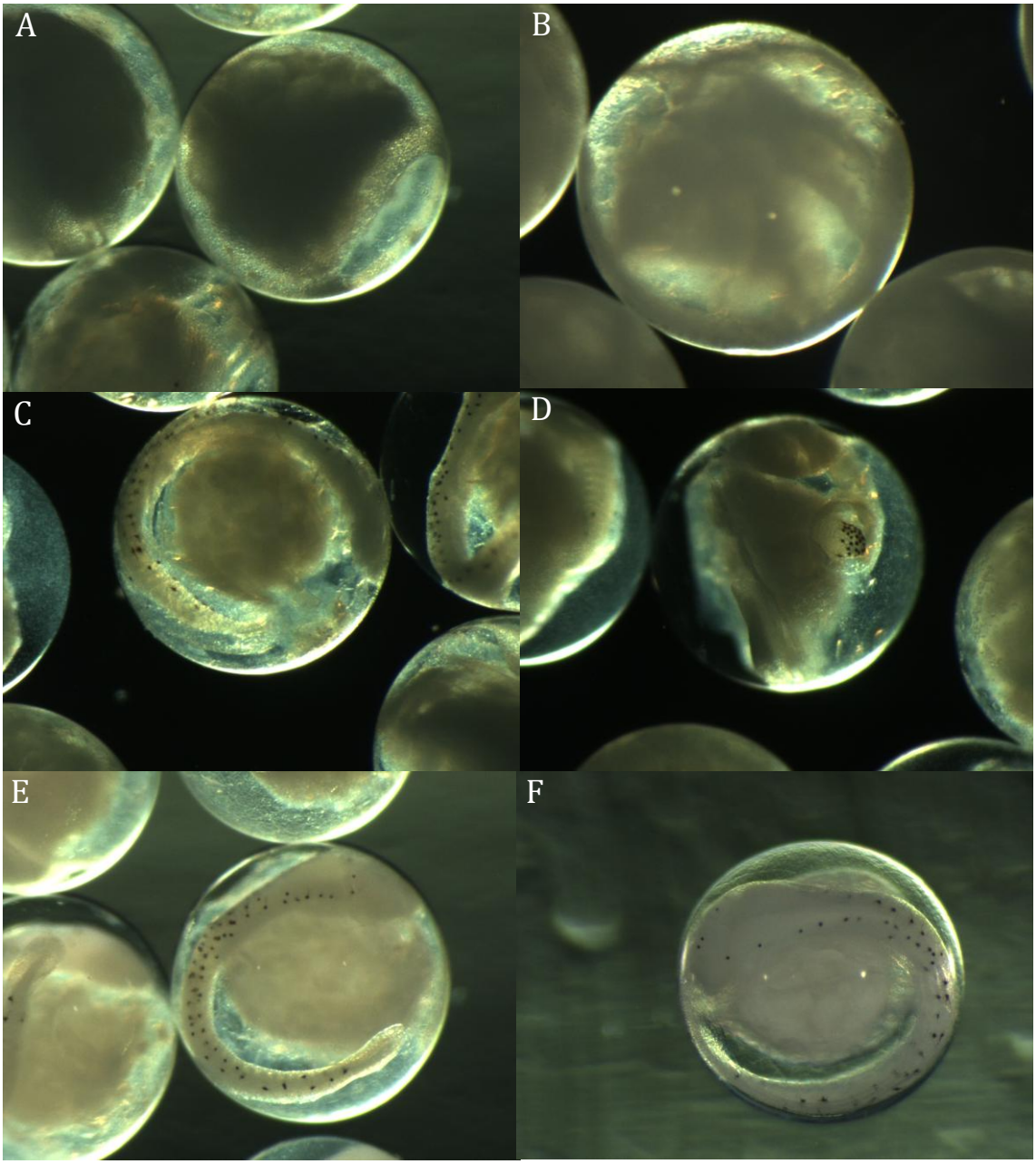

Figure 2. Eggs of jack mackerel (*Trachurus declivis*) collected in shelf waters off NSW in October 2002 (range = 0.93-0.97 mm diameter). A, stage 4; B, stage 6; C, D, stage 8; E, stage 9; F, stage 11. Photographs show segmented yolk sac (C – F), single, pigmented oil globule (D) and pigment along dorsal surface of embryo (C, E, F).

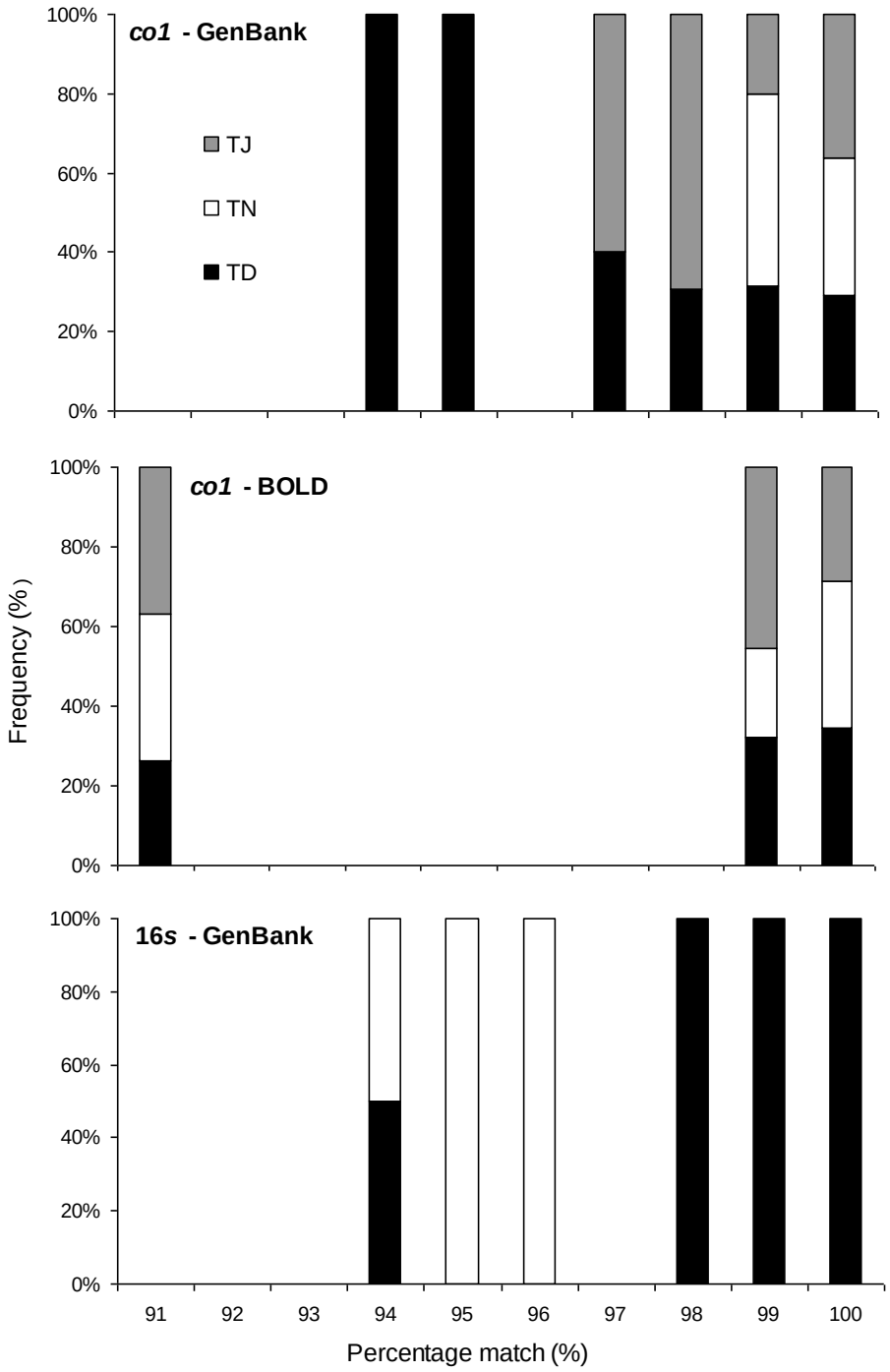

Figure 3. Species matches obtained for *COI* and 16*s* genomic regions of the mtDNA extracted from *Trachurus* eggs collected in shelf waters between southern Qld and central NSW in October 2002. Top two plots show matches of the *COI* gene obtained from GenBank and BOLD public databases. Abbreviations: TJ, *T. japonicus*; TN, *T. novaezelandiae*; TD, *T. declivis*; the few matches with *T. trachurus* were omitted from the plots.

3.2 Developmental stages of jack mackerel eggs

All except stage 1 eggs of jack mackerel were available for this study. Stage 1 includes newly-spawned and fertilized eggs with the characteristic single oil globule and up to 64 individual cells which can be counted (Cunha *et al*., 2008). Developmental stages 2-11 can be separated using features such as formation of blastodisc, closure of blastopore, and formation and growth of embryo prior to hatching (Fig. 2).

Stage 2 is characterized by the formation of the blastodisc (=blastodermal cap) at the animal pole. The blastodisc continues growing downwards around the segmented yolk. Stage 3 commences with appearance of the germinal ring, a thickening of the blastodisc margin, and ends with the formation of the embryonic shield at the top of the blastodisc. This shield later becomes the embryo axis. By Stage 4 the embryonic axis is clearly defined, though neither head nor tail is yet discernible (Fig. 2A). Enveloping of the yolk through the downwards developing of germinal ring (= epiboly) continues through stage 4, later ending by stage 6 with the closure of the blastopore, i.e. completion of epiboly. The head and tail regions become visible by stage 5, and both are clearly discernible by stage 6 (Fig. 2B). At this stage the embryo covers over half of the yolk mass and a few myomeres are apparent. Pigment starts to appear along the dorsal surface of the embryo by stage 6, gradually increasing in intensity through to stage 11. By stage 7 the embryo's enlarged tail begins to separate from the yolk mass, the primordial eyes are visible over the head melanophores form around the single oil globule. By stage 8 the embryo covers almost three quarters of the yolk mass, and its tail is clearly separated from the yolk (Fig. 2C). From stage 9 (Fig. 2E) through to stage 11 (Fig. 2F), i.e. prior to hatching, pigment continues to appear over the head, body and tail, and the tail continues to grow around the yolk mass until it reaches past the head. Throughout the last six stages the single, pigmented oil globule is clearly visibly roughly in the middle of the yolk between the head and tail regions but it migrates towards the head region before hatching (Fig. 2F-D).

3.3 Spatial distribution of jack mackerel eggs

Eggs of jack mackerel during the October 2002 and 2003 surveys occurred predominantly between 32.5^{o}S (Sugarloaf Pt) and 37.0^{o}S (just north of Cape Howe) along southern NSW (Figs 1, 4). Abundances during the 2002 survey were greatest off Jervis Bay (1,030 eggs per m^2), at a station just inshore of the shelf break (35.0^{o}S; 151.07^{o}E). Captures of 100-250 eggs per m^2 were also recorded off Sugarloaf Pt, just south of Jervis Bay and slightly north of Cape Howe in October

2002, and only off Sugarloaf Pt in October 2003 (225 eggs per m^2) and just south of Jervis Bay in February 2004 (194 eggs per m^2; Fig. 5). For the purpose of estimating spawning biomass, the spawning area of jack mackerel in October 2002 was estimated in 21,327 km^2 (Table 3.1; Fig. 4).

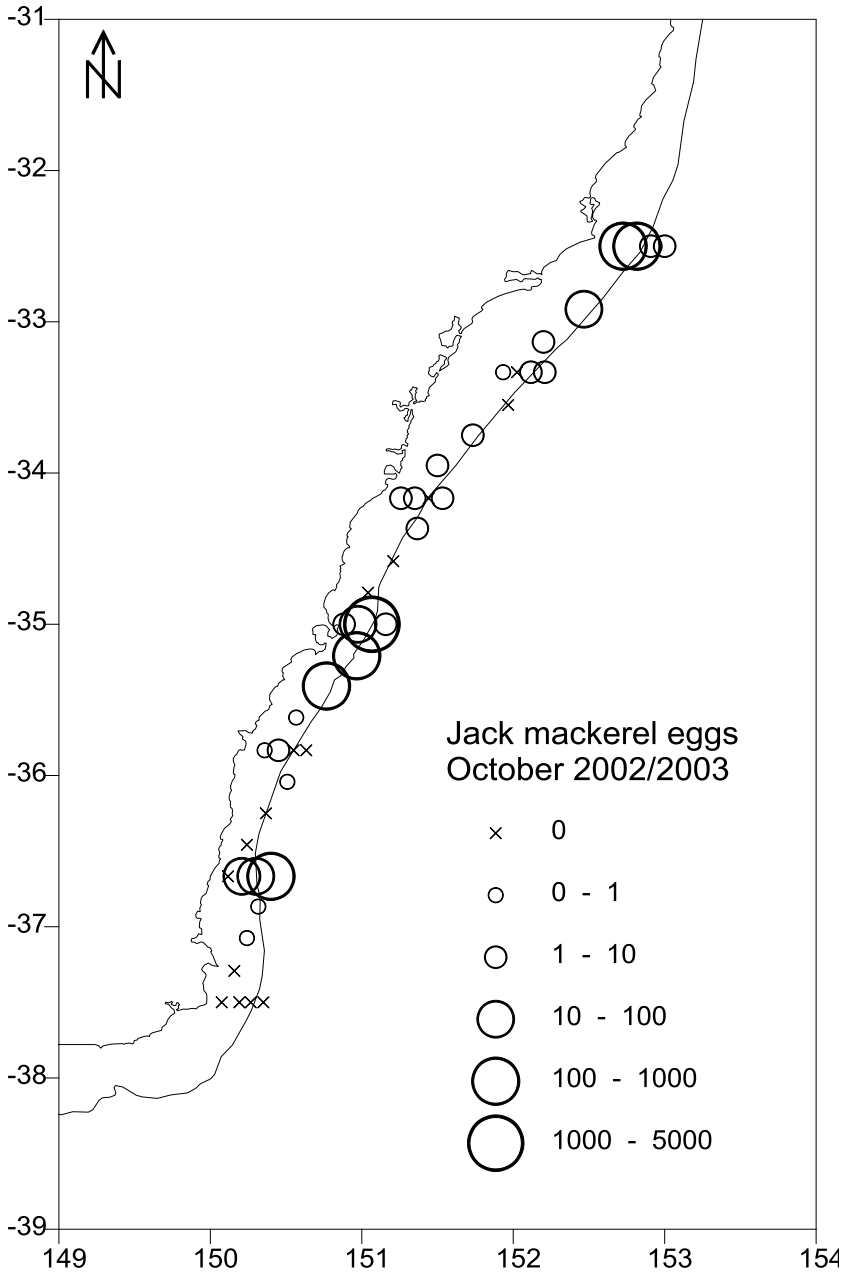

Figure 4. Distribution of pelagic eggs of jack mackerel (*Trachurus declivis*) along shelf waters of southern NSW during October 2002 and October 2003 (data combined for the two surveys).

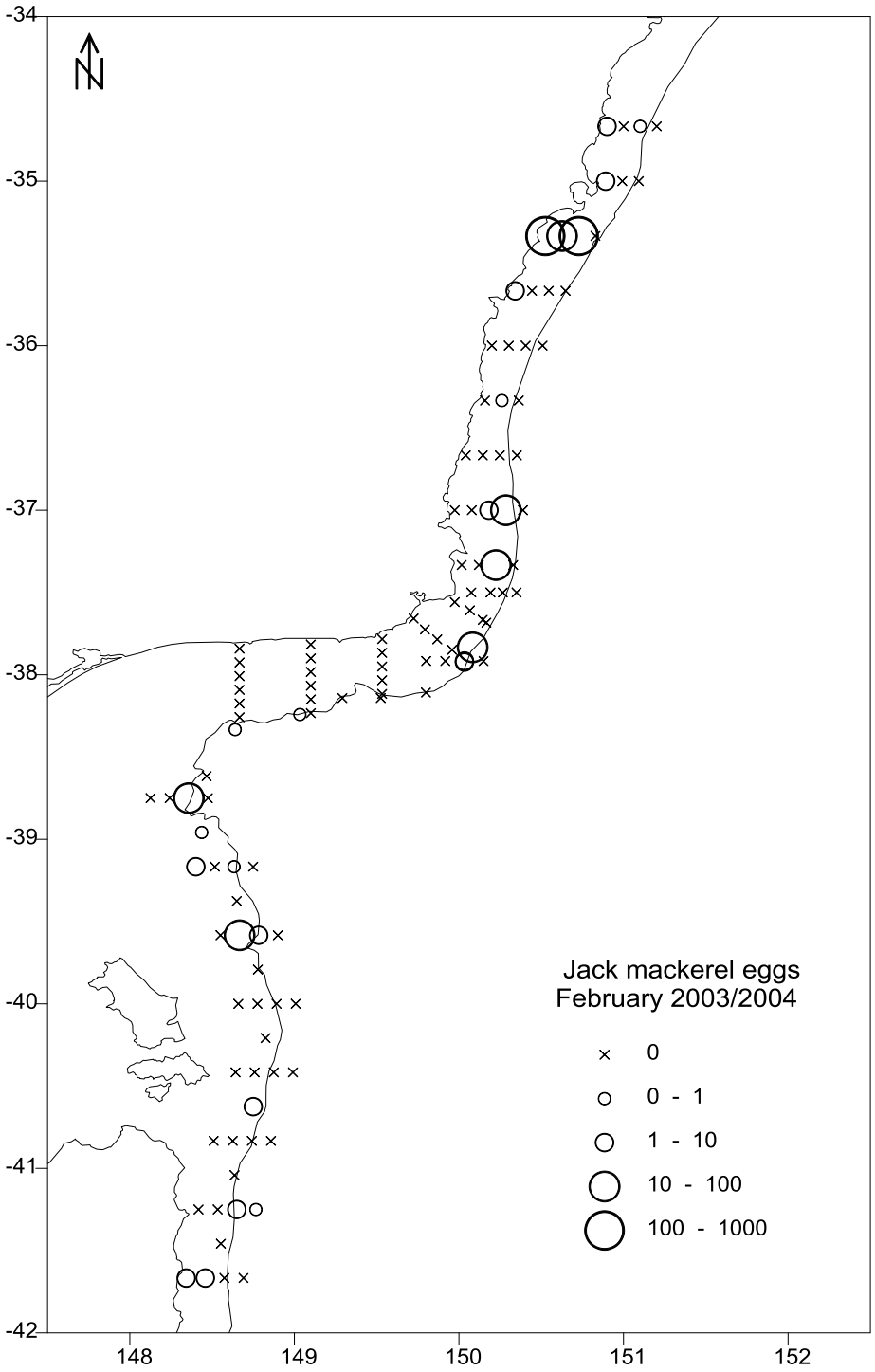

Figure 5. Distribution of pelagic eggs of jack mackerel (*Trachurus declivis*) along shelf waters of southern NSW through to central eastern Tasmania during February 2003 and February 2004 (data combined for the two surveys).

3.4 Spawning conditions

Quotient analysis of jack mackerel egg abundances obtained during October 2002 and October showed an optimal spawning temperature at 18.5°C (Fig. 6). It is likely that the high quotient value obtained for that temperature interval (7) may reflect high abundances of jack mackerel eggs off Sugarloaf Pt and Jervis Bay (Fig. 4). As with the quotient analysis, the bubble plot of all positive stations from the October 2002-2003 surveys showed groups of high egg abundances in waters with average temperatures/salinities of 16.5-18.5°C/35.5–35.6 (Fig. 7). Jack mackerel eggs from the February 2003 and February 2004 surveys (Fig. 5) were collected in waters with mean temperatures (to 50m) mostly within the 17.2-20.1°C range.

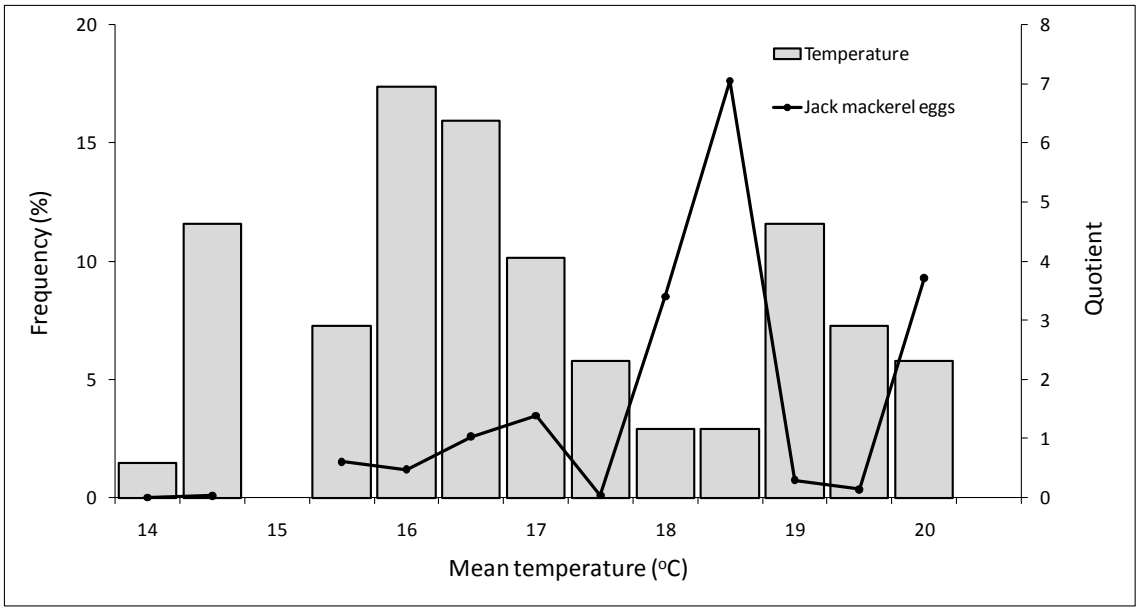

Figure 6. Quotients of abundance of jack mackerel eggs (numbers per m^2) by mean temperature (°C) from combined data obtained along shelf waters off southern NSW in October 2002 and October 2003. Bars correspond to percentage frequency of occurrences of water column temperatures at each positive station averaged for the first 50m.

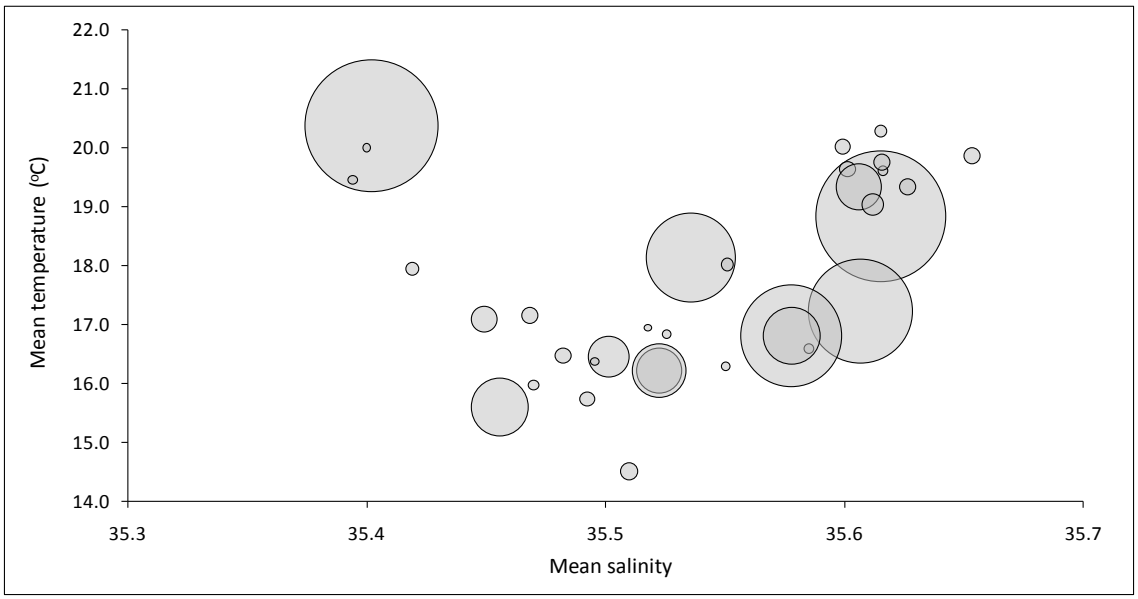

Figure 7. Bubble plot showing abundances of jack mackerel eggs (numbers per m^2) collected at different combinations temperatures and salinities (average top 50 m values at each positive station) during the October 2002 and October 2003 surveys along shelf waters off southern New South Wales; bubble sizes are proportional to egg abundances.

3.5 Adult parameters

Average mature female weight (W = 311.4 g), batch fecundity (F = 62,947) and sex ratio (R = 0.346) derive from jack mackerel caught off eastern Bass Strait and Tasmania in 2002 (Table 3.1). Ranges of these parameters for 2001-2004 were W = 286.7 – 332.9 g; F = 57,068 – 73,555; and R = 0.346 – 0.454. Ranges of each of these adult parameters, including spawning fraction (S) values from literature on *Trachurus* spp., i.e. 0.1 – 0.3, (see section *2.4.4*), were subsequently employed to calculate limits of spawning estimate biomass of jack mackerel off southern NSW.

3.6 Daily egg production

Egg abundances (m^2) plotted against respective ages (days) followed the typical exponential mortality model described for eggs of other small pelagic fishes (Fig. 8). Weighted mean P_0 estimates for jack mackerel (eggs/0.05m^2 day^{-1}) varied between the two egg aging models and statistical estimation methods, being slightly higher for the egg abundance-at-age data derived from the aging model developed for the Chilean mackerel both for NLS (4.93 vs. 3.36) and GLM (3.92 vs. 3.80). In addition, NLS-derived P_0 values obtained for both aging models were characterised by larger CVs compared to those obtained for the GLM (Table 3.1).

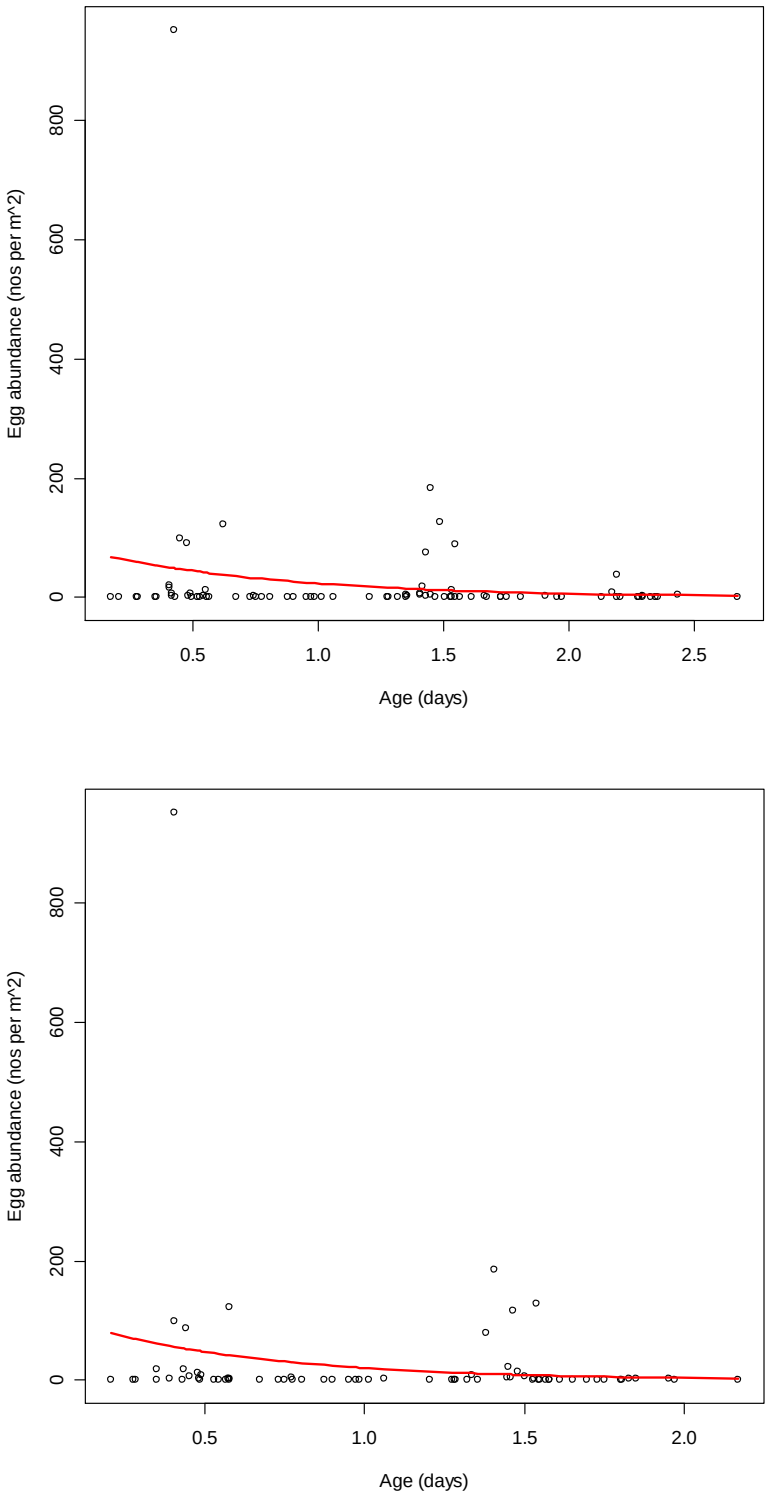

Figure 8. Mortality model for jack mackerel eggs caught in 2002 off southern New South Wales (extreme data omitted) for cohorts aged using the temperature-dependent egg incubation models of Cunha *et al*. (2008) (top) and Sepulveda *et al*. (2009) (bottom). Curves correspond to the superimposed fitted mortality curve derived from GLM with negative binomial error distribution.

Table 3.1. Adult input parameters, weighted daily egg production (P_0) and spawning biomass estimates (tonnes) for jack mackerel (*Trachurus declivis*) off southern New South Wales in October 2002. Assignment of cohort ages is based on temperature-dependent egg incubation models obtained for *Trachurus* spp. from Portugal (Cunha *et al*., 2008) and Chile (Sepulveda *et al*., 2009). Weighted daily egg production (P_0) was estimated from egg abundance-at-age data subjected two discrete statistical methods, namely NLS and GLM. Adult parameters derive from biological data collected of *T. declivis* captured off eastern Tasmania (Tas) and eastern Bass Strait between 2001 and 2004. Sex ratios for jack mackerel caught in 2003 and 2004 off eastern Tas were considered unlikely (0.72 and 0.90) and hence were not employed in biomass estimations; values of spawning fraction (S) were obtained from relevant literature.

Parameter		
No. stations	36	
DEPM survey area / spawning area (km²)	23,934 / 21,327	
2002 average weight females (W; g)	311.4	
2002 average batch fecundity (F)	62,947	
2002 sex ratio (R)	0.346	
Spawning fraction (S) - literature	0.20	
Method to assign ages to staged eggs	*T. trachurus* (Portugal) Cunha *et al.* (2008)	*T. murphyi* (Chile) Sepulveda *et al.* (2009)
Non-linear least squares (NLS)		
Weighted P_0 (eggs/0.05m²/day) (CV)	3.36 (0.77)	4.93 (0.91)
Mortality (day^{-1}) (CV)	-1.17 (0.97)	-1.68 (0.98)
2002 spawning biomass (t)	**114,943**	**168,817**
GLM – negative binomial error distribution		
Weighted P_0 (eggs/0.05m²/day) (CV)	3.80 (0.59)	3.92 (0.68)
Mortality (day^{-1}) (CV)	-1.30 (0.32)	- 1.30 (0.44)
Akaike Information Criterion	458.2	414.0
2002 spawning biomass (t)	**130,082**	**134,218**
Estimated spawning biomass limits		
Range W (g) (Tas; 2001 – 2004)	286.7 – 332.9	
Range F (Tas; 2001 – 2004)	57,068 – 73,555	
Range R (Tas; 2001, 2002)	0.346 – 0.454	
Range S (literature)	0.1 – 0.3	
Biomass (t) – NLS-derived P_0	46,013 – 271,076	67,580 – 398,129
Biomass (t) – GLM-derived P_0	52,074 – 306,778	53,729 – 316,532

3.7 Spawning biomass estimates

Spawning biomass estimates for jack mackerel within the defined spawning area off southern NSW were ~115,000-169,000 t based on NLS-computed P_0s, and ~130,000-134,000 t based on GLM-computed P_0s (Table 3.1). These estimates are based on egg abundance-at-age data obtained with temperature-dependent incubation models published for two geographically-remote *Trachurus* species, average adult reproductive parameters obtained from jack mackerel samples caught off eastern Bass Strait/Tasmania in 2002, and spawning fraction information from current literature on *Trachurus*. Biomass ranges estimated as a proxy of confidence limits were ~46,000-398,000 t for NLS-computed P_0s, and ~52,000-316,000 t based on GLM-computed P_0s. These figures are based on egg abundance-at-age data derived from the two temperature-dependent incubation models, and the extreme average values of adult parameters obtained from the above jack mackerel samples as well as literature (Table 3.1).

4. Discussion

4.1 Identification of jack mackerel eggs

Ichthyoplankton samples examined for this study were collected along shelf waters between Wide Bay (Qld) and Cape Howe (NSW), and contained eggs of various species including *Trachurus* spp. Eggs of this genus are easily distinguished from those of other non-carangid taxa using diameter and specific morphological features, particularly the segmented yolk and the single, pigmented oil globule (Ahlstrom and Ball, 1954; Robertson, 1975; Ahlstrom and Moser, 1980; Cunha *et al*., 2008). In addition, results of initial molecular tests targeting two specific regions of the mitochondrial DNA (mtDNA) to verify identifications confirmed the presence of *Trachurus* eggs in samples. However, molecular tests undertaken to separate eggs of jack mackerel from those of the co-occurring yellowtail scad had proved impossible given uncertainties in species matches from all positive runs (see below). Consequently, eggs of the two species in the few mid-NSW stations were they occurred together were separated using the slightly smaller average diameter of yellowtail scad eggs (0.75-0.80 cf. 0.93-1.04 mm), as described for material collected in New Zealand shelf waters (Robertson, 1975; Crossland, 1981).

Extraction and sequencing of *COI* and 16*s* rRNA gene regions from randomly-selected eggs collected at several sites along the survey area returned high percentage matches with four *Trachurus* species which included yellowtail scad

and jack mackerel, as well as *T. japonicus* and *T. trachurus* which are absent in Australian waters (Gomon *et al*., 2008). Furthermore, such mix was obtained regardless of gene region used, or public database visited to compare sequences (GenBank or BOLD). Such results were surprising, and indicate that targeting either mtDNA gene region to discriminate between yellowtail scad and jack mackerel eggs may not the best option at this stage. However, it is interesting to note that adult museum voucher specimens of the two species across Australian waters separate well using *COI* despite a few yellowtail scad tissue samples grouping together with jack mackerel (A. Graham, CSIRO Hobart, *pers. comm*.). This grouping is believed to indicate some degree of hybridization across yellowtail scad and jack mackerel populations, with the two species sharing *COI* haplotypes (B. Ward, CSIRO Hobart, *pers. comm*.). While there is no information regarding 16*s* to separate these mackerels, it is likely that both species may also share 16*s* haplotypes as it is the case of three *Trachurus* species from the Atlantic and Mediterranean waters (Karaiskou *et al*., 2003). The latter work found that the mackerel tested shared 10 different haplotypes for 16*s* compared to 22 for *cytochrome b*, another protein-coding gene in the mtDNA region commonly employed to differentiate adult mackerels (Cardenas *et al*., 2005) as well as eggs (Garcia-Vazquez *et al*., 2006). Results from 16*s* analyses by Karaiskou *et al*. (2003) led to their conclusion that the 16*s* gene sequence was more conservative than that of *cytochrome b*, i.e. subjected to slower evolution rate, thereby making the latter gene region a more suitable marker for the genetic separation of the three European mackerels. The same gene region was successfully sequenced along with *COI* to verify the identity of eggs of blue mackerel (Neira and Keane, 2008).

The ambiguity of the molecular tests run during this study demonstrates the poor discriminatory power of the two chosen molecular markers to separate jack mackerel eggs from those of yellowtail scad, while shedding some light on the inherent genetic similarities between these closely-related mackerels. In the context of the latter, it is relevant that a recent comprehensive phylogenetic study of the 11 recognised species placed within the genus *Trachurus* using *cytochrome b* and D-loop sequences revealed five discrete groups, one of which comprised a southwest Pacific group containing *T. japonicus*, *T. novaezelandiae* and *T. declivis* (Cardenas *et al*., 2005). The study also showed this group consistently clustering separately from the other four groups, and concluded that all three were well-defined species representing a monophyletic lineage. While neither *cytochrome b* nor D-loop gene regions were tested during this study to distinguish eggs of jack mackerel and yellowtail scad, the latter was successfully sequenced to verify the identity of eggs of redbait (Neira *et al*., 2008b), and consequently remains a promising alternative in future molecular studies along with *cytochrome b*.

During this study PCR amplifications and subsequent mtDNA sequencing failed in around 34% of the eggs tested. Such failure rate was thought to be attributed to various factors, including quality/quantity of DNA in eggs, poor extraction techniques, quality of PCR reagents, type and suitability of Primers used, and type of fixative and/or preservation period. The former two are probable key factors, especially if eggs have been left for long periods without fixative and the extraction process does not include using specific kits to maximize the extraction of small DNA quantities, as in the case of fish eggs. Primer suitability to target selected gene regions appears also to be an important factor, along with using the correct annealing temperature. In terms of the fixative, all tested eggs had been fixed and preserved in 98% ethanol which constitutes the recommended medium for DNA analyses over formalin-preserved tissues (Karaiskou *et al*., 2007). In addition, the relatively high PCR amplification success of the eggs tested (66%) leading to generally complete *COI* and 16*s* sequences demonstrates that preservation period (ca. 9 years) may not be an important factor.

4.2 Egg distribution and spawning area estimate

Results of this study indicate that spawning of jack mackerel during October 2002 and October 2003 took place along most NSW shelf waters, with no eggs identified northwards of Sugarloaf Point in northern NSW (32.5^{o}S). Eggs were found well within the shelf area bathed by a mixed water body (MIX; 18.5-19.8^{o}C) comprising the warm, low-nutrient East Australian Current (EAC; 20.6-22.3^{o}C) north of 32.5^{o}S and Tasman Sea water to the south (TAS; 16.0-17.0^{o}C), with the MIX-TAS interface being identified at 34.3^{o}S in October 2002 and 33.7^{o}S in October 2003 (Keane and Neira, 2008; Neira and Keane, 2008). The absence of jack mackerel eggs north of 32.5^{o}S is likely to be linked to the presence of the EAC identified for that region in both surveys (Neira and Keane, 2008). The observation that jack mackerel may prefer to spawn in cooler waters is supported by quotient analysis of egg abundances which showed temperatures of 18.5^{o}C as those most suitable for spawning, at least during October.

Jack mackerel eggs were also collected between Jervis Bay (35.3^{o}S) and St Helens in eastern Tasmania (41.7^{o}S), including north-eastern Bass Strait, in February 2003 and February 2004, though in significantly lower concentrations compared to the October surveys. The finding of scattered eggs in low concentrations off eastern Tasmania during February is not surprising given that peak spawning of jack mackerel along that region takes place between December and late January (Jordan *et al*., 1995). In addition, reported sea surface temperatures across shelf waters off eastern Tasmania during summer spawning are almost identical to

those reported in this study (mean to 50m) for late summer, i.e. 17.6ºC to 20.6ºC (Jordan *et al*., 1995). Since there is no information for southern NSW waters comparable to that available for eastern Tasmania, the presence of jack mackerel eggs in October 2002 and October 2003 is not indicative of whether the surveys were conducted before, during or after peak spawning of the species in that region.

Given the spatial distribution of jack mackerel in south-eastern Australia (Gomon *et al*., 2008), findings of this study pose three major issues that would need to be considered in future biomass estimations: (1) spawning appears to take place progressively southwards, starting at the northern-most region of the species distribution in early spring and continuing along eastern Tasmania through to mid-summer; (2) the region defined as spawning area in October 2002 excludes eastern Tasmania, implying that it only represents a fraction of the entire spawning area of jack mackerel in south-eastern Australia; and (3) biomass estimates based on egg production confined to the northern spawning distribution of the species, as in this study, are highly likely to be negatively biased, i.e. they underestimate the actual biomass of the south-eastern stock.

4.3 Spawning biomass estimate and caveats

Spawning biomass estimates reported here for the jack mackerel off southern NSW (~140,000 t) are largely imprecise and, as such, need to be taken with due caution. Main reasons for this uncertainty lays in the data assigned to each of the parameters required by the daily egg production method (DEPM), including the lack of reproductive data for jack mackerel from south-eastern Australia, and the absence of a species-specific temperature-dependent incubation model to assign ages to field-caught eggs. In addition, surveys conducted in October 2002/2003 and February 2003/2004 were timed to coincide with spawning of blue mackerel, with the sampling effort designed to delineate the spatial extent of spawning of this small pelagic species rather than applying the DEPM. A summary of the main issues concerning each parameter is presented below.

4.3.1 Assignment of age to staged eggs

No temperature-dependent incubation model is presently available to assign ages to field-caught eggs of jack mackerel from south-eastern Australia. Consequently, stage-to-age models developed for two geographically-distant *Trachurus* spp. were applied in this study to complete this task, i.e. for horse mackerel (*T. trachurus*) from Atlantic-Iberian waters using a GLM with Poisson distribution and log-link function (Cunha *et al*., 2008), and Chilean mackerel (*T. murphyi*) from eastern

Pacific waters using Lo's (1985) deterministic least squares model (Sepulveda *et al*. 2009). Whilst built to age eggs of two other *Trachurus* species, the application of these models seem justified as the average temperatures reported here for jack mackerel spawning (18.5°C) fall within the ranges employed in the egg incubation trials of *T. trachurus* (12-19°C; Cunha *et al*., 2008) and *T. murphyi* (14, 17 and 19°C; Sepulveda *et al*., 2009). However, it is highly likely that applying either model would affect the jack mackerel egg abundance-at-age data required to estimate mean daily egg production (P_0) and in turn the resultant biomass estimates.

Besides the lack of a species-specific temperature-dependent incubation model, no information is available on peak spawning time (hh:mm) of jack mackerel in south-eastern Australia, which is required to compute egg ages from water temperature and sampling time. Given the absence of such information, spawning time was set at 23:30 hrs following work on Chilean mackerel, which is closer to 00:00 hrs reported for Pacific mackerel (*T. symmetricus*) (Macewicz and Hunter, 1993).

4.3.2 Spawning area (A)

The region defined during this study as spawning area (ca. 21,300 km^2), which was based on the spatial distribution of jack mackerel eggs along southern NSW, represents only a fraction of the entire spawning area of this pelagic species in south-eastern Australia. Previous research on this species has demonstrated that spawning extends along eastern Tasmania (Jordan *et al*., 1995) and into eastern Bass Strait (Neira, 2005). Therefore, the exclusion of eastern Tasmania during this study reinforces the conclusion that the biomass estimates reported here are negatively biased.

4.3.3 Spawning fraction (S)

Spawning fraction (*S*), the fraction of females spawning per day, constitutes perhaps the most difficult parameter to estimate in DEPM work (Hunter and Lo, 1997). As *S* has not been estimated for jack mackerel or yellowtail scad in Australasian waters, values were assumed to be 0.10-0.30 females day^{-1} based on published information on *T. trachurus* and *T. symmetricus* (Macewicz and Hunter, 1993; Karlou-Riga and Economidis, 1997; Goncalves *et al*., 2009). These estimates were quite different than those reported by Sepulveda *et al*. (2009) for *T. murphyi* from the eastern Pacific (~0.07), and were subsequently not employed during calculations in this study. It is worth noting that *S* estimates depend on female size and therefore age (Claramunt *et al*., 2007), and that precision of this parameter is

highly improved if adult sampling is carried out during peak spawning season (Stratoudakis *et al*., 2006).

4.3.4 Mean weight females (W)

No *W* data are available for female jack mackerel from NSW. Consequently average *W* data was estimated from weights of mature females (i.e. ovary stages III-V) captured along eastern Tasmania and Bass Strait within the November-January period from 2001 to 2004, therefore during the spawning season in those years (data archived at FACC – IMAS). Besides being from a different region, is unlikely that appropriate weight corrections would have been applied to any of the mature females employed to account for the positive weight bias from ovary hydration, as required by the DEPM.

4.3.5 Batch fecundity (F)

As with *S*, there is no batch fecundity (*F*) data available for jack mackerel or yellowtail scad in Australasian waters. Consequently, it was assumed reasonable to estimate average *F* of jack mackerel using the fecundity/mean female weight relationship published for *T. trachurus* (Karlou-Riga and Economidis, 1997), with *W* being the average weight of female jack mackerel caught off eastern Tasmania during 2001-2004. Given the lack of histological and gravimetric ovarian data for spawning female jack mackerel, it is reasonable to conclude that the average *F* estimated in this study is highly imprecise. It is worth noting, however, that the average value of 205 oocytes/g applied to female jack mackerel during this study falls within the 200-209 oocytes/g range reported for *T. trachurus* (Abaunza *et al*., 2003; Goncalves *et al*., 2009).

4.3.6 Sex ratio (R)

Fraction of jack mackerel females by weight (*R*) employed during this study was estimated from specimens captured in 2001 and 2002. Ideally, however, average *R* should have been estimated from males and females caught simultaneously with egg surveys during the peak spawning season over the entire assumed spawning area. The same logically pertains to average *W* and the other two adult parameters, i.e. *S* and *F*.

4.3.7 Egg production at spawning time (P_0)

Weighted P_0s of jack mackerel in October 2002 were estimated employing two distinct statistical methods (NLS and GLM) using egg-at-age abundance data derived from two different stage-to-age models. Results were surprisingly similar for the GLM runs (3.80 and 3.92 eggs/0.05m^2/day) compared to NLS runs (3.36 and 4.93 eggs/0.05m^2/day). However, the improved CVs associated with the GLM-derived P_0s suggest that this statistical procedure provides a better fit to the jack mackerel egg data. This observation parallels recent DEPM work on redbait from eastern Tasmania, which likewise found GLM to fit better the egg data for this mid-water species (Neira and Lyle, 2011). However, whether GLM adequately fits these data in jack mackerel or other *Trachurus* species need to be tested further. More importantly, there are no comparable P_0 accounts for *Trachurus* apart from total production figures reported for north-east Atlantic *T. trachurus* (Dransfeld *et al*., 2005) that were estimated using a different method to that employed here for jack mackerel.

Considering the high likelihood that jack mackerel spawns from southern NSW to southern Tasmania, the P_0 estimates reported here for southern NSW in October only represent eggs spawned by a fraction of the south-eastern Australian stock. As such, biomass estimates would correspond to those for that area at that time, and would certainly be negatively biased as they exclude eastern Tasmania. In addition, since there is no information on whether peak spawning of jack mackerel takes place in spring and/or summer, estimating P_0 at the beginning of the spawning season (e.g. October) in any given year may not be as representative as during peak season due to the expected lower spawning fraction (S) i.e. presence of less actively reproductive females. These factors, combined with the smaller spawning area, would certainly result in a spawning biomass lower to that based on data from peak spawning (Stratoudakis *et al*., 2006).

4.4 Recommendations

Ideal conditions to guarantee a successful DEPM application for estimating spawning biomass of small pelagic fishes are clearly stated and explained in various publications (e.g. Lasker, 1985; Hunter and Lo, 1997; Stratoudakis *et al*., 2006; Cubillos *et al*., 2007; Neira and Lyle, 2011). The main issue pertains to when and where to sample, with a plan that should ideally follow a design incorporating simultaneous adult and egg surveys covering as much of the suspected spawning area as possible. In the case of jack mackerel, much work is needed to precisely pinpoint when and where to sample. This work includes (1) reproductive biology,

perhaps following a similar approach to that of redbait off eastern Tasmania (Ewing and Lyle, 2009); and (2) egg surveys during peak spawning season, based on outcomes of reproductive analyses, i.e. annual trend in gonadosomatic index, including pilot surveys to assess likely extent of spawning area. The latter can be designed following basic reproductive work already carried out on jack mackerel from eastern Tasmania (Marshall *et al*., 1993; Jordan, 1994; Jordan *et al*., 1995).

Reasonable progress in the area of reproductive biology both of jack mackerel and yellowtail scad can be made by funding specific, time-limited projects, as in the case of university Honours/Masters theses. With appropriate supervision and laboratory facilities, these projects are cost-effective and often successful providing samples are readily available from the fishery.

As with the main parameters needed for DEPM, developing a temperature-dependent incubation model for jack mackerel eggs, as well as yellowtail scad, is crucial to the process of assign ages to eggs based on developmental stages. This step is fundamental in the computing of mean daily egg production and hence spawning biomass estimates. Along with this task, additional molecular research is needed to verify the identity of eggs of these two closely-related species, especially in shelf regions where they overlap during spawning. Given the uncertainty in the identifications obtained using the mtDNA gene regions of *COI* and 16*s* rRNA, the next step is to test both *cytochrome b* and D-loop sequences to verify egg identities (Cardenas *et al*., 2005).

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7. Appendix

Results from molecular analyses carried out on two regions of the mtDNA (*COI* and 16*s*) of eggs identified as *Trachurus* spp., with level of sequence matching (%) to each of four valid species in the genus. Taxonomic identifications derive from GenBank and BOLD online databases (see section 2.2 for details). Species abbreviations: TD, *T. declivis*; TJ, *T. japonicus*; TN, *T. novaezelandiae*; TT, *T. trachurus*. Eggs tested (n = 50) were individually selected from >1,000 collected with vertical plankton samples taken along shelf waters off southern Queensland to mid New South Wales in October 2002.

Sample data						*COI*				16*s*	
						GenBank		BOLD		GenBank	
Vial	Transect	Station	No. eggs	Label	Stage	Species	%	Species	%	Species	%
2	AS 2-3	3	1	E2	10	TD	83	TN	91.5	TD	99
								TJ	91.5	TN	96
								TD	91.5		
3	AS 2-3	3	1	E3	5					TD	99
										TN	96
4	AS 5-6	3	1	E4	10	TN	100	TN	100.0	TD	99
						TD	100	TJ	100.0	TN	96
								TD	100.0		
5	AS 5-6	3	1	E5	2					TD	94
										TN	95
6	9	1	1	E6	2					TD	99
										TN	96
7	9	1	1	E7	2					TD	98
										TN	95
8	9	1	1	E8	2					TD	100
										TN	96
9	9	1	1	E9	7					TD	99
										TN	96
10	AS 9-10	2	1	E10-A	10	TD	100	TD	100.0	TD	100
										TN	94
11	AS 9-10	2	1	E11-B	10	TJ	97	TD	100.0	TD	99
						TD	94	TJ	100.0	TN	96
12	AS 9-10	2	1	E12-C	6	TJ	100	TD	100.0	TD	99
						TD	97			TN	96
13	AS 9-10	2	1	E13-D	2	TJ	98	TJ	100.0	TD	99
						TD	96			TN	96
14	AS 9-10	2	1	E-14E	2	TJ	99	TN	99.7	TD	99
						TD	99	TD	99.7	TN	96
15	AS 9-10	2	1	E15-F	2	TD	100	TD	100.0	TD	100
						TJ	100	TN	99.8	TN	96
16	AS 9-10	2	1	E16-G	2					TD	99
										TN	96
17	8	1	1	E17-A	2					TD	99
										TN	96
18	8	1	1	E18-B	NoM						

Appendix. Results from molecular analyses carried out on two regions of the mtDNA (*COI* and 16*s*) of eggs identified as *Trachurus* spp, cont….

Sample data						*COI*				16*s*	
						GenBank		BOLD			
Vial	**Transect**	**Station**	**No. eggs**	**Label**	**Stage**	**Species**	**%**	**Species**	**%**	**Species**	**%**
19	AS 10-11	3	1	E19-A	9	TD	99	TN	100.0		
						TN	99	TD	100.0		
20	AS 10-11	3	1	E20-B	6	TD	98	TN	99.7	TD	99
						TJ	98	TJ	99.7	TN	96
								TD	99.7		
21	12	3	1	E21-A	6	TD	99	TN	100.0	TD	99
						TJ	99	TD	100.0	TN	96
22	12	3	1	E22-B	6	TD	100	TN	100.0	TD	99
						TJ	100	TJ	100.0	TN	96
								TD	100.0		
23	12	3	1	E23-C	3	TD	100	TD	100.0	TD	100
										TN	96
24	12	3	1	E24-D	3	TJ	98	TD	98.9	TD	99
						TD	98	TJ	98.7	TN	95
25	12	3	1	E25-E	2	TJ	100	TJ	98.9	TD	98
						TD	99	TD	98.9	TN	96
26	12	3	1	E26-F	2					TD	99
										TN	96
27	12	3	1	E27-G	2	TJ	100	TJ	98.7	TD	99
						TD	99	TD	98.7	TN	96
						TT	99	TN	98.7		
28	12	3	1	E28-H	4	TN	99	TN	99.3	TD	99
						TD	99	TJ	99.3	TN	96
								TD	99.3		
29	12	3	1	E29-I	4	TN	100	TT	99.6	TD	99
						TD	100	TN	99.6	TN	96
								TJ	99.6		
30	12	3	1	E30-J	4	TN	99	TN	100.0	TD	99
						TD	99	TJ	100.0	TN	96
						TJ	99	TD	100.0		
38	9	2	1	E38-A	3					TD	99
										TN	96
42	9	2	1	E42-E	8					TD	99
										TN	96
43	9	2	1	E43-F	9					TD	99
										TN	96
44	9	2	1	E44-G	9					TD	99
										TN	96

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