FRDC FINAL REPORT

EVALUATION OF EGG PRODUCTION AS A METHOD OF ESTIMATING SPAWNING BIOMASS OF REDBAIT OFF THE EAST COAST OF TASMANIA

F.J. Neira, J.M. Lyle, G.P. Ewing, J.P. Keane and S.R. Tracey

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2004/039 Evaluation of egg production as a method of estimating spawning biomass of redbait off the east coast of Tasmania

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OBJECTIVES

1. To estimate the critical reproductive parameters for redbait along the east coast of Tasmania, particularly spawning fraction and batch fecundity.
2. To develop and validate methods for identifying and staging the eggs and larvae of redbait.
3. To estimate the location and extent of spawning areas of redbait on the east coast of Tasmania, including a quantification of the levels of egg production of this species.
4. To evaluate the use of the daily egg production method for estimating the spawning biomass of redbait on the east coast of Tasmania.
5. To produce a minimum biomass estimate of redbait in Zone A of the Small Pelagic Fishery.

NON-TECHNICAL SUMMARY

During the mid-1980s a major purse-seine fishery for small pelagic fishes, mainly jack mackerel (*Trachurus declivis*), developed off Tasmania. Catches exceeded 40,000 t in the late 1980s but were prone to large inter-annual fluctuations, due mainly to variation in the availability of surface schooling fish. By 2000 purse-seine fishing had effectively ceased, at which time industry commenced trialling mid-water trawling for small pelagics. Redbait (*Emmelichthys nitidus*), rather than jack mackerel, comprised the bulk of the trawl catch.

Since the late 1980s, the fishery has been managed using a combination of input and output controls, including a total allowable catch (TAC). The initial TAC was set at 42,000 t, based on the highest purse-seine annual catch. The TAC was decreased to 34,000 t (combined species TAC) for the 2002-03 and subsequent fishing seasons but without any scientific basis. At the same time the fishery had changed from a purse-seine fishery for jack mackerel to a mid-water trawl fishery primarily targeting redbait. Management of the fishery, which now forms part of the Commonwealth Small Pelagic Fishery (SPF), is currently under review but will be based on output controls (quotas).

The primary objective of the present study was to evaluate the suitability of the daily egg production method (DEPM) for redbait. The DEPM is a fishery-independent method currently used worldwide to provide spawning biomass estimates of small

pelagic fishes that are serial or batch spawners, i.e. release batches of pelagic eggs into the water column throughout the spawning season. The method assumes that the biomass of a fish population can be estimated from the daily production of eggs over the spawning area, the weight and proportion of females that spawned on a given day, and the average fecundity of each reproductively active female. As such, there are several characteristics relating to the reproductive biology, spawning dynamics and development of eggs that are prerequisites for the method.

Redbait reproductive biology was examined from fish caught off eastern and south-western Tasmania. Redbait are batch spawners with asynchronous oocyte development and indeterminate fecundity, spawning over a discrete 2-3 month period during spring. Size at maturity varied markedly between eastern and south-western Tasmania, with south-west coast fish attaining 50% maturity at sizes nearly 100 mm larger than off the east coast. Correspondingly, ages at maturity also differed by region, with east coast redbait maturing at around 2 years of age compared to 4 years in the south-west. Examination of functional oocyte groupings and histology of post-ovulatory follicles indicated that females spawned once every three days, and that peak spawning occurred before midnight. The relationship between batch fecundity and ovary-free body weight was linear, with fecundity increasing at a rate of 186 oocytes per gram of weight.

Attributes of the reproductive biology and spawning dynamics of redbait indicated that the species was suitable for the application of the DEPM. Adult DEPM input parameters estimated for east coast redbait in 2005 and 2006 were, respectively: sex ratio (R) = 0.30, 0.44; female weight (W) = 71.7, 78.3 g; batch fecundity (F) = 10,894, 11,441; and spawning fraction (S) = 0.32, 0.32.

Reared eggs and field-collected material were employed to describe the development of the pelagic eggs and larvae of redbait. Hydrated oocytes from adults trawled from spawning grounds were fertilized and reared to the yolk-sac larval stage, and the data employed to build a temperature-dependent egg incubation model. Embryogenesis lasted 96, 84 and 54 hours at mean temperatures of 13.1, 14.4 and 16.5^{o}C, respectively, and was divided into seven stages based on extent of epiboly until blastopore closure (stages I-III) and embryo growth (stages IV-VII). Morphological identification of eggs collected during ichthyoplankton surveys was validated using a molecular primer/ probe combination capable of isolating a 120 base-pair segment of the mtDNA d-loop gene region unique to redbait. The probe was tested using real-time polymerase chain reaction (PCR) amplification of DNA, producing an 80-100% agreement across all egg stages. Variability of mean egg ages (y) by temperature (t) and stage (i) was best described by the deterministic stage-to-age model

$$y = 35.911\, e^{-(0.155t + 0.262i)}\, i^{(2.436)}.$$

The development of this incubation model to assign ages to staged field-caught eggs of redbait represented a significant achievement in terms of its application to estimate spawning biomass using the DEPM.

Spawning habitat of redbait was described from egg, larval and environmental data collected over shelf waters between north-eastern Bass Strait and the lower south-west

coast of Tasmania in October 2005 and 2006. Egg data were further analysed to estimate the spawning areas off eastern Tasmania, while daily egg abundance-at-age data were employed to compute mean daily egg production (P_0) and instantaneous mortality (Z) estimates. Eggs occurred along the entire area sampled in 2005 (15,650 km^2; 38.8-43.5^oS). By contrast, 96% of the eggs caught in the much larger area sampled in 2006 (21,351 km^2) came from the shelf off eastern Tasmania (40.5-43.5^oS) while very few occurred south of 43.5^oS along the southern to south-west coasts (145.5-147.7^oE). The distribution and abundance of redbait eggs and larvae indicated that spawning takes place mostly along a 5 nm corridor over the shelf break, in average mid-water temperatures of 13.5-14.0^oC. This observation was supported by the significantly greater abundances of day-1 eggs at shelf break than at either shoreward or offshore stations. Estimated spawning areas (% of total survey area) were 13,220 km^2 (84.5%) in 2005 and 8,695 km^2 (40.7%) in 2006.

Mean P_0 and Z were computed for two data scenarios by fitting a least squares non-linear regression model (NLS) and a generalised linear model (GLM), with the latter providing a better description of the data. Excluding eggs assigned ages ≤ 4 hours and ≥ 98% of incubation time, GLM-derived P_0 (eggs/0.05m^2 day^{-1}) was estimated as 4.04 both in 2005 (CV 0.14) and 2006 (CV 0.19), with Z of 0.37 (CV 0.24) and 0.50 (CV 0.34) for 2005 and 2006, respectively. Total egg production per spawning area (eggs x 10^{12}) was 1.26 in 2005 and 1.05 in 2006. Biomass estimates based on the preferred model (GLM with extreme egg cohorts excluded) were 86,990 t (CV 0.37) in 2005 and 50,782 t (CV 0.19) in 2006.

In conjunction with previous ichthyoplankton surveys, our data support the proposition of a discrete eastern spawning stock that splits around southern Tasmania and extends north into southern NSW. Given the geographical extent of this stock and the fact that the entire spawning area was not surveyed in this study, spawning biomass estimates are almost certainly conservative.

This study has conclusively established that redbait is a suitable species for the application of the DEPM, with the results providing a sound scientific basis on which to base harvest levels. Additional work is nevertheless required in various technical areas of biomass estimation. Potential improvements in terms of acquisition of egg and adult reproductive data to augment precision of the DEPM application, including testing of alternative model scenarios, are highlighted. More importantly, however, future surveys will need to cover the entire shelf distribution of redbait to ensure that spawning biomass estimates truly reflect current stock abundance.

OUTCOMES ACHIEVED

The primary outcome of this project has been to provide a foundation for the sustainable management of redbait early in the development of the fishery. Specifically, this study has demonstrated the suitability of the DEPM to determine spawning biomass of redbait, thereby providing a sound basis for the implementation of a harvest strategy developed around a precautionary approach to exploitation.

Adult and ichthyoplankton data from this and previous studies provide evidence of a single spawning redbait stock off south-eastern Australia which is likely to extend between mid-southern NSW and south-eastern Tasmania. This observation is generally consistent with the Commonwealth's move towards stock-based management for the Small Pelagic Fishery (SPF) that proposes eastern and western management zones, separated at 146°30'E off southern Tasmania.

The spawning biomass for redbait off eastern Tasmania was estimated to be between 50,000 – 90,000 t, though given that surveys almost certainly did not cover the entire area occupied by spawning stock, these estimates are conservative. Nevertheless, this project has provided a basis for management to set scientifically-defensible catch limits.

Ultimately, whether fishery-independent assessment occurs will be determined by the trade-offs between the costs of data collection (industry investment into research) set against the potential risks to the stocks and ecosystem of not having reliable biomass estimates and hence lower allocations to industry. This project has provided a firm basis for industry and management to make decisions about the future development of the redbait fishery.

KEYWORDS: Redbait, *Emmelichthys nitidus*, Daily Egg Production Method (DEPM), spawning cycle, egg and larval development series, genetic identification of fish eggs, plankton survey, Small Pelagic Fishery, biomass assessment.

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BACKGROUND

FISHERY SYNTHESIS

A major purse-seine fishery for small pelagic fishes developed off Tasmania in the mid-1980s, with catches peaking at over 40,000 t in the late 1980s (Pullen, 1994a). The fishery was based on surface schooling jack mackerel (T*rachurus declivis*) with catches prone to large inter-annual variations that effectively resulted in the purse-seine fishery ceasing in 2000.

To reduce dependence on availability of surface schooling fish, a six-month trial of mid-water pair trawling was conducted off the east coast of Tasmania in 2001-02, resulting in a total catch of over 5,000 t, of which nearly 90% was redbait (*Emmelichthys nitidus*). At the end of 2002, a large multipurpose mid-water trawl vessel commenced fishing operations off the east coast, capturing >7,000 t of small pelagics by the end of the 2002-03 fishing year. Redbait again dominated the catches, followed by jack mackerel and blue mackerel (*Scomber australasicus*). Redbait are primarily frozen whole for use as feed for farmed southern bluefin tuna (*Thunnus maccoyi*), and are also used along with the other small pelagics in the production of fish meal for use in the aquaculture industry.

Since the late 1980s, the fishery has been managed using a combination of input and output controls, under a total allowable catch (TAC). The initial TAC was set at 42,000 t and was based on the highest annual catch from the purse-seine fishery (Jordan *et al*., 1992). The TAC was decreased to 34,000 t (combined species TAC) for the 2002-03 and subsequent fishing seasons but without any scientific basis. Besides this, the fishery has changed dramatically from a purse-seine fishery for jack mackerel to a mid-water trawl fishery targeting mostly redbait (with jack mackerel as a by-product). The management of the fishery, now part of the Commonwealth Small Pelagic Fishery (SPF), is currently under review and will be managed using output controls (quotas).

PREVIOUS RESEARCH

During the late 1980's and early 1990's, considerable research effort was directed at describing the fisheries biology of jack mackerel. Projects were initiated to (a) evaluate tools for assessment of the jack mackerel stocks; (b) describe factors contributing to inter-annual variability in the availability of jack mackerel; and (c) collect information on the early life history and reproductive biology of the species (Jordan *et al*., 1992, 1995). Research outputs included greater understanding of interactions between local oceanography and the availability of surface schools jack mackerel (Harris *et al*., 1992; Williams and Pullen, 1993), and data on the reproductive biology and early life history of jack mackerel (Harris *et al*., 1992; Young and Davis, 1992; Marshall *et al*., 1993; Williams and Pullen, 1993; Jordan, 1994; Jordan *et al*., 1995). However, no successful method of assessing the size of the jack mackerel resource was developed, despite

attempts to use a combination of aerial surveys of surface schooling fish, and hydroacoustic surveys of surface schools and sub-surface spawning schools on the shelf break (Jordan *et al*., 1992). It was recognised that the evaluation of methods to estimate abundance of jack mackerel remained a priority, particularly to support the review of the catch allocation. In contrast to jack mackerel, very little research attention was directed at redbait until a recent compilation of available information for the species (Welsford and Lyle, 2003).

REDBAIT

Redbait is a geographically widespread pelagic fish species distributed over the continental shelf break of Africa, Australia and South America. They are also found in association with seamounts, islands and mid-oceanic ridges in the south-west Atlantic, Indian and south Pacific Oceans (Heemstra and Randall, 1977; Last *et al*., 1983; Gomon *et al*., 1994; Hoese *et al*., 2007). Redbait are known to be an important component of the pelagic ecosystem as prey for species such as tunas and billfishes, seabirds and mammals (Brothers *et al*., 1993; Gales and Pemberton, 1994; Young *et al*., 1997; James and Stahl, 2000; Hedd and Gales, 2001). Despite being fished commercially throughout its range for human consumption, bait and fish meal, little is known of the biology and ecology of this species. Furthermore, there have been no formal stock assessments or biomass estimates for redbait or other species in the family anywhere in the world (Welsford and Lyle, 2003). Redbait and other species in the family Emmelichthyidae are targeted outside Australia by trawling operations, with the majority of catch taken by vessels from New Zealand, and small amounts by states formerly in the USSR (Russian Federation, Georgia and Ukraine) and South Africa. Excluding Australia, worldwide catches of redbait have been in the range 1,800 - 3,000 t per annum, most reported from New Zealand (Anonymous, 2001).

The largest landing of redbait recorded from the Tasmanian purse-seine fishery was almost 1,300 t in 1986-87 (Pullen, 1994b). Recent mid-water trawl catches of redbait off Tasmania have averaged around 7,000 t and are therefore higher than those reported elsewhere in the world. Given the short history of the fishery there is no time series of fishery-dependant information for redbait off Tasmania, nor fishery-independent biomass estimates to support the TACs for this or other small pelagic species.

THE DAILY EGG PRODUCTION METHOD

The daily egg production method (DEPM) is a fishery-independent method currently used extensively by several countries to provide biomass estimates of small pelagic fishes that are serial or batch spawners, i.e. release batches of pelagic eggs into the water column throughout the spawning season (Parker, 1985). The method assumes that the size of the spawning biomass of a population can be estimated from the:

- daily production of eggs over the entire surveyed spawning area,
- weight and proportion of females that spawned the previous night, and
- average number of eggs spawned by each female.

In Australia, this method has been employed to estimate the spawning biomass of

pilchard in Western Australia (Fletcher *et al*., 1996) and South Australia (Ward *et al*., 1998), and it has recently been evaluated for blue mackerel in south-eastern Australia (Ward and Rogers, 2008). Elsewhere, the DEPM has been used extensively to estimate spawning biomass of several small pelagic fish species, including northern anchovy (*Engraulis mordax*) and Pacific sardine (*Sardinops sagax*) in California (Lasker, 1985; Lo, 2001; Lo and Macewicz, 2002), anchovy (*E. ringens*) and common sardine (*Strangomera bentincki*) in Chile (Cubillos *et al*., 2007), and horse mackerel (*Trachurus trachurus*) in Portugal (Cunha and Stratoudakis, 2000; see also review by Stratoudakis *et al*., 2006).

NEED

The introduction of large-scale mid-water trawl operations (2001) to target small pelagic species in Zone A of the Small Pelagic Fishery (SPF) has produced catches of redbait that have no precedent locally and there is real potential for further rapid expansion. Little information is available on the biology or population dynamics of redbait, and there are no assessments of stock size on which to base TACs.

The need to provide a scientific basis for the estimation of TACs remains a high research priority for the SPF. Significantly, provision of minimum or conservative biomass estimates for redbait will enable the sustainability of current harvest levels to be evaluated and contribute to the setting of future TACs. Whether on-going biomass estimation using DEPM is justified, given the high cost of such assessments, may also be investigated in the light of uncertainties arising from the estimation procedure and potential risks to the stocks and ecosystem.

The development of a method for estimating redbait biomass is urgently required to support the setting of scientifically defensible TACs. Fishery-dependent methods of assessing fish stocks are generally unsuitable for small pelagic species due to their schooling behaviour and targeted nature of the fishing operations. Furthermore, due to the very recent development of mid-water trawling targeting redbait, no time series of data are available to detect changes in redbait stocks off Tasmania. The DEPM has been applied successfully for biomass estimation of a variety of small pelagic fish species, and may therefore be suitable for estimating redbait biomass. There are of course obvious advantages in generating biomass estimates as early as possible in the development of this fishery if fishery impacts are to be detected and managed.

Significant recent research investment has been directed at an assessment of the DEPM for blue mackerel (FRDC Project 2002/061, Ward and Rogers, 2008), a species that co-occurs with redbait off eastern and southern Australia. Although linkages were maintained between projects in terms of research objectives, expertise and resources, redbait have a distinctly different spawning season and thus the timing of blue mackerel egg surveys were not appropriate for redbait. As a consequence the present project has been specifically designed to address the feasibility of applying the DEPM to redbait.

OBJECTIVES

1. To estimate the critical reproductive parameters for redbait along the east coast of Tasmania, particularly spawning fraction and batch fecundity.

2. To develop and validate methods for identifying and staging the eggs and larvae of redbait.

3. To estimate the location and extent of spawning areas of redbait on the east coast of Tasmania, including a quantification of the levels of egg production of this species.

4. To evaluate the use of the daily egg production method for estimating the spawning biomass of redbait on the east coast of Tasmania.

5. To produce a minimum biomass estimate of redbait in Zone A of the Small Pelagic Fishery.

Note: this report has been developed as series of stand-alone papers for submission to scientific journals, and therefore there is repetition in some of the background information.

CHAPTER 1: REPRODUCTIVE BIOLOGY OF REDBAIT AND ESTIMATION OF ADULT REPRODUCTIVE PARAMETERS FOR THE DAILY EGG PRODUCTION METHOD

G.P. Ewing, J.M. Lyle and D.C. Welsford

Objective 1: To estimate the critical reproductive parameters for redbait along the east coast of Tasmania, particularly spawning fraction and batch fecundity.

Redbait off Tasmania have a discrete 2-3 month spawning season during the austral spring between September and November. The species is a batch spawner with asynchronous oocyte development and indeterminate fecundity, reproductive traits suitable for the application of the daily egg production method to estimate spawning biomass. Functional groupings of oocytes and post-ovulatory follicles were derived from fine-scale classification and analysis of concurrent oocyte stages and were used to derive a histological criterion for estimating spawning fraction that implied that redbait females spawn once every three days. The relationship between batch fecundity and weight was linear, with fecundity increasing at a rate of 186 oocytes per gram of ovary-free weight. Overall mean batch fecundities ranged between 11,001 and 27,162 for eastern and south-western Tasmania respectively, the marked difference reflecting the substantially larger fish present off the south-west coast. Size at maturity varied markedly by region with south-west coast fish attaining maturity at around 100 mm larger than those from the east coast. Sizes at 50% maturity in males were 146 mm for east coast and 244 mm for south-west coast fish, and 157 mm for east coast and 261 mm for south-west coast females. Ages at maturity also differed regionally, with east coast fish maturing at around 2 years and south-west coast fish at 4 years of age. The reasons for the dramatic differences in size and age at maturity between fish from the east and southwest coasts warrant further investigation.

Key adult parameters of mean mature female weight, batch fecundity, female sex ratio and spawning fraction were estimated separately for eastern and south-western Tasmania for 2005 and 2006.

1.1 INTRODUCTION

It is essential to correctly identify the spawning strategy of a species to be able to estimate egg production (Lasker, 1985). The daily egg production method (DEPM) is applicable to species that are asynchronous batch spawners with indeterminate fecundity. The spawning strategy of a species is established by examination of the sequence of oocyte developmental stages, whereby asynchronous oocyte development is characterised by the presence of all stages of oocyte development without a dominant cohort until hydration. Indeterminate fecundity refers to strategies where potential annual fecundity is not fixed before the onset of spawning and *de novo* vitellogenesis occurs (Hunter *et al.,* 1992). Indeterminate fecundity is characterised by a continuous range of the stages of oocyte development from unyolked to yolked, increased rates of atresia at the end of the spawning season and an increase in the mean diameter of advanced oocytes over the spawning season (Walker *et al.,* 1994; Murua and Saborido-Rey, 2003).

Spawning fraction is a critical parameter for asynchronous batch spawners and has a significant influence on biomass estimates from DEPM (Hunter and Lo, 1997; Stratoudakis *et al.,* 2006). Sampling for spawning fraction estimates must be random and the proportion of the population spawning on a single day is generally estimated from the proportion of ovaries with post-ovulatory follicles (POFs) that indicate spawning in the prior 24 hours (Hunter and Goldberg, 1979). A key describing the deterioration and resorption processes of POFs with respect to time is necessary to infer a 24-hour batch and is generally species-specific. Decay rates have also been observed to vary with water temperature, with POFs decaying more quickly in warmer waters. This key can be achieved by sampling spawning fish in captivity (Leong, 1971; Fitzhugh and Hettler, 1995; Macchi *et al.,* 2003), or by sampling spawning aggregations over a 24 hour period (Goldberg *et al.,* 1984; Hunter and Macewicz, 1985).

Sampling to generate a fecundity relationship should encompass the full range of sizes of spawning fish, and for asynchronous batch spawners should encompass the duration of the spawning season (Murua and Saborido-Rey, 2003). The gravimetric method of estimating batch fecundity measures the number of oocytes per gram of ovary tissue from a macroscopic count of a single batch of oocytes from weighed sub-samples. These are then multiplied up to the number of oocytes per gram of fish weight using the gonad weight (Hunter *et al.,* 1985), and a model fitted to describe fecundity as a function of fish weight. However, this method may be difficult for asynchronous spawners if there is insufficient distinction between cohorts of eggs just prior to spawning (Murua and Saborido-Rey, 2003). The gravimetric method also necessitates histological examination of ovaries to ensure that spawning has not commenced prior to sampling.

Sex ratio is an essential parameter for the DEPM as it permits the inclusion of males in the biomass estimate. Sex ratio, mean female weight and subsequent mean batch fecundity are all calculated from mature fish and, as such, it is critical to be able to

distinguish mature from immature individuals. In practice, this can be difficult based solely on macroscopic appearance outside of the spawning season.

As with any of the attributes of the reproductive strategy of a fish species, the timing and duration of the spawning season as well as the size and age at the onset of maturity are species-specific adaptations to maximise the probable survival of offspring. However, conspecific variation has also been observed in response to regional oceanographic factors (Silva *et al.,* 2006) and fishing effort (Olsen *et al.,* 2005; Ziegler *et al.,* 2007).

Redbait (*Emmelichthys nitidus* Richardson 1845) is one of two species of emmelichthyids reported off Tasmania, the other being rubyfish (*Plagiogeneion macrolepis*) (Last *et al.,* 1983; May and Maxwell, 1986; Gomon *et al.,* 1994). Despite being widely distributed throughout the Southern Hemisphere (Heemstra and Randall, 1977; Markina and Boldyrev, 1980; Melendez and Cespedes, 1986; Oyarzun and Arriaza, 1993; Parin *et al.,* 1997), information on the reproductive strategy of redbait is scant. Roschin (1985) reported that redbait taken from seamounts in the Indian Ocean had a brief spawning season (August) during which spawning was restricted to a portion of the mature population and sex ratios were approximately 1:1. Rubyfish has a similar geographical distribution to redbait and is reported to spawn during winter-spring in the southern Indian Ocean, maturing at around 240mm standard length (Mel'nikov and Ivanin, 1995).

In this chapter we (i) describe the reproductive strategy of redbait off Tasmania and assess its suitability for estimating spawning biomass using the DEPM, and (ii) provide the necessary adult input parameters for the model. This included spawning strategy, spawning fraction, mean batch fecundity, mean female weight and sex ratio by spawning season and by region. Other reproductive traits investigated by region were size and age at maturity, and the timing and duration of the spawning season.

1.2 METHODS

1.2.1 Spawning season sampling

Redbait were sub-sampled from mid-water trawl catches taken by the commercial fishing vessel *Ellidi* off the Tasmanian shelf break, mostly in depths of 100 to 180 m. Adult sampling for the DEPM was paired with plankton sampling in eastern Tasmania in October 2005, and eastern and south-western Tasmania in October 2006 (Table 1.1). Whilst sampling from the commercial fishing vessel was advantageous, the size of the mid-water trawl net coupled with the logistics of catching redbait, including the necessity for trawls of sufficient duration to run down schools (typically >3-5 hours), prohibited a fully structured research sampling regime. In practice, redbait were only caught when fish marks were observed on the vessel's echosounder or sonar, while attempts to catch fish in the absence of marks typically resulted in nil catches. As a consequence, very little fishing was conducted during the daytime when schools tended to be dispersed. Such logistic and commercial considerations precluded extensive paired sampling between the research vessel undertaking the plankton survey (Chapter 3) and the fishing vessel, as would ideally be the case in a DEPM sampling regime. Consequently, opportunistic supplementary sampling of commercial fishing operations was conducted during the spawning seasons of 2004, 2005, 2006 and 2007 (September to November inclusive) off eastern and south-western Tasmania (Fig. 1.1, Table 1.1). Supplementary samples were used to boost sample sizes for reproductive parameters and to ascertain the duration of the spawning season.

A random sample of between 100 and 200 fish was frozen from each shot for later examination. These fish were thawed and fork length (FL, ± 1mm), total weight (± 1g), sex, gonad stage and weight (± 0.1g) were recorded. Macroscopic gonad staging criteria are listed in Table 1.2. During 2004 and 2005, length and gonad stage were recorded from a further random sample of around 30 females from every shot. The gonads from these fish were removed and preserved in FAACC (10% formalin, 5% glacial acetic acid and 1.3% calcium chloride). During 2006, a random sample of at least 30 mature females was collected from each shot and preserved whole in FAACC (with a slit from vent to ventral cradle to assist preservation). Additional females with hydrated oocytes were also preserved in FAACC but these fish were kept separate from the random samples and used to supplement samples used for fecundity estimation.

1.2.2 Spawning seasonality

In addition to spawning season sampling, random samples of at least 100 fish per shot were collected opportunistically from commercial fishing outside the spawning season over the 2003 to 2006 period. These fish were frozen on board and later thawed and measured for fork length (FL, ± 1 mm), total weight, sex, macroscopic gonad stage and gonad weight.

The spawning season of redbait was identified using temporal trends in gonad macroscopic stage and gonadosomatic index (GSI) calculated by the following equation:

$$GSI = \left[\frac{G_{wt}}{(W - G_{wt})} \right].100$$

where G_{wt} is gonad weight and W is total body weight.

The duration of the spawning season was further investigated by examining trends in the rate of oocyte atresia from histological sections of gonads of randomly sampled fish within the spawning season.

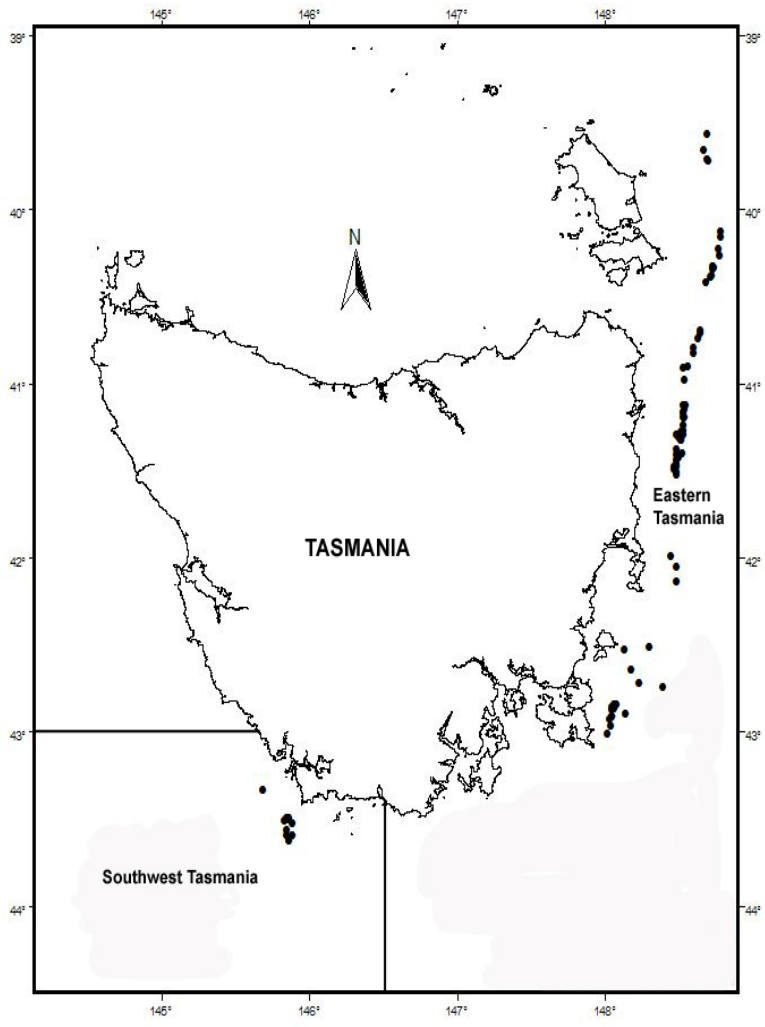


Fig. 1.1 Map of Tasmania showing adult sampling locations (solid black circles) by region.

Table 1.1 Summary of redbait collected during spawning season sampling by region and year.
* Samples from shots which were paired with DEPM plankton samples.

Region	Year	Month	No. males frozen	No. females frozen	No. females FAACC	No. shots
Eastern Tasmania	2004	Oct	352	389	98	5
		Nov	195	280	135	3
	2005	Oct*	836	687	268	7
	2006	Sep	233	102	119	5
		Oct*	424	441	365	6
		Oct	1,078	694	172	9
	2007	Sep		90		2
	Total		**3,118**	**2,683**	**1,157**	**37**
South-western Tasmania	2004	Nov	234	345	107	3
	2005	Oct	26	128	50	1
	2006	Oct*	79	228	105	2
	2007	Sep		128		2
	Total		**339**	**829**	**262**	**8**
Grand total			**3,457**	**3,512**	**1,419**	**45**

1.2.3 Spawning mode

A combination of microscopic examination of ovarian development coupled with modal progression in the size of oocytes was used to characterise spawning mode.

Ovaries that had been preserved in FAACC were blotted dry and weighed (± 0.1g). A transverse portion of gonad tissue was dissected from around the midpoint, and processed using standard histological techniques to yield 5µm sections which were stained with haematoxylin and eosin dyes. A subsample of sections covering all macroscopic gonad stages was examined using a compound microscope at around 100X magnification, and oocyte developmental stages and condition of post ovulatory follicles described and categorised (Tables 1.3, 1.4). At least three sections from every randomly sampled and preserved gonad were examined for the presence of each oocyte and POF category. The presence of atretic oocytes was also recorded.

A sample of around 100 mg of tissue was removed from FAACC preserved female gonads of three fish from each of the main oocyte developmental stages (Table 1.3). The diameter of every oocyte in each of these samples was measured using Leica image analysis software (IM1000) and oocyte size frequencies plotted.

1.2.4 Spawning fraction

Three criteria, based on the presence and/or absence of various advanced oocyte and POF categories, were used to generate three spawning fractions from the set of preserved gonads from randomly sampled mature females. The first spawning criterion (SC-1) sought to identify early spawning activity and considered a spawning batch to be fish with late migratory nucleus and/or early hydrated oocyte categories (3e-4c) present but excluded fish with fresh POF categories. The second criterion (SC-2) sought to identify fish in the process of spawning and only included fish with hydrated oocytes

(4a-d) and/or fresh POF (POFa-c) categories. The third spawning criterion (SC-3) sought to identify fish that had recently spawned and only included fish with fresh POF categories (POFa-c).

Diurnal cycles of spawning activity were explored through an examination of the relative proportions of individual and coincident oocyte developmental stages from histological samples with respect to the mid-time of the sampled trawl. As some trawls extended over periods exceeding 6 hours the precise time that fish were sampled is approximate only. The accuracy of each of the three spawning criteria in reflecting spawning fraction was assessed with reference to the diurnal trend in oocyte development.

Table 1.2 Criteria for assigning macroscopic stages to the gonads of redbait, based on West (1990). Mean gonadosomatic indices for each stage are indicated.

Stage		Mean GSI	Appearance of gonads
Female			
I	Immature / virgin	0.58	Ovaries thin and thread-like translucent and nearly colourless. Oocytes are not visible.
II	Maturing virgin or recovering spent	3.98	Ovaries beginning to enlarge, are more rounded and appear translucent pinkish. Oocytes are not visible.
III	Maturing	4.03	Ovaries maturing and filling approximately two-thirds of the body cavity. Ovaries are yellow or pale orange and ova are visible, small and opaque.
IV	Hydrated	5.55	Ovaries at maximum size and filling body cavity. Translucent hydrated oocytes distributed throughout the ovary between yolked oocytes.
V	Spawning	4.43	Pressure on the ventral sides of the fish causes extrusion of hydrated oocytes in a fluid stream.
VI	Spent	0.59	Ovaries slack and bloodshot. Small proportion of hydrated oocytes present.
Male			
I	Immature / virgin	0.36	Testes are small and straplike with a smooth appearance and translucent.
II	Maturing virgin or recovering spent	0.63	Testes are small, opaque, straplike, angular in transverse section and off-white in colour.
III	Maturing	3.52	Testes larger and white in colour. White milt can usually be squeezed from the central sinus from a sectioned testi.
IV	Spawning	5.25	Testes even larger, white in colour. Milt is released with little or no pressure on the abdomen.

Table 1.3 Categorisation system for oocyte developmental in redbait

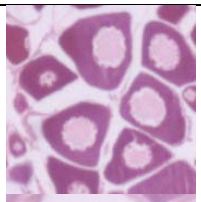
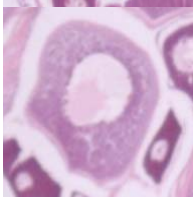
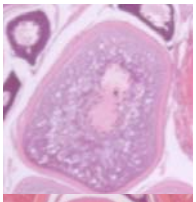
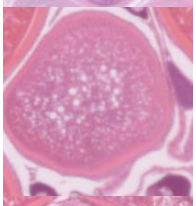
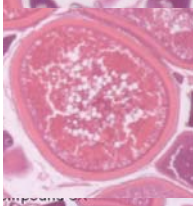
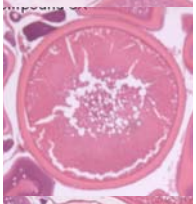
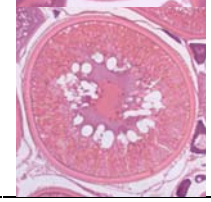
Category	Description	Image	Stage
1b Perinucleus	• Nucleus increases in size and lightens • Area of cytoplasm small • Zona radiata forms		Early oocyte stages
1c Late perinucleus	• Nucleus increases further in size • Area of cytoplasm increases • Zona radiata widens		
1d Pre-vitellogenesis	• Nucleus increases further in size • Vacuoles evident around nuclear membrane • Zona radiata widens further		
2a Early cortical alveoli	• Cortical alveoli and very small oil droplets forming • Nuclei possibly evident		
2b Late cortical alveoli	• Cortical alveoli and very small oil droplets forming • Nuclei possibly evident		
3a Yolk granular	• Yolk granules evident • Oil droplets still very small form • Nuclei possibly evident		
3b Oil droplet	• Yolk granules evident • Oil droplets small, numerous and yet to coalesce • Nucleolus and nuclear membrane clear		

Table 1.3 Continued

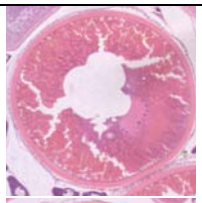
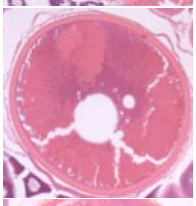
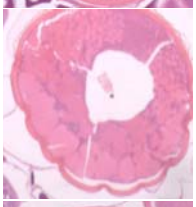
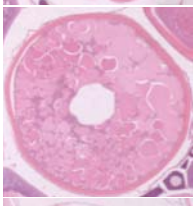
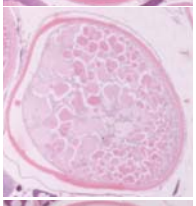
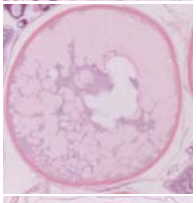
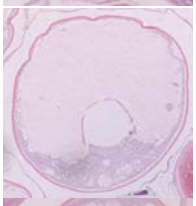
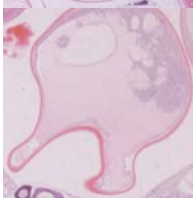
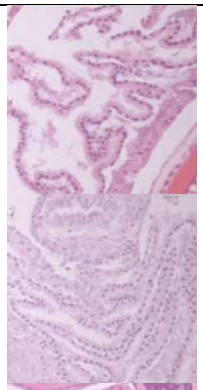
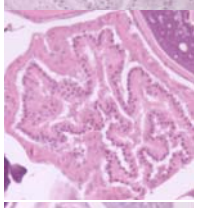
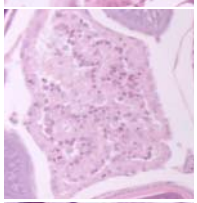
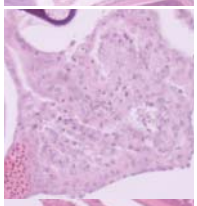
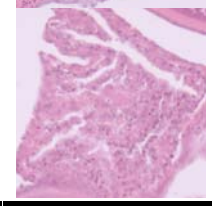
Category	Description	Image	Stage
3c Early migratory nucleus	• Yolk granules evident • Oil droplets almost or completely coalesced • Nuclei evident but yet to migrate		Migratory nucleus stage
3d Migratory nucleus	• Yolk granules evident - yolk platelets yet to form • Nuclei migrated or migrating, but not dispersed		
3e Late nuclear migration	• Yolk platelets forming • Nuclei migrated but only partially dispersed		
3f Post nuclear migration	• Yolk platelets very clear • Nuclei dispersed		
4a Very early hydration	• Oocyte transparent • Small lumen between oocyte and follicle • Yolk platelets very clear		Early hydration stage
4b Early hydration	• Oocyte transparent • Small lumen between oocyte and follicle • Yolk platelets still visible		
4c Early hydration	• Oocyte transparent • Detached from follicle • Yolk platelets not discernable		
4d Hydrated	• Oocyte transparent and distorted by preservation • Detached from follicle • Next most advanced oocytes are at yolk vesicle formation stage		Fully hydrated stage

Table 1.4 Categorisation system for the decay of post ovulatory follicles (POFs) in redbait.

Category	Description	Image	Stage
POFa Very fresh POFs	• Follicular lumen still open from evacuated oocyte • Granulosa cells distinct • Intact string formation of granulosa cells		Fresh POFs
POFb Fresh POFs	• Follicular lumen less open • Granulosa cells distinct • Intact string formation of granulosa cells		
POFc Less-fresh POFs	• Follicular lumen further contracted • Granulosa nuclei distinct • String formation of granulosa cells starting to decay		
POFd Not-fresh POFs	• Follicular lumen almost gone • Granulosa nuclei still distinct • String formation of granulosa cells present but disorganised		Old POFs
POFe Old POFs	• No follicular lumen • Gaps appearing from decay process • Few granulosa nuclei are discernable		
POFf Very old POFs	• No follicular lumen • Gaps appearing • Granulosa nuclei rare or indiscernable		

1.2.5 Fecundity estimation

Fish with a complete and discernable batch of eggs were identified by the presence of hydrated oocytes and the absence of fresh POFs in histological sections. From each of these fish, three transverse samples, of around 100 mg of tissue each, were removed from around the middle of the ovary, weighed (± 1mg) and the total number of hydrated oocytes counted. From these counts, three estimates of total batch fecundity were obtained for each female using the gravimetric method (Hunter *et al.,* 1985) by multiplying the number of hydrated oocytes per gram of ovary segment by the total ovary weight. Fish were rejected if the CV between the three fecundity estimates

exceeded 10%. A batch fecundity relationship was estimated using linear regression of mean batch fecundity (from the three fecundity estimates) and ovary-free weight for each fish.

1.2.6 Mature female weight

Females randomly sampled during the spawning season, and with macroscopic gonad stage III or greater, were used to estimate the mean weight of mature females by year and region. The population mean weight of mature females was calculated from sample means weighted by sample size using the equation:

$$W = \sum_{i=1}^{s} \left(\overline{W}_i * \frac{n_i}{N} \right)$$

where $\overline{W}_i$ is the mean mature female weight, n_i is the number of fish in sample i, s is the number of samples, and N is the total number of fish in all samples. For each shot a linear regression of total weight against ovary-free weight was used to convert mean mature female weight to an ovary-free weight. Ovary-free weight was applied to the batch fecundity relationship to generate mean population batch fecundity by shot. Relative batch fecundities were an expression of mean batch fecundity by total fish weight. Coefficients of variation for mean mature female weights and mean batch fecundities were calculated using the methodology described in Cubillos *et al*. (2007).

1.2.7 Sex ratio

Male and females randomly sampled during the spawning season, and with macroscopic gonad stage III or greater, were used to estimate sex ratio of mature redbait by year and region using the equation:

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

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

The population mean sex ratio was weighted by sample size using the equation:

$$\overline{R} = \sum_{i=1}^{s} \left(R_i * \frac{n_i}{N} \right)$$

where R_i is the sex ratio and n_i is the number of fish in sample i, s is the number of samples and N is the total number of fish in all samples. Coefficients of variation for mean sex ratios were calculated using the methodology described in Cubillos *et al*. (2007).

1.2.8 Size and age at sexual maturity

Size at 50% maturity (L_{50}) by sex and region was estimated from fish randomly sampled during the spawning season by fitting a logistic model to the percentages of sexually mature fish (stage III or greater), grouped into 10 mm size classes. The logistic curve is represented by the equation:

$$P_{ML} = \frac{1}{\left[1 + e^{(a+bL)}\right]}$$

where P_{ML} is the proportion of mature fish from each size class, L is size class, and a and b are constants fitted by minimising the sum of squared residuals. Length at 50% maturity was estimated by the equation:

$$L_{50} = -\frac{a}{b}$$

Ages were assigned to a sub-set of randomly sampled fish based on the protocols outlined in Appendix 3 and age at 50% maturity (t_{50}) estimated by sex and region by fitting a logistic model to the percentages of sexually mature fish from the aged dataset (grouped into one year age classes). The logistic curve is represented by the equation:

$$P_{MA} = \frac{1}{\left[1 + e^{(a+bt)}\right]}$$

where P_{MA} is the proportion of mature fish in each size class, t is the age class and a and b are constants fitted by minimising the sum of squared residuals. Age at 50% maturity was estimated by the equation:

$$t_{50} = -\frac{a}{b}$$

1.3 RESULTS

1.3.1 Spawning seasonality

Trends in male and female GSIs indicate that redbait have a discrete spawning season extending over a 2-3 month period during spring. The GSIs from east coast redbait rose sharply in August, peaking in September - October before declining to resting levels by January (Fig. 1.2). A similar pattern was evident for south-western Tasmania, although the GSI peak occurred between October – November, i.e. one month later.

Macroscopic staging of females confirmed that the seasonal increase in GSIs was attributed to reproductive activity. Fish with maturing gonads (stage III) dominated east coast samples in August and by September over half of the fish examined had hydrated oocytes (stage IV) (Fig. 1.3). Fish with hydrated oocytes were present through to November and spent fish (stage VI) were evident between November and January, implying that limited spawning activity may have extended to December and January. Very few running ripe fish were observed in these samples, possibly an artefact of freezing making such gonads difficult to distinguish from those with hydrated oocytes. Samples collected from between January and August were dominated by fish with undeveloped or resting gonads (>90%). A similar pattern of gonad stage development was evident off south-western Tasmania. Spawning season GSIs for south-western Tasmania were consistently lower than those for fish from eastern Tasmania (Fig. 1.2), presumably in response to the lower proportion of actively spawning fish (≥ stage III) in the samples (Fig. 1.3).

The occurrence of oocyte atresia in histological sections from fish sampled off eastern Tasmania during 2004 increased from 11% in fish sampled in late October to 36% of the fish sampled in November. These observations support macroscopic staging by implying that the peak in spawning activity was over by mid-November.

1.3.2 Spawning mode

The size frequency of oocytes in stage II gonads was dominated by perinucleus stage oocytes with modal diameters of around 75 μm and pre-vitellogenic stage oocytes with diameters at around 200 to 300μm (Fig. 1.4). Mature ovaries showed a continuous range of diameters from early vitellogenic (approximately 300 μm) through to the migratory nucleus stage which displayed a mode at around 650 μm (stage III). Early hydrated oocytes displayed a mode at around 800 μm while fully hydrated oocytes separated clearly from early hydration stages with a mode at around 950 μm (stage IV). The coincident cohorts of developing oocytes in these size frequency distributions indicate an indeterminate spawning strategy.

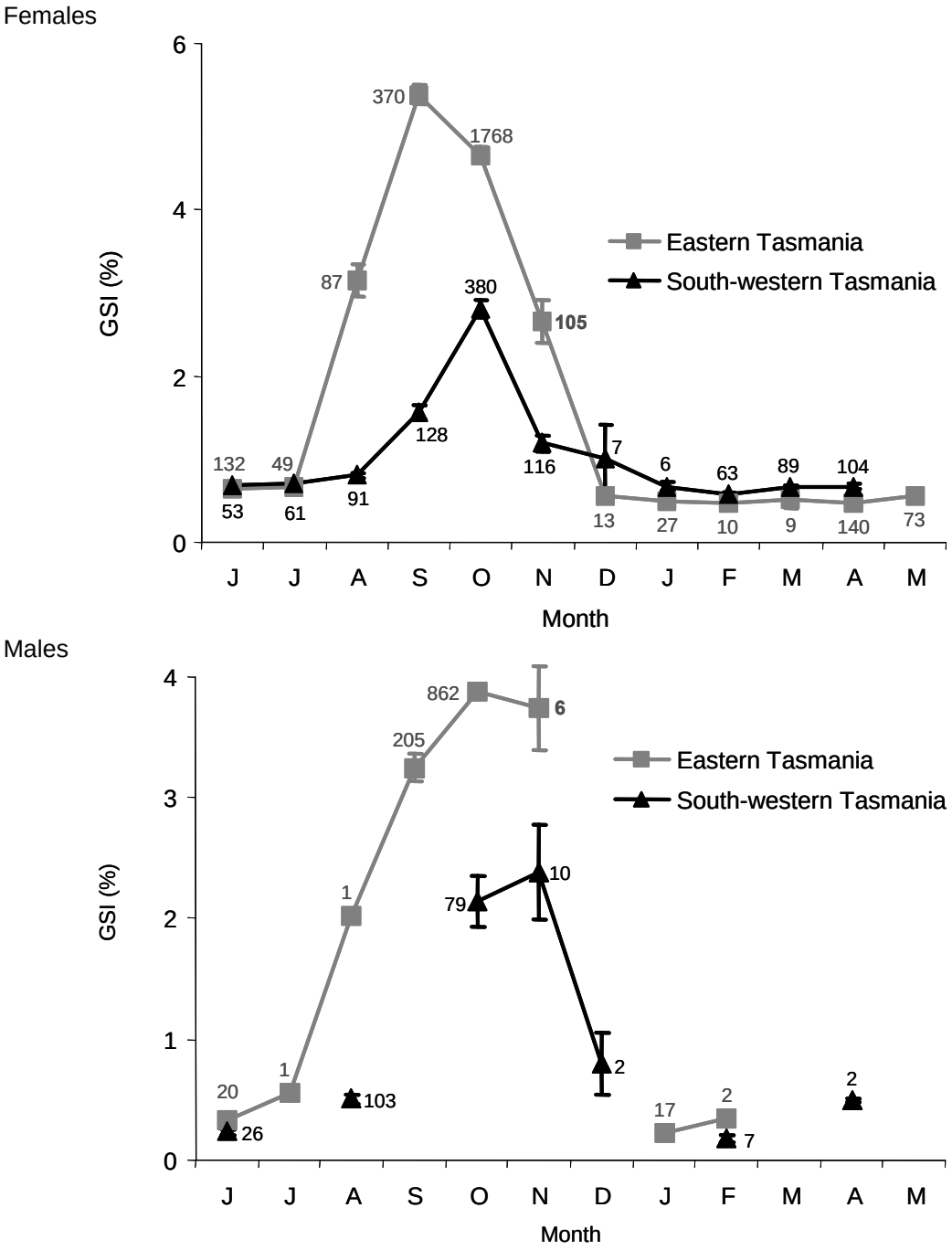
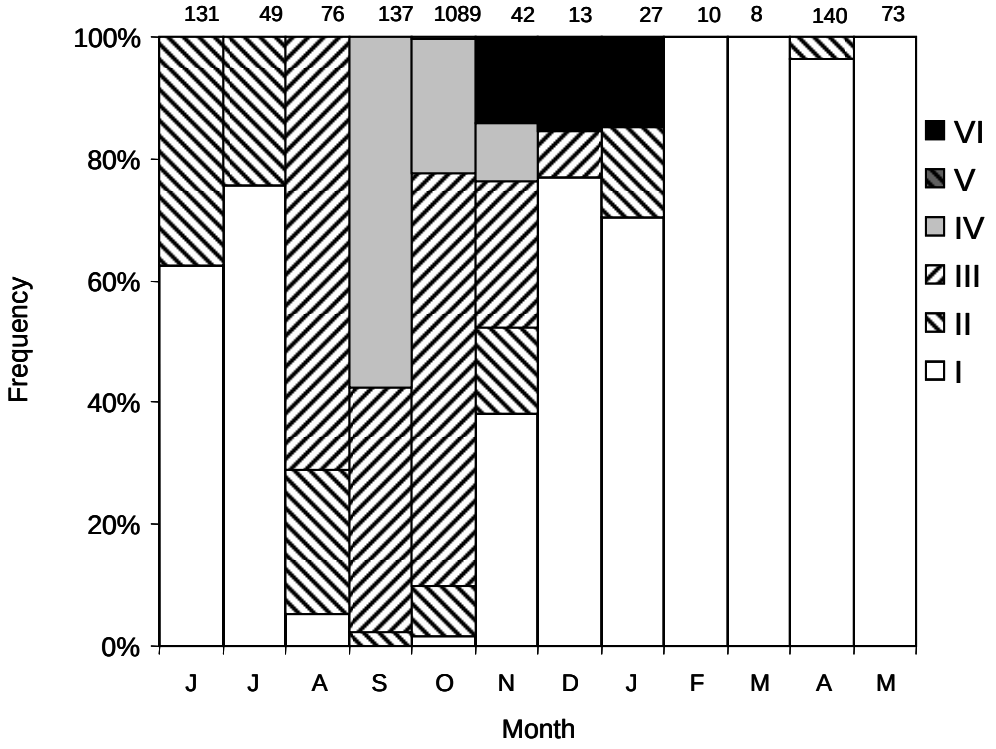


Fig. 1.2 Monthly distribution of mean GSI by sex and region. Numbers represent sample size and error bars are standard error.

Eastern Tasmania



South-western Tasmania

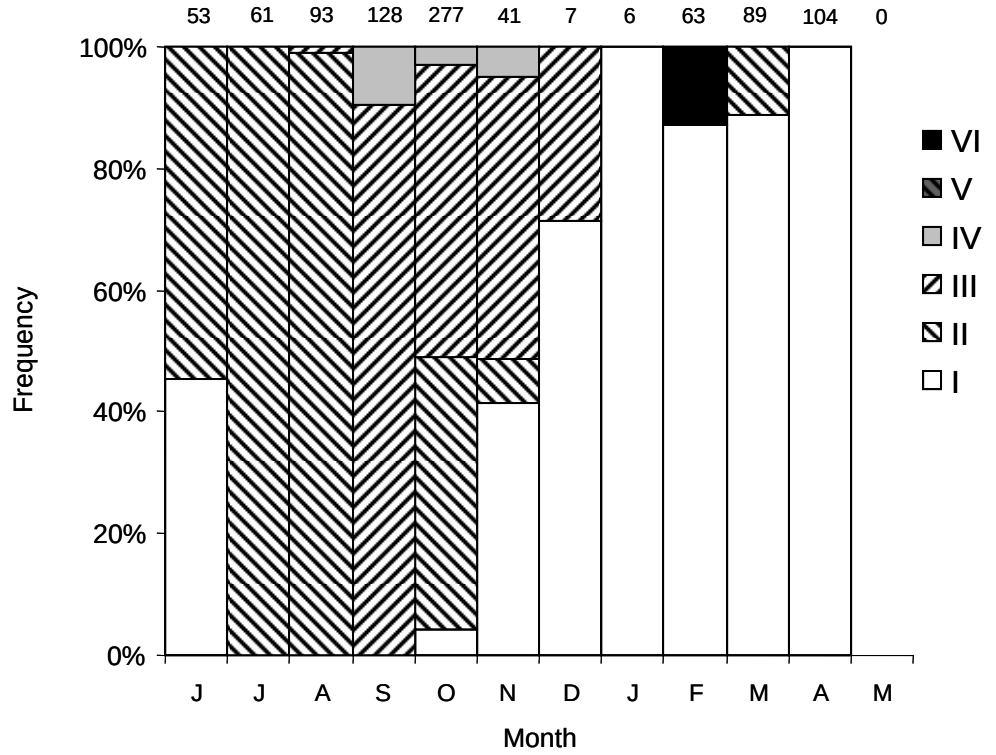


Fig. 1.3 Monthly distribution of female macroscopic gonad stages by region. Numbers represent sample sizes.

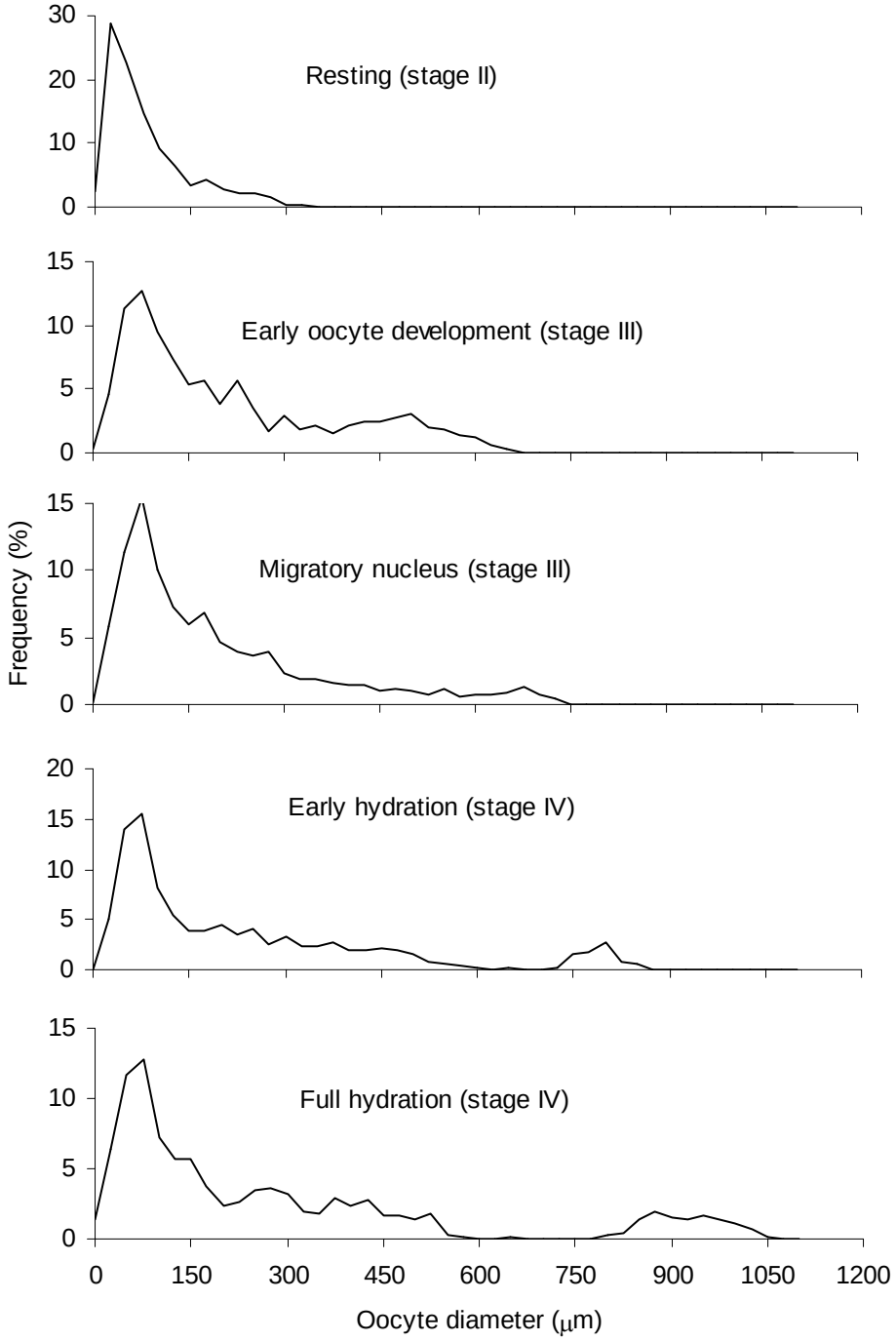


Fig. 1.4 Oocyte size frequency distributions for oocyte development stages from resting (gonad stage II) to fully hydrated (gonad stage IV).

1.3.3 Spawning fraction and timing of spawning

Histological processing of ovaries yielded interpretable sections with a gradient of decay in POFs easily identifiable. Estimates of spawning fraction for the 2005 and 2006 spawning seasons are detailed in Table 1.5. The 2004 spawning season sampling and 2005 south-western coast sampling did not yield sufficient fish with advanced stages of oocyte development to estimate spawning fractions.

Depending on the spawning criteria applied, overall mean spawning fraction estimates were 0.2 (SC-1), 0.25 (SC-3) and 0.3 (SC-2), implying a spawning frequency of once every 3-5 days. The validity of these criteria was evaluated by examining the process of oocyte development. Unyolked, pre-vitellogenic, cortical alveolar and oil droplet phases (early oocyte stage, categories 1b to 3b) were present in every gonad examined histologically, and were found to be the only categories present in 26% of gonads (Table 1.6). Of the more advanced oocyte stages, migratory nucleus stage oocytes (categories 3c to 3f) occurred on their own or coincided with early hydrated oocytes (categories 4a to 4c) or with old POFs (POFd to POFf) but were never observed with fresh POFs (categories POFa to POFc). Similarly, early hydrated oocytes did not coincide with the occurrence of fresh POFs. Further, despite fully hydrated oocytes (category 4d) coinciding with fresh POFs (in 4.5% of samples), they were never present with migratory nucleus oocytes.

The diurnal progression of oocyte development for stages indicative of reproductive activity, i.e. early hydration to the occurrence of fresh POFs, is provided in Figure 1.5. Fish with ovaries containing early hydrated oocytes were most prevalent in the early morning period (03:00 - 12:00) (Fig. 1.5). No samples were available for the afternoon period but by early evening (18:00 - 21:00) fish with fully hydrated eggs and fresh POFs were recorded indicating active spawning during this period. Catches sampled between 21:00 and 03:00 were dominated by fish with fresh POFs, with the proportion of fish with fully hydrated oocytes falling sharply after midnight (00:00). No fish with fresh POFs were recorded after 09:00 whereas old POFs were prevalent (>30%) in catches taken at all times of the day (data not shown). This pattern implies that fresh POFs may only persist for less than 24 hours whereas old POFs endure for at least 24 hours and possibly longer.

Table 1.5 Spawning fraction based on different criteria for redbait sampled during 2005 and 2006 off eastern and south-western Tasmania.

SC-1 is based on ovaries with late migratory nucleus stage and/or early hydrated oocytes present (no fresh POFs); SC-2 included fish with hydrated oocytes and/or fresh POFs present; and SC-3 included fish with fresh POFs present (no hydrated oocytes). N is the number of fish; value in parentheses is the coefficient of variation on the mean. Means are weighted for sample size.

Year	Region	Shot	Date	N	SC-1	SC-2	SC-3
2005	Eastern	1	14/10/05	34	0.09	0.63	0.24
	Tasmania	2	15/10/05	9	0.22	0.11	0.00
		3	15/10/05	36	0.33	0.33	0.17
		4	15/10/05	13	0	0.85	0.85
		5	16/10/05	32	0	0.37	0.47
		6	16/10/05	18	0.16	0.00	0.00
		7	17/10/05	46	0.11	0.20	0.29
		8	18/10/05	41	0.03	0.15	0.15
				229	**0.116 (0.41)**	**0.315 (0.23)**	**0.27 (0.23)**
2006	Eastern	1	28/09/06	30	0.13	0.29	0.34
	Tasmania	2	12/10/06	48	0.13	0.15	0.06
		3	12/10/06	48	0.04	0.08	0.15
		4	13/10/06	50	0.40	0.32	0.08
		5	13/10/06	47	0.17	0.48	0.40
		6	14/10/06	26	0.31	0.54	0.08
		7	15/10/06	47	0.34	0.34	0.17
		8	20/10/06	30	0.27	0.27	0.27
		9	21/10/06	29	0.25	0.48	0.34
		10	22/10/06	31	0.23	0.52	0.55
		11	23/10/06	29	0.22	0.37	0.22
		12	24/10/06	21	0.19	0.10	0.27
				436	**0.222 (0.16)**	**0.321 (0.14)**	**0.232 (0.19)**
	South-western	1	30/10/06	44	0.41	0.34	0.09
	Tasmania	2	31/10/06	40	0.28	0.32	0.18
				84	**0.345 (0.19)**	**0.333 (0.02)**	**0.131 (0.32)**
	All data			**749**	**0.186 (0.16)**	**0.319 (0.12)**	**0.245 (0.15)**

Table 1.6 Matrix indicating the presence (rows) and co-occurrence (columns) of oocyte and post ovulatory follicle (POF) stages in the combined sample of mature female gonads.
Presence is indicated by shading. N is the number of samples in each column or row.

Stage	**Co-ocurrence of stages**											**N**	**%**
Early oocyte	■	■	■	■	■	■	■	■	■	■	■	1023	100
Migratory nucleus		■	■								■	158	15.4
Early hydrated			■	■	■							155	15.2
Fully hydrated					■	■	■					99	9.7
Fresh POFs							■	■	■			151	14.8
Old POFs									■	■	■	374	36.6
N	268	84	41	85	29	24	46	72	33	308	33	**1023**	
%	26.0	8.2	4.0	8.3	2.8	2.3	4.5	7.0	3.2	30.1	3.2		

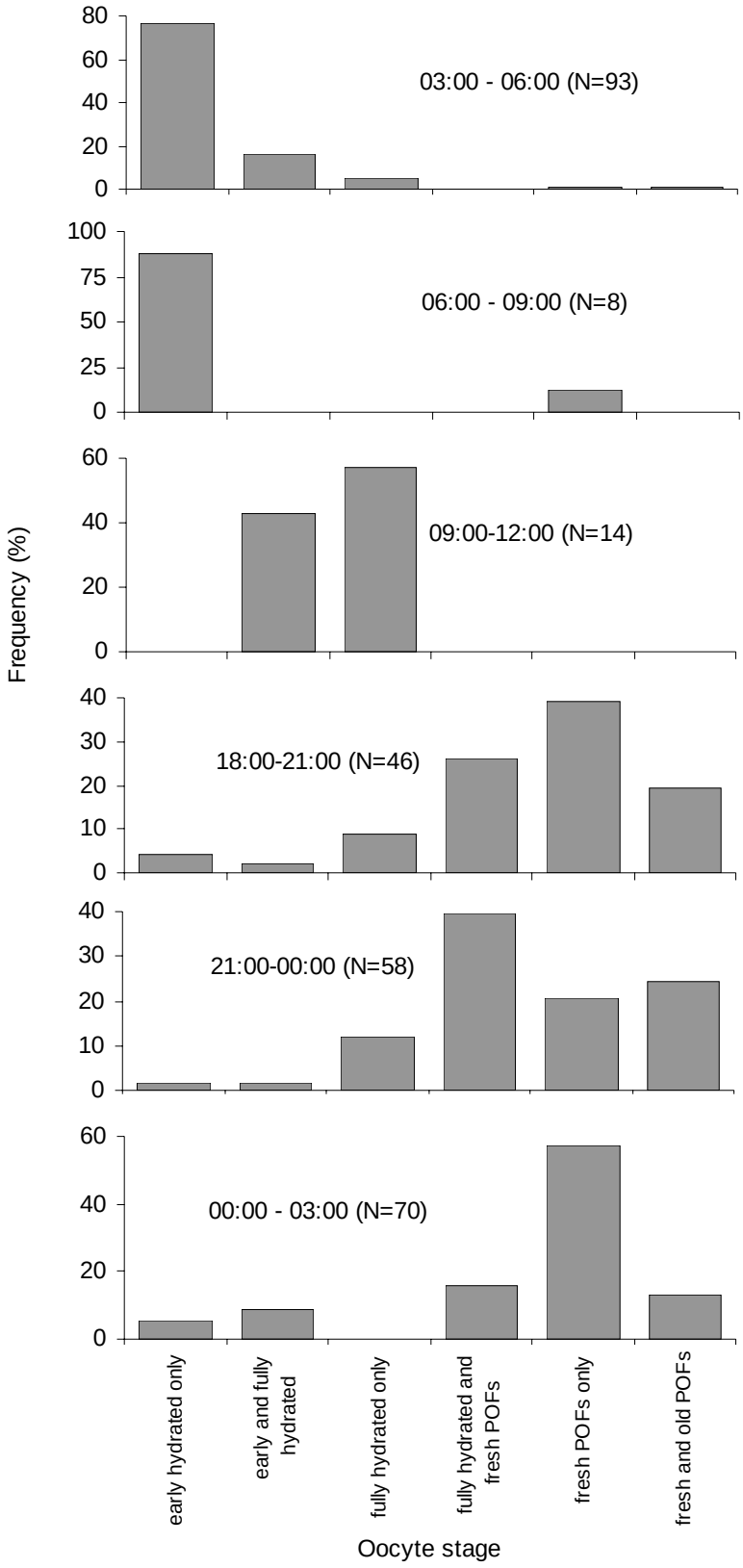


Fig. 1.5 Relative proportion of selected advanced oocyte stages by time of day for redbait. Note: no samples were available between 12:00 and 18:00 hours. N is sample size.

1.3.4 Fecundity and mean batch size

Hydrated oocytes were easy to discern from other oocyte stages within ovaries due to their considerably larger diameter (Fig. 1.4) and greater transparency. Batch fecundity relationships were generated from fish sampled from eastern and south-western Tasmania in 2005 and 2006, while sampling in 2004 did not yield sufficient fish with hydrated oocytes to contribute to the relationship. Of 117 fish examined for fecundity, three were rejected due to a CV of >10% based on replicate samples. Individual batches ranged from 1,365 (210 mm FL) to 77,950 hydrated oocytes (300 mm FL). The relationship between batch fecundity and gonad-free weight was linear with fecundity increasing at a rate of 186 oocytes per gram of ovary-free weight (Table 1.7; Fig. 1.6).

Table 1.7 Batch fecundity regression parameters for redbait by spawning season.
N is sample size

Year	N	Fecundity regression parameters		
		Intercept (SE)	Slope (SE)	R^2
2005	13	-3917.3 (2382)	163.38 (18.1)	0.88
2006	101	-3060.5 (1148)	198.93 (12.4)	0.72
Both years	**114**	**-2479.1 (1044)**	**186.43 (10.8)**	**0.73**

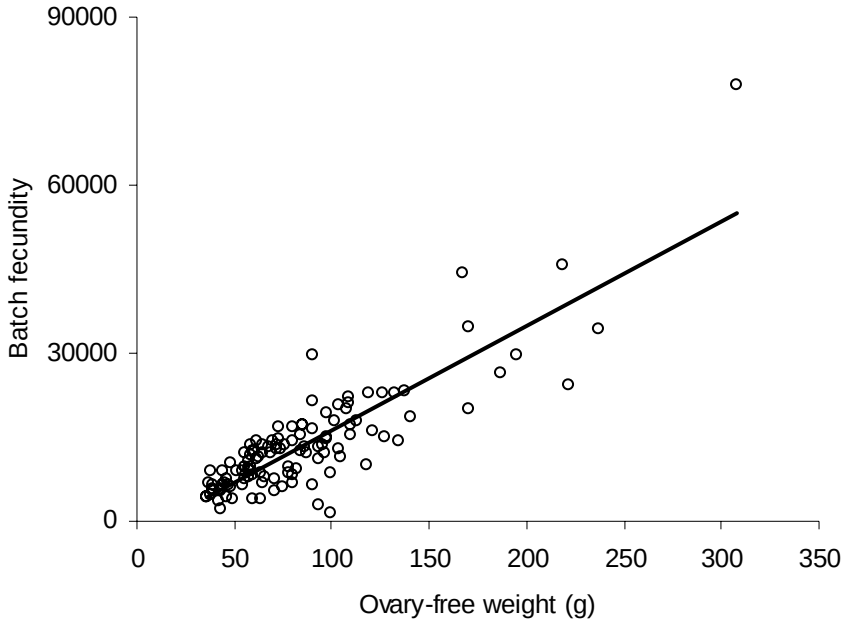


Fig. 1.6 Relationship between batch fecundity and ovary-free body weight for redbait sampled during the spawning seasons of 2005 and 2006.

Mean mature female weights from randomly collected samples (corrected for sample size) are presented by region and spawning season in Table 1.8. Mean weights were consistently higher for fish sampled from south-western Tasmania, with markedly larger fish sampled in 2005. However, samples sizes for this region were relatively low, with the 2005 sample derived from a single trawl.

Mean batch fecundities derived from the combined batch fecundity relationship by spawning season and region ranged between about 9,500 – 11,400 (mean 11,000) for eastern Tasmania and 21,200 – 47,500 (mean 27,200) for south-western Tasmania, the marked regional difference reflecting the larger fish sampled in the latter region.

Table 1.8 Mean mature female weights (g) and mean batch fecundities by region and spawning season.
Means are corrected for sample size. N is the number of fish examined.

Region	Year	N	Mean mature female wt.		Mean batch fecundity	
			Mean	CV	Mean	CV
Eastern Tasmania	2004	1,145	64.71	0.14	9,584	0.19
	2005	507	71.73	0.19	10,894	0.24
	2006	1,507	78.28	0.06	11,441	0.09
	All years	**3,159**	**72.31**	**0.06**	**11,001**	**0.09**
South-western Tasmania	2004	579	140.62	0.08	23,737	0.09
	2005	164	270.11	-	47,527	-
	2006	229	130.66	0.04	21,239	0.05
	All years	**972**	**160.12**	**0.14**	**27,162**	**0.16**

1.3.5 Sex ratio

Mean sex ratios for mature redbait are presented by region and spawning season in Table 1.9. By weight, mature males dominated in eastern Tasmania in 2005 and 2006, whereas females were dominant in 2004 and in each of the south-western Tasmanian samples.

Table 1.9 Mean sex ratios of mature redbait by region and spawning season.
Means are calculated from total weights and are corrected for sample size. N is the number of fish examined.

Region	Year	N	Ratio of mature females by weight	
			Mean	CV
Eastern Tasmania	2004	1,145	0.57	0.05
	2005	822	0.30	0.19
	2006	1,934	0.44	0.05
	All years	**3,901**	**0.45**	**0.06**
South-western Tasmania	2004	579	0.61	0.04
	2005	140	0.82	-
	2006	170	0.63	0.24
	All years	**889**	**0.64**	**0.07**

1.3.6 Size and age at sexual maturity

Logistic parameters for maturity ogives and 50% sexual maturity values for size (L_{50}) and age (t_{50}) are presented in Table 1.10. Proportions of mature fish by size and age, as well as logistic curves by sex and region, are provided in Figures 1.7 and 1.8. Females attained maturity at larger sizes than males in both regions although age at maturity was generally similar between the sexes within a given region. There were, however, marked differences in sizes and ages at maturity between the regions, with both sexes maturing at around 100 mm larger and 2 years older off south-western Tasmania compared with the eastern Tasmania.

Table 1.10 Size and age at sexual maturity logistic parameters and 50% maturity (L_{50}) values by sex and region.

Region	Sex	Size at maturity				Age at maturity			
		N	a	b	L_{50} (mm)	N	a	b	t_{50} (yrs)
Eastern Tasmania	female	760	-16.81	0.11	157	141	-3.29	1.66	2.0
	male	594	-13.58	0.09	147	170	-3.20	1.58	2.0
South-western Tasmania	female	654	-12.68	0.05	261	133	-2.09	0.52	4.1
	male	128	-12.47	0.05	244	111	-3.00	0.62	4.8

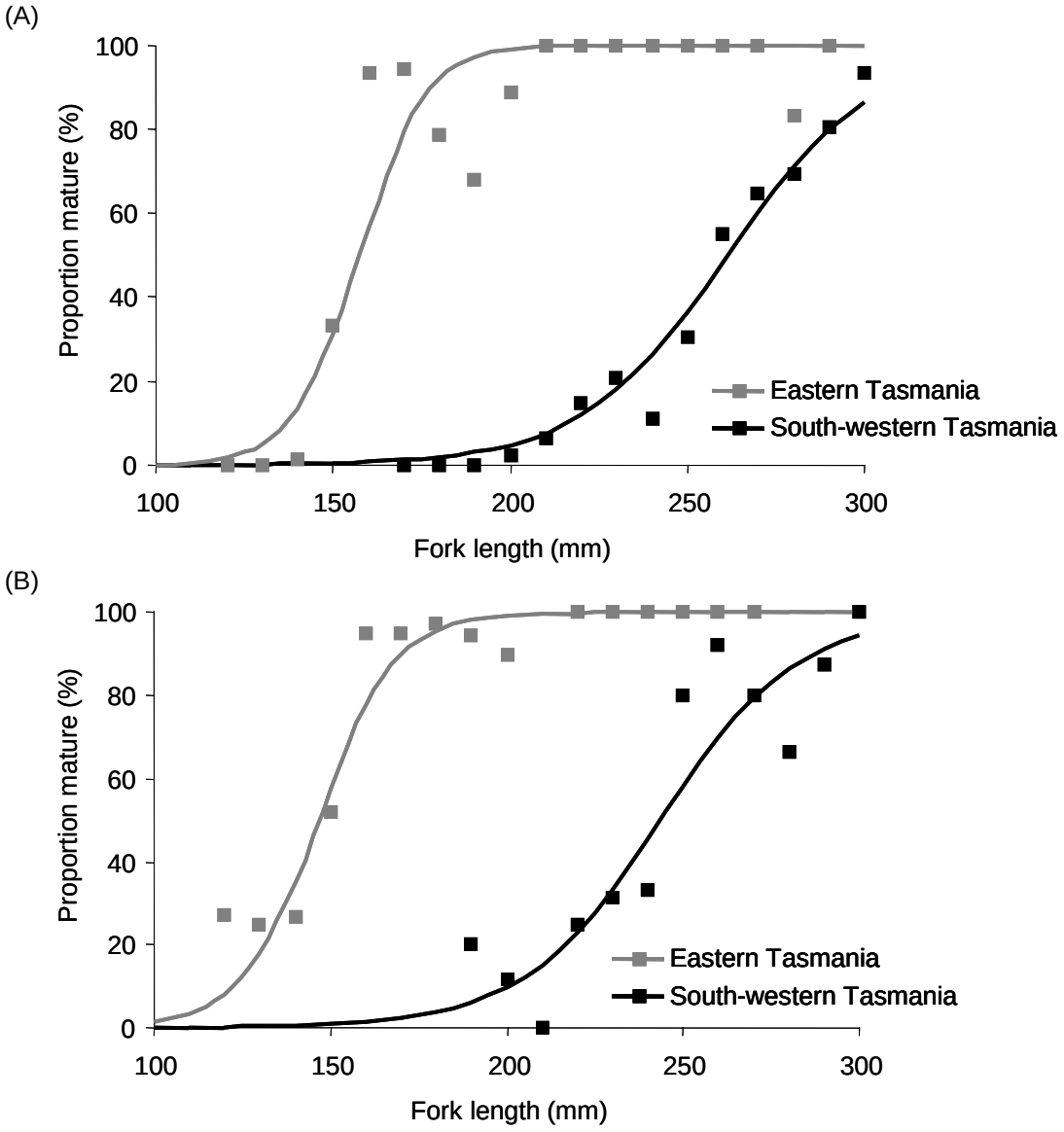


Fig. 1.7 Proportion of mature female (A) and male (B) redbait by length class and region, with logistic ogives fitted.

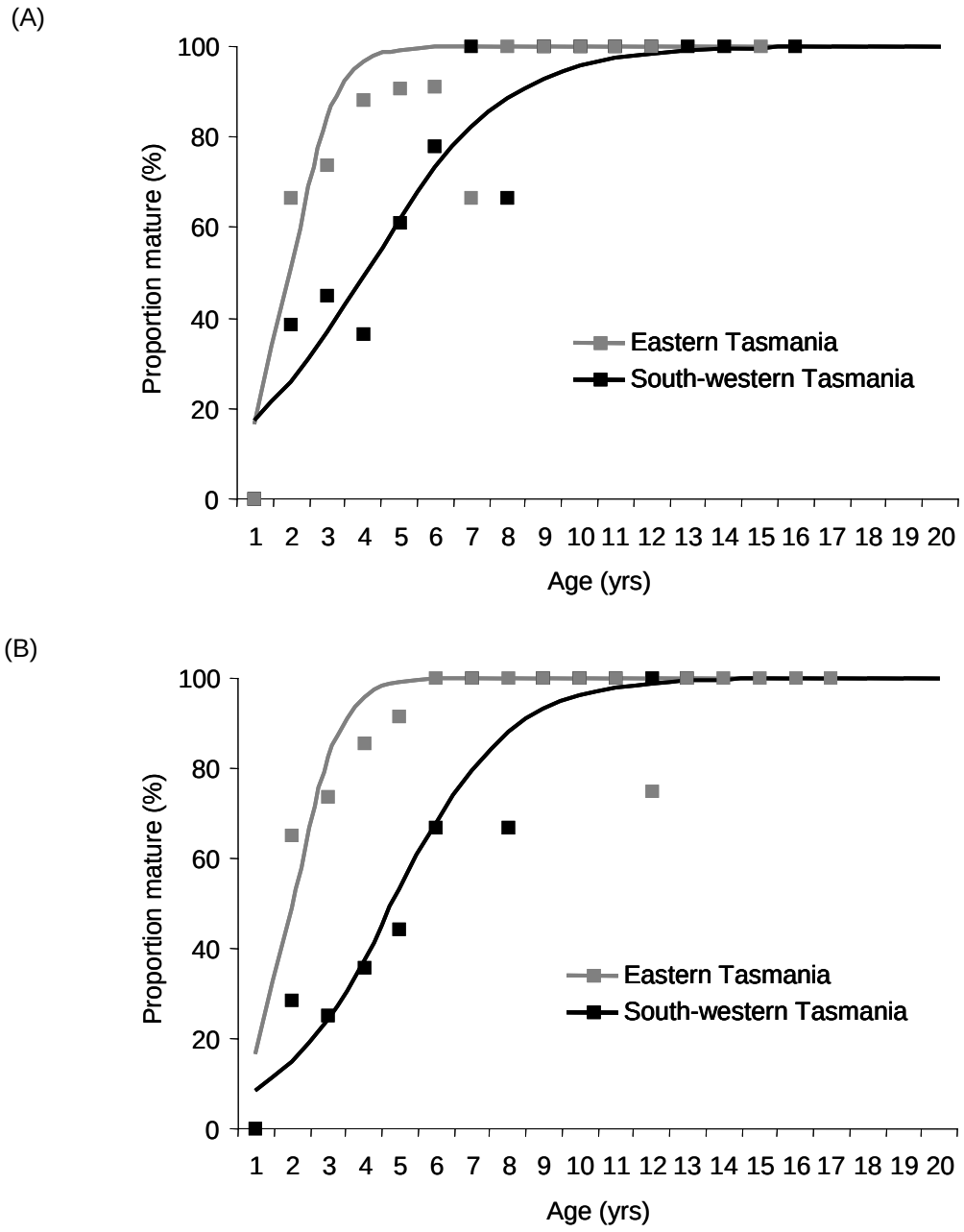


Fig. 1.8 Proportion of mature female (A) and male (B) redbait by age class and region, with logistic ogives fitted

1.4 DISCUSSION

1.4.1 Spawning season

Annual trends in GSI and macroscopic gonad stages indicated that redbait from eastern Tasmania spawn during between September and November, with peak activity during September and October. The effective cessation of spawning in November was supported by an increased rate of atresia in the ovaries of fish sampled during that month. Undeveloped or immature gonads dominated at other times of the year, reflecting in part a difficulty in distinguishing between resting and immature gonad stages when sampled outside the spawning season. Relatively small sample sizes were available from south-western Tasmania and few hydrated individuals were observed in samples. Further and more intensive sampling in this area is required to better define the spawning season for the region, noting that GSIs peaked in October rather than September in the south-west implying possible regional differences in the timing of peak spawning activity. Regional differences in spawning seasonality relating to localised environmental conditions have been reported for a number of species, including *Engraulis japonicus* (Funamoto *et al.,* 2004) and *Istiophorus platypterus* (Hernandez and Ortega, 2000).

1.4.2 Spawning mode

This study has established that redbait is an asynchronous batch spawner with indeterminate fecundity. The concurrent presence of multiple cohorts of oocytes during the spawning season and the presence of old POFs alongside migratory nucleus stage oocytes represent key diagnostic features for this spawning mode. Other small pelagic species also exhibit this reproductive strategy, including representatives of the clupeid, engraulid and scrombrid families (Hunter *et al.,* 1985; Alheit, 1993; Murua *et al.,* 2003; Murua and Saborido-Rey, 2003; Mackie *et al.,* 2005).

1.4.3 Spawning frequency and timing

Three commonly used criteria were used to identify a daily batch of eggs, namely (i) early spawning activity (late migratory nucleus to early hydration), (ii) active spawning (hydrated oocytes and fresh POFs) and (iii) recently completed spawning (fresh POFs), yielding spawning periodicities of around five, three and four days, respectively. Given the magnitude of the effect that this range of values would have on DEPM-based estimates of spawning biomass, it was desirable to develop a more detailed understanding of the process of ovulation in redbait to determine which criteria most accurately described a daily spawning batch. The sequence of oocyte development and ovulation in relation to the co-occurrence of development stages and time of day revealed that hydration was initiated in the early morning period, with spawning underway by evening and more or less completed by midnight, implying a mid-evening spawning peak. Unfortunately, no sampling was conducted early in the afternoon, due to reliance on commercial catch sampling, so it was not possible to determine the

precise time that spawning commenced. However, commencement of spawning around dusk is common among pelagic fishes (Thresher, 1984).

Since no fresh POFs were recorded in samples after about 09:00, it is probable that this POF stage persisted for well under 24 hours, possibly around 12 hours, whereas old POFs were present throughout the day, implying that they endured for periods in excess of a day. Longevity of POFs is normally estimated from induced spawning of captive fish (Leong, 1971; Fitzhugh and Hettler, 1995; Macchi *et al.,* 2003), and more recently from 3D reconstructions of POFs (Korta *et al.,* 2004). However, by examining the sequence of oocyte development and ovulation throughout the day we have been able to gain important insights into POF ages and were able to assess the relative accuracy of the spawning fraction criteria. For example, a criterion requiring just old POFs could overestimate a daily batch as it is likely that they persist for longer than a day. Alternatively, the criterion specifying only fresh POFs (SC-3) would underestimate a daily batch since these stages appear to last for less than a day. However, given that hydrated oocytes were complementary with fresh POFs within the diurnal cycle, a combination of hydrated oocytes and/or fresh POFs should identify a daily spawning batch irrespective of the time of day that the fish were sampled. This criterion has been used to generate estimates of spawning fraction in sardines (Macewicz *et al.,* 1996). Further, as both hydrated oocytes and fresh POFs were likely to persist less than a day, there is a lower likelihood of over-estimating spawning fraction. Thus, we conclude that the most likely spawning frequency for female redbait was once every three days. Further evidence supporting this conclusion was provided by the prevalence of fish with no advanced oocyte stages present and/or with old POFs only, irrespective of the time of day they were sampled. Collectively, these groups accounted for around 60% of samples, implying that for almost two-thirds of the oocyte developmental cycle, ovaries contained no advanced oocytes or fresh POFs.

A spawning periodicity of once every three days is unusually frequent, but not unprecedented. For example, while clupeids, engraulids and some perciforms generally have longer periodicities (Fletcher *et al.,* 1996; Macewicz *et al.,* 1996; Macchi and Acha, 2000; Quintanilla and Perez, 2000; Ganias *et al.,* 2003; Murua and Motos, 2006), perciforms such as *Micropogonias furnieri* (Macchi *et al.,* 2003), *Scomberomorus commerson* (Mackie *et al.,* 2005), *Kaiwarinus equula* (Yoneda *et al.,* 2002) and *Istiophorus platypterus* (Hernandez-Herrera *et al.,* 2000) share redbait's short spawning periodicity. Claramunt *et al.* (2007) concluded that for sardines and anchovies larger individuals spawned more frequently than smaller individuals. Although based on limited data, this phenomenon was not evident in this study, since south-western samples yielded similar spawning fractions despite the prevalence of larger fish.

1.4.4 Fecundity

Relative batch fecundity across species has been shown to vary with environmental conditions (DeMartini, 1991; Funamoto and Aoki, 2002), as well as geographically (Abaunza *et al.,* 2008) and both intra- and inter-seasonally (Alheit, 1993). Despite these limitations, relative batch fecundity is useful for comparisons of fecundity between species, particularly if average female weights and peak spawning times are used

(Alheit, 1993). The relative batch fecundity of redbait of around 190 eggs per gram of gonad-free weight is low in comparison with other small pelagics such as anchovies (~500) and sardines (~300) (Hunter *et al.,* 1985; Alheit, 1993; Fletcher *et al.,* 1996; Funamoto and Aoki, 2002; Macchi and Pajaro, 2003; Ganias *et al.,* 2004; Cubillos *et al.,* 2007). However larger pelagic such as scombrids and carangids share a similar relative batch fecundities to redbait of around 150 to 210 (Karlou-Riga and Economidis, 1997; Yamada *et al.,* 1998; Abaunza *et al.,* 2003). Relative fecundity within species has been shown to increase with increasing size, but appears to decrease with increasing size across pelagic species and redbait, despite their size, align more closely with larger pelagic species.

One of the primary advantages of a batch spawning strategy is an increase in the likelihood that larvae will be released into favourable conditions by "spreading the risk" over a longer period (Alheit, 1988). Consequently, indeterminate spawners generally have a protracted spawning season lasting three to 12 months (Alheit, 1988, 1989; Davis and West, 1993; Macewicz and Hunter, 1993; Militelli and Macchi, 2004; Karlou-Riga and Economidis, 1997). Redbait, however, have a relatively short spawning season of around 2-3 months. It is conceivable then that the reproductive strategy of redbait, i.e. high spawning fraction and low fecundity, is an adaptation that enables this species to take advantage of the short annual pulse in productivity that occurs in Tasmanian coastal waters in the austral spring. This pulse in coastal productivity is driven by an annual phytoplankton bloom from rising water temperatures, increased daylight hours and increased nutrients from vertical mixing on the coastal shelf from strong seasonal westerly winds (Harris *et al.,* 1987, 1988, 1991, 1992). It is plausible that sufficient nutrition is provided by high coastal productivity during the spawning season to support such rapid oocyte development, thereby reducing the necessity for a more protracted spawning season.

1.4.5 Size and age at maturity

There were marked regional differences in size and age at sexual maturity, with males and females from south-western Tasmania maturing at some 100 mm larger and two years older compared to redbait from eastern Tasmania. Interestingly, size at maturity observations for redbait from south-western Tasmania were more consistent with those reported for other emmelichthyids (Nor *et al.,* 1985; Mel'nikov and Ivanin, 1995). Onset of maturity has been related to fish condition (Livingston *et al.,* 1997; Morgan, 2004), though a comparison of Fulton's condition index between samples from the eastern and south-western Tasmania did not yield a significant difference ($P = 0.11$, $t_{stat} = -1.60$, $N = 3{,}120$). Butler *et al.* (1996) reported a latitudinal trend in age and size at maturity of Pacific sardine (*Sardinops sagax*) on the Californian coast, but also considered that the trend could be due to gear selectivity. Variation in size and age at maturity has also been observed in other sardine stocks (e.g. van der Lingen *et al.,* 2006) and has been attributed to density-dependant effects from fishing depletion. Given the early stage of development of the fishery for redbait around south-eastern Australia, it is unlikely that fishing will have impacted sufficiently to elicit a density-dependent response from the exploited population.

1.4.6 Implications for stock structure

Reproductive tactics are an interaction between genetic heritage and environmental influences, and conspecific variation provides evidence of geographic and/or reproductive isolation that has been used for the identification of fish stocks (Begg *et al.,* 1999). The reproductive tactics observed in the south-western population of redbait, i.e. slightly later spawning season and older maturity, may be indicative of a level of discreteness from the eastern population. It is noteworthy that no commercial quantities of redbait have been taken off southern Tasmania and ichthyoplankton surveys conducted during the peak spawning season also produced very low abundances of redbait eggs and larvae within that region (Chapter 3). Alternatively, the reproductive tactics of redbait may be particularly responsive to environmental conditions such as generally higher water temperatures in eastern Tasmania due to the influence of warm water associated with the East Australian Current (Chapter 3).

It is also plausible that the observed regional differences were caused by sampling biases such as fishing in slightly deeper water in south-western Tasmania. There is strong anecdotal evidence that redbait school by size, and that in eastern Tasmania smaller redbait are targeted by fishing in shallower inshore waters (TAFI, *unpublished data*). Hence, it is possible that south-western samples were taken from an area where schools of redbait were composed of larger fish, and the larger size at maturity simply reflects the absence of small fish in the samples. Commercial catch sampling provides some support for this observation, with consistently larger fish taken off the south-west coast (>99% between 140 – 280 mm, with a strong mode at 190 mm) compared with the east coast (>99% between 110 – 210 mm, with modes at 120 and 170 mm) (TAFI, *unpublished data*). A survey of both regions over a wider spatial scale may yield samples more representative of the reproductive tactics and population size structure.

1.4.7 Conclusions

Redbait reproductive biology was successfully described, despite limitations in obtaining samples from commercial fishing operations. Redbait are batch spawners with asynchronous oocyte development and are a suitable species for the application of the DEPM to estimate spawning biomass. The methodology employed to empirically derive the histological criteria to estimate spawning fraction eliminated both the need for costly aquaria-based spawning experiments and the uncertainty inherent in accepting POF ageing criteria from other species. Two key questions arise from the differences in reproductive tactics observed between eastern and south-western Tasmania. Firstly, to what extent are the differences an artefact of sampling and a product of schooling behaviour and, secondly, to what extent are these populations isolated from each other. Further research is warranted to investigate the degree of discreteness between redbait populations from eastern and south-western Tasmania, and the extent to which the timing and duration of the spawning season, as well as spawning periodicity, respond to fluctuations in food availability and environmental conditions.

CHAPTER 2: DEVELOPMENT OF REDBAIT EGGS AND LARVAE, INCLUDING A TEMPERATURE DEPENDENT EGG INCUBATION MODEL

F.J. Neira, J.P. Keane, J.M. Lyle and S.R. Tracey

Objective 2: To develop and validate methods for identifying and staging the eggs and larvae of redbait.

Reared eggs and field-collected material were employed to describe the development of the pelagic eggs and larvae of redbait. Hydrated oocytes from adults trawled from spawning grounds off eastern Tasmania were fertilized and reared to the yolk-sac larval stage, and the data employed to build a temperature-dependent egg incubation model. Embryogenesis lasted 96, 84 and 54 hours at mean temperatures of 13.1, 14.4 and 16.5^{o}C respectively, and was divided into seven stages based on extent of epiboly until blastopore closure (stages I-III) and embryo growth (stages IV-VII). Eggs (1.00-1.05 mm diameter) are spherical with a smooth chorion, small perivitelline space and prominent, unsegmented yolk with a single, posteriorly-located oil globule (0.18-0.20 mm diameter) that becomes pigmented from stage III. Embryos have two distinct snout melanophores, and a paired melanophore row laterally along the trunk and tail. Morphological identification of eggs collected during surveys in October 2005 and 2006 was validated using quantitative PCR amplification of the mtDNA d-loop gene region unique to redbait, producing an 80-100% agreement across all seven stages. Newly-emerged larvae (1.9-3.3 mm) possess a prominent yolk sac with the posteriorly-located, pigmented oil globule, mouth not yet functional and unpigmented eyes. Notochord flexion occurs between 5 and 8 mm while fins are formed by 12 mm. Larvae examined (3.3-17.4 mm) are lightly pigmented and possess percoid features such as an elongate to moderate body, coiled, triangular-shaped gut, preopercular spines and 24-25 myomeres; two prominent pigment patches opposite each other dorsally and ventrally along the tail are diagnostic. Variability of mean egg ages (y) by temperature (t) and stage (i) was best described by the deterministic stage-to-age model $y = 35.911\ e^{-(0.155t + 0.262i)}\ i^{(2.436)}$. Developmental changes and model outputs paralleled those reported for laboratory-reared eggs of known clupeoids and scombrids, whereby hatching time and transition periods between stages decrease with increasing temperatures. The suitability of the incubation model to assign ages to staged field-caught eggs of redbait is discussed in terms of its application to estimate spawning biomass using the daily egg production method.

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2.1 INTRODUCTION

The application of the daily egg production method (DEPM) to estimate spawning biomass of batch spawning fishes with pelagic eggs requires ample knowledge of reproductive biology and spawning dynamics of the target species. This includes mean daily egg production (*P*) over the spawning area which relies on the accurate identification of eggs derived from purposely-designed surveys (Hunter and Lo, 1997; Stratoudakis *et al*., 2006). The parameter *P* is estimated from an egg mortality model which requires prior assignment of ages to field-caught eggs based on development stage and ambient water temperature (Lo, 1985; Lo *et al*., 1996; Bernal *et al*., 2001). Such an incubation model is developed by artificially rearing eggs over a range temperatures present within the spawning habitat of the target species.

In this chapter we describe the early life history stages of redbait based predominantly on eggs and larvae caught along eastern Tasmanian shelf waters during the main spawning period (October), as well as eggs artificially reared through to the yolk-sac larval stage. Egg identifications were confirmed using molecular genetic analyses. Larvae, which also include specimens from shelf waters in various locations in south-eastern Australia, are compared to those of other emmelichthyids. Eggs reared at a range of water temperatures equivalent to that where redbait spawns off eastern Tasmania were examined to describe main development stages, and the age-by-stage data employed to assemble a temperature-dependent egg incubation model. The resultant model is compared to that of other small pelagics for which DEPM is routinely applied.

2.2 MATERIALS AND METHODS

2.2.1 Sources of field-caught eggs and larvae

Redbait eggs and larvae examined for this study derive from 287 spring-summer plankton samples collected across south-eastern Australia (140.1-151.1°E; 35.0-44.5°S) during 1984-2006, including museum specimens (Table 2.1; Fig. 2.1). Most, however, come from shelf waters between eastern Bass Strait around to Port Davey in south-western Tasmania in October 2005 and 2006, and southern New South Wales (NSW) in October 2002 and 2003 (Jervis Bay to Eden). The surveys off Tasmania were designed to coincide with the October peak spawning period of redbait (Chapter 1), as required for DEPM-based biomass estimates. The sampling area off eastern Tasmania and NSW included shelf waters between 15.0 nautical miles (nm) inshore of the shelf break (200 m depth contour) and 7.5 nm offshore of the break (Fig. 2.1) (refer to Neira and Keane [2008] for details on NSW surveys). Archived museum larval redbait caught at various locations around shelf waters of Tasmania in 1984-85 and 1999 were also examined (Table 2.1).

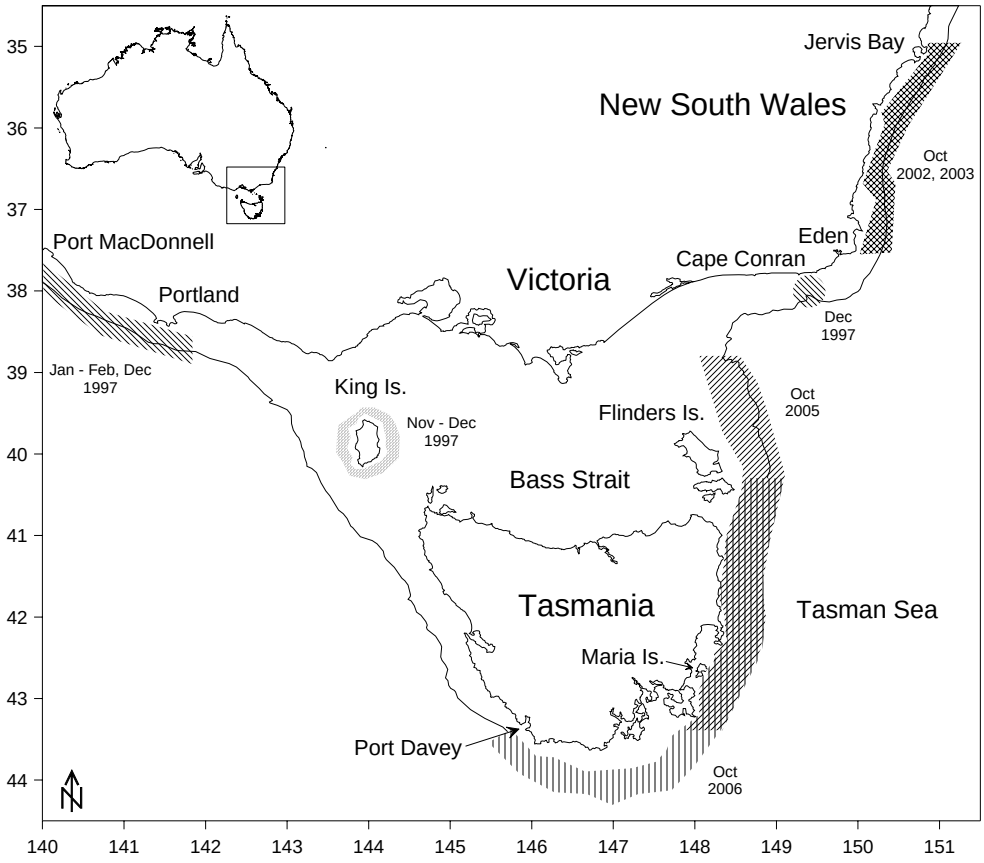

Fig. 2.1 Collection areas in south-eastern Australia of redbait eggs and larvae examined for this study. Refer to Table 2.1 for exact dates of surveys.

Table 2.1 Summary of available field-caught redbait eggs and larvae from south-eastern Australia examined for this study.

Localities are shown in Fig. 2.1. Details of nets used are provided when available; dash (-) means eggs were not available from these samples. Abbreviations: AMS – Australian Museum (sample catalogue number); NSW – New South Wales; Tas – Tasmania; NE – north-east; SE – south-east; SW – south-west.

Survey date	Shelf region/locality	Samples	Sample type	Sampler	Eggs	Larvae
20 Oct 1984	Eastern Tas (AMS I. 39851-001, -002)	2	Oblique	Conical net	-	101
26 Nov 1984	Eastern Tas (AMS I. 39853-001)	1	Oblique	Conical net	-	10
15 Feb 1985	Eastern Tas (Maria Is) (AMS I. 39852-001, 002)	1	Oblique	Conical net	-	5
20 Nov – 19 Dec 1996	Eastern Bass Strait – southern NSW	1	Surface	Bongo	-	1
31 Jan – 1 Feb 1997	Northern Bass Strait	3	Vertically-stratified	BIONESS[a]	-	3
19 – 27 Mar 1997	Eastern Tas	1	Oblique	Bongo	-	1
25 Nov – 8 Dec 1997	King Island	3	Surface	Bongo	-	3
12 – 16 Dec 1997	Northern Bass Strait	3	Vertically-stratified	BIONESS[a]	-	3
27 Feb 1999	Eastern Tas (AMS I. 39899-001)	1	Oblique	Bongo	-	1
12 – 22 Oct 2002	Southern NSW	23	Vertical	Bongo[b]	60	10
1 – 8 Oct 2003	Southern NSW	15	Vertical	Bongo[b]	988	51
12 – 17 Oct 2005	NE Bass Strait to SE Tas	97	Vertical	Bongo[b]	9,280	311
10 – 31 Oct 2006	Eastern, southern and SW Tas	133	Vertical	PAIROVET[c]	1,146	68

[a]Four 1 m^2 mouth nets each for 0-25, 25-50, 50-75 and 75-100 m depth stratum;

[b]0.6 m diameter, 3 m long, 300 and 500 μm mesh nets;

[c]0.25 m diameter, 1.5 m long 300 μm mesh nets.

2.2.2 Field procedures

Field-caught redbait eggs and larvae available for this study were caught with a variety of samplers equipped with 300-500 μm mesh nets (Table 2.1). Most eggs and larvae originated from day-night, non-stratified vertical samples taken either with a bongo or a modified PAIROVET sampler (Smith *et al*., 1985), whereas larvae from northern Bass Strait originated from daytime vertically-stratified samples collected with an opening-closing BIONESS (F.J. Neira, *unpublished data*). For the vertical samples, nets were encased within a weighted stainless-steel frame that was lowered to within ~5-7 m of the seabed or to a maximum of 200 m, whichever the deeper, and immediately brought

on board. Sampling depth during surveys was regulated using an echosounder (Tasmania) or a Scanmar depth sensor fitted to the net frame (NSW). On each sampling occasion samples from the two hard net codends were combined and fixed immediately in either 98% ethanol (October 2002, 2003 and 2005) or 10% formalin-seawater (October 2006). All redbait eggs and larvae were sorted under a dissecting scope, and stored in 70-98% ethanol. Environmental data, including temperature by depth, were obtained simultaneously with each plankton sample using CTD profilers.

2.2.3 Artificial rearing of eggs and larvae

Rearing of redbait eggs was successfully achieved through to the late yolk-sac larval stage using eggs extracted from adults trawled from the spawning grounds off eastern Tasmania in October 2006, in the same area of the egg survey. Hydrated oocytes were gently stripped from females immediately after capture and fertilized with sperm. Eggs were then transferred in batches of up to 100 into 250 ml containers preconditioned with full-strength seawater and aeration, and immersed within three temperature-controlled baths prepared on board the fishing vessel. Each bath was set with up to 10 rearing containers and maintained at average temperatures of 13.1, 14.4 and 16.5^{o}C through the periodic addition of ice and/or chilled water. Temperature within each bath was recorded with a continuous temperature-data logger (to nearest 0.01^{o}C) and backup thermometers (to nearest 0.5^{o}C). All dead eggs observed during the trials were removed and water in rearing containers replaced periodically by adding preconditioned seawater. Up to 33 eggs were taken at intervals of 1.5-6.0 hours from each container until hatching, fixed with 5% buffered formalin-sea water, and later used to distinguish the key development stages by time (hours). These stages, together with average temperature readings taken from each container at the same time of egg removal, were subsequently employed to assemble the temperature-dependent egg development model (see below). In all, 926 redbait eggs were reared and subsequently fixed during the trials, 379, 323 and 224 from the 13.1, 14.4 and 16.5^{o}C baths, respectively.

2.2.4 Identification of field-caught eggs and larvae

Field-caught eggs were initially identified as redbait using a suite of morphological characters observed in formalin-fixed eggs reared from spawning adults caught during research surveys. The identity of 216 of these eggs caught during October 2005 off eastern Tasmania was subsequently tested using molecular genetic analyses. Tested eggs were selected to cover the entire survey area and full range of development stages. For the analyses, a molecular primer/probe combination capable of isolating a 120 base-pair segment of the mtDNA d-loop gene region unique to redbait was developed. The probe was tested using real-time polymerase chain reaction (PCR) amplification of DNA extracted from (1) muscle tissue of adult redbait; (2) unfertilised eggs stripped from spawning females; (3) muscle tissue of adult *Plagiogeneion macrolepis* (rubyfish), the only other emmelichthyid species known to occur in Australia; and (4) muscle tissue of *Latris lineata* (striped trumpeter; family Latridae), whose eggs are likely to co-occur with those of redbait based on spawning season.

In the laboratory, each egg was stored individually in 96-well PCR plates and immersed in a 14 µL of 0.1 M Tris-HCl pH8 solution. The contents of each plate were frozen to -30ºC before being macerated using sterile wooden tooth picks, and then subjected to another freeze/thaw cycle prior to the PCR. Quantitative real-time PCR (qRT-PCR) was carried out on a RotorGene 2000 (Corbett Research, Sydney, Australia) using a SYBR green detection protocol (Karsai *et al*., 2002). The PCR profile consisted of a 30s denaturation at 95ºC, then 40 cycles of 10s at 95ºC, and 45s at 59ºC. To calculate DNA concentration in ng μL^{-1}, a standard series was developed using a known concentration of redbait DNA extracted from muscle tissue. Six ten-fold dilutions from an initial concentration of 60 ng μL^{-1} were used to generate a series of standards. The amount of product amplified from each egg was subsequently determined at the end of each cycle by the RotorGene software. A reaction with a concentration of less than the most dilute standard ($6x10^{-5}$ ng μL^{-1}) was assumed to be either not redbait or a false negative, that is, insufficient DNA template. Eggs of teleosts other than redbait were also included in genetic runs to test for potential false-positive results.

Larvae were initially identified to family using a set of morphological characters unique to Emmelicththyidae, and specifically to *Emmelichthys* (Konishi, 1988, 2000), including body shape and pigment pattern. Larvae were subsequently identified as redbait employing the series method, whereby the largest larva is identified to species using fin meristics unique to this species and then linked to successively smaller larvae using morphological and pigment characters (Neira *et al*., 1998).

2.2.5 Descriptions of eggs and larvae

Morphological characters and terminology regarding the development of eggs through to the late yolk-sac larval stage follow Ahlstrom and Moser (1980) and Kendall *et al*. (1984). Assigned development stages are intended to show recognizable milestone changes in morphology, and were based on eggs artificially reared at 13.1^{o}C, fixed in 5% formalin and preserved in 70% ethanol. Egg measurements, i.e. diameter of chorion and oil globule, were made to the nearest 0.01mm with a Leica MZ7.5 stereo-microscope fitted with an eyepiece micrometer. Photographs of eggs and larval stages were obtained with a Leica DC300F camera attached to the scope and recorded using Leica IM50 imaging software.

Terminology and measurements pertaining to larvae follow Neira *et al*. (1998). The term "larva" comprises the period between hatching and the attainment of full external meristic characters (e.g. fins, scales), and includes the yolk-sac, preflexion (= post-yolk exhaustion), flexion and postflexion stages. Measurements taken in each of 56 larvae examined for the description were made from images captured under the stereo-microscope and recorded to the nearest 0.01 mm using imaging software. Unless stated otherwise, all larval lengths correspond to body length (BL, mm), i.e. notochord length (snout tip to notochord tip) in yolk-sac, preflexion and flexion larvae, and standard length (snout tip to posterior hypural margin) in postflexion larvae. Measurements of head length (HL), yolk-sac length, body depth (BD) and preanal length (PAL) were expressed as percentage of BL, whereas eye diameter (ED) was expressed as percentage

of HL; ranges and means (± 95% C.I.) of all relative measurements except yolk-sac length are provided for the preflexion, flexion and postflexion stages.

2.2.6 Temperature-dependent egg development model

Each of the 926 reared redbait eggs was assigned to one of seven development stages, and given a specific age (hours) corresponding to the time spent post-fertilization in each temperature treatment prior to fixation. Incubation data were then arranged in a matrix whereby each cohort and temperature treatment was assigned a percentage based on the number of eggs per development stage, including zeros, with the sum of all staged eggs per cohort and treatment adding to 100%. This format considers the proportion of eggs of different stages within a specific age cohort to account for the fact that some eggs reared after *y* hours in the t^{o}C bath may have reached the next stage faster than others. The traditional egg incubation model developed for northern anchovy by Lo (1985) was then fitted to these data from temperature-dependent experiments to compute mean egg ages at different temperatures and stages. This deterministic stage-to-age model assumes that the age of an egg is best represented by a mixed exponential and power function of temperature and stage:

$$y_{i,t} = a\, e^{(bt + ci)}\, i^{d}$$

where $y_{i,t}$ corresponds to the mean age (hours) of stage *i* at mean incubation temperature t^{o}C, and *a*, *b*, *c* and *d* are coefficients common to all stages and temperatures; coefficient estimates were obtained using log transformation of the mean age for each stage at each temperature and the model fitted by least squares (Lo, 1985). Model-predicted mean egg ages by development stage were plotted for a range of temperatures likely to be encountered by redbait during the spawning period off eastern Tasmania, i.e. 9-17^{o}C from September to November, together with observed mean egg ages estimated from the three incubation trials. All model runs were performed using R (R Development Core Team, 2007).

2.3 RESULTS

2.3.1 Description of eggs

The pelagic eggs of redbait are spherical, transparent and measure 1.00-1.05 mm in diameter (Fig. 2.2). They possess a smooth chorion, a very small perivitelline space, and a prominent, unsegmented yolk with a distinct 0.18-0.20 mm diameter oil globule. The globule becomes lightly pigmented with small melanophores after ~34 hours post-fertilisation, and is located near the tip of the embryo's tail in late-stage eggs and posteriorly in the yolk sac of newly-emerged larvae (Fig. 2.2j). Pigment develops progressively over the embryo, particularly along the trunk and tail, and over the head. Diagnostic characters of mid to late-stage eggs include a row of stellate melanophores laterally along the trunk and tail of embryos, starting just posterior to the developing eyes, and two distinct stellate melanophores over the snout (Fig. 2.2h).

Quantitative real-time PCR (qRT-PCR) confirmed the identity of 194 (90%) of the eggs initially identified as redbait using morphology (Table 2.2). Depending on development stage, the percent agreement between morphologically and genetically identified eggs varied between 80 and 100%, the lowest success rate being in the more advanced stages (V-VI). Additionally, all 24 eggs identified as not being redbait using morphological features tested negative following the qRT-PCR.

Table 2.2 Number of eggs by development stage identified as redbait using morphological features (n = 216) and subsequently confirmed as rebait using quantitative real-time polymerase chain reaction (qRT-PCR), and the percentage (%) corroboration between the two methods.

Development stage	Identification method		Percent (%) agreement
	Morphology	qRT-PCR	
II	91	82	90
III	12	11	92
IV	39	39	100
V	30	24	80
VI	27	22	81
VII	16	16	100
Total	**216**	**194**	**90**

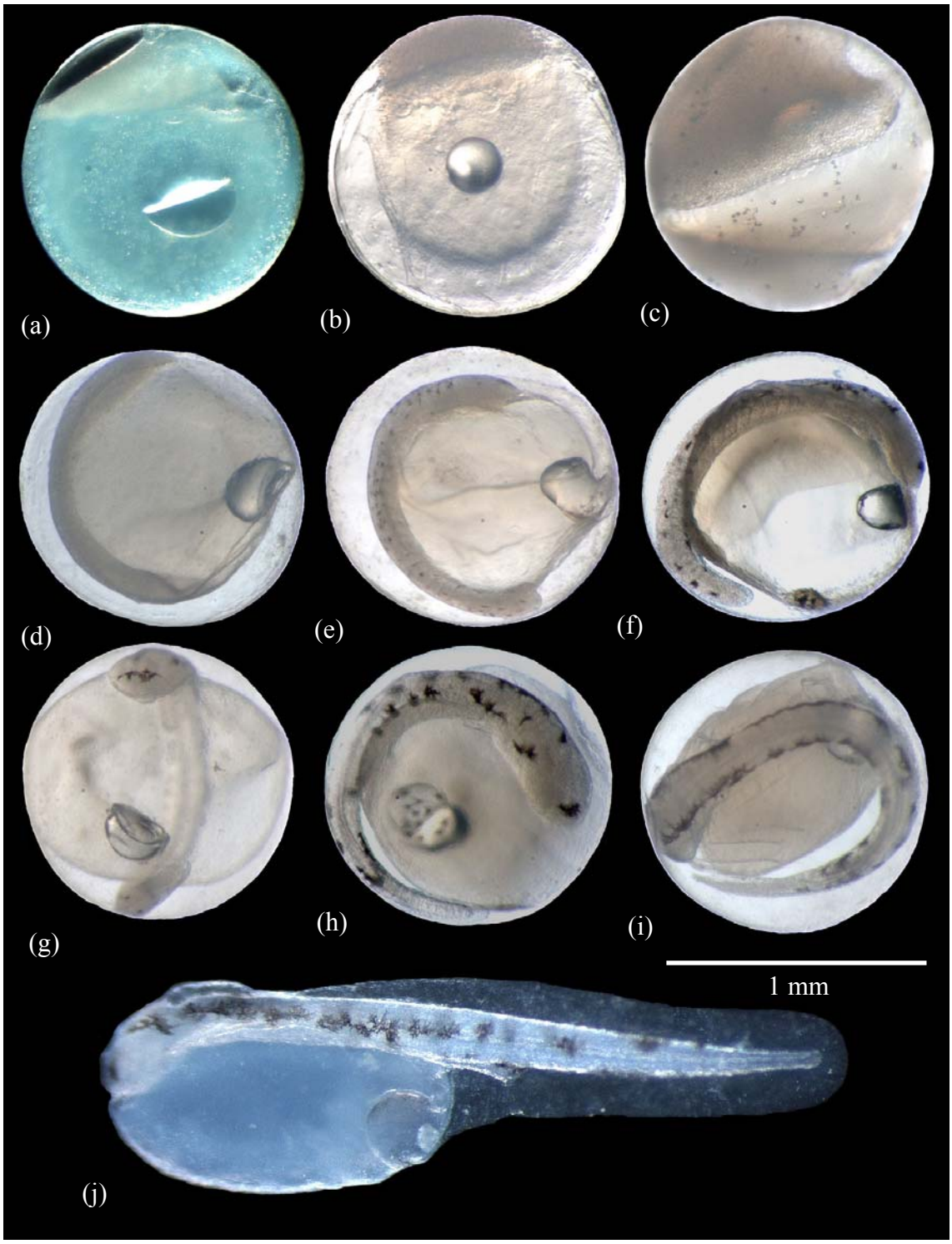

Fig. 2.2 Development stages of redbait eggs reared from known adults at 13.1°C (a-i) and newly-emerged yolk-sac larva (j). (a) Stage I (0.0-1.5 h); (b) Stage II (1.5-32 h); (c) Stage III (22.8-48.0 h); (d) Stage IV (40.0-60.0 h); (e) Stage V: (60.0-66.0 h); (f), (g) Stage VI (73.8-90.0 h); (h), (i) Stage VII (84.0-96.0 h); (j) 1.96 mm yolk-sac larva emerged after 84 h of rearing at 14.4°C; 1mm scale bar applies only to eggs. Photos by FJN.

2.3.2 Egg development stages

The development of redbait eggs was divided into seven stages (I-VII), based on the artificially-reared material (Fig. 2.2a-i). A detailed description of each development stage is provided below, together with the approximate duration of each stage (hours) at a mean temperature of 13.1^{o}C.

Stage I: unfertilized (no evident cleavage) to first cell divisions (0.0-1.5 h; Fig. 2.2a). Few cells evident over animal pole in newly fertilized eggs, and a single, distinct oil globule. Stage II: first cleavages to blastoderm covering <1/3 of yolk (1.5-32.0 h; Fig. 2.2b). Blastodermal cells (blastomeres) obvious over animal pole early during stage; blastoderm develops later into a cup-shaped structure with no discernible cells. Blastodisc edge squeezes yolk at top of animal pole after about 10 h. Oil globule visible on vegetative pole, off centre from animal axis when viewed laterally. Stage III: blastoderm covers >1/3 to 2/3 of yolk (22.8-48.0 h; Fig. 2.2c). Germ ring (thickened edge of blastodisc around yolk) starts to develop, signifying beginning of embryonic axis formation (embryonic shield). Germ ring becomes more pronounced as epiboly continues, and embryonic shield becomes noticeable. Stage IV: epiboly completed (40.0-60.0 h; Fig. 2.2d). Blastopore closes at posterior tip of embryonic axis (tail primordium). Embryonic shield evident as an opaque, ribbon-like structure over yolk margin, extending <1/2 circle around yolk. Stage V: 1/2-3/4 circle embryo (60.0-73.8 h; Fig. 2.2e). Head and tail regions of embryo discernible, while tail remains attached to yolk mass. Somite divisions (myomeres) start to appear. Tiny stellate melanophores peppered over trunk and tail of embryo, as well as few over oil globule. Stage VI: tail separation (73.8-90.0 h; Fig. 2.2f, g). Posterior region of embryo's tail separates from yolk and grows towards oil globule. Head begins to differentiate but eye cups not yet evident. Myomeres are clearly visible. Additional melanophores develop laterally along embryo's trunk and tail; pigment noticeable over snout but not over otic region. Stage VII: full tail separation to hatching (84.0-96.0 h; Fig. 2.2h, i). Tail well separated from yolk mass, tip reaching past oil globule and approaching left optic cup. Embryo pigment intensifies, including melanophores over snout and otic region, and a paired row of melanophores develops laterally along trunk and tail; optic cups remain unpigmented.

2.3.3 Description of larvae

Morphology. The 66 redbait larvae examined and measured for the description ranged between 1.9 and 17.4 mm BL, and included 10 yolk-sac, 26 preflexion, 19 flexion and 11 postflexion stages (Table 2.3). Body length of reared larvae at hatching ranged from 1.9 to 3.3 mm, while the smallest field-caught preflexion larva with functional mouth and no yolk sac measured 3.3 mm (Fig. 2.3). Newly-emerged larvae have a prominent yolk sac with a posteriorly-located, lightly pigmented oil globule, a mouth that is not yet functional and embryonic eyes with unpigmented cups (Fig. 2.2j). The horizontal length of the yolk sac decreased from 1.2 mm in the 1.9 mm (0 day old) larva (61.7% BL) to 0.9 mm in the 3.3 mm (2.5 days old) larva (28.6% BL). Notochord flexion occurs in the 5-8 mm range, which encompasses the smallest flexion (5.3 mm) and postflexion (7.4 mm) larvae examined; largest preflexion larva measured 7.1 mm while all larvae >7.4 mm had undergone notochord flexion. Larvae are initially elongate becoming moderate

in depth (BD 29.1-30.9%) after flexion (Table 2.3; Fig. 2.3). The head is relatively small before flexion but becomes large during flexion (HL 35.9-36.1%). The eyes are round and large, about 30-40% HL in diameter. The mouth is large from the late preflexion stage, with a noticeably angled and pronounced lower jaw. The premaxilla reaches to below mid eye in all stages. Small villiform teeth form along the premaxilla and dentary by the late preflexion and postflexion stages, respectively. The gut is moderately long to long (PAL 49.2-62.6%), initially straight but starts to coil by 4.6 mm becoming triangular and compact after flexion. There are 24-25 myomeres. Scales had not yet formed in the largest postflexion larva examined (17.4 mm).

Head spination comprises preopercular, interopercular, opercular, supracleithral and posttemporal spines. Small anterior preopercular spines develop from 4.4 mm, reaching five during late flexion and occasionally six after flexion. Five to seven posterior preopercular spines appear during late flexion, 10 after flexion. Other spines, including up to four interopercular, develop during the flexion/early postflexion stages.

Table 2.3 Range of body length (BL, mm) and range (mean values ± 95% C.I.) of main body proportions (given as percentage of BL) of field-caught redbait larvae from south-eastern Australia.

Table excludes measurements on 10 artificially reared yolk-sac larvae.

	Preflexion (*n = 26*)	**Flexion** (*n=19*)	**Postflexion** (*n=11*)
BL (mm)	3.25 – 7.07	5.33 – 7.45	7.93 – 17.38
HL (%BL)	10.67 – 33.14 (22.38 ± 3.19)	30.67 – 39.80 (35.87 ± 1.16)	32.57 – 39.78 (36.11 ± 1.59)
ED (%HL)	30.48 – 60.00 (40.30 ± 3.02)	30.07 – 35.44 (33.14 ± 0.73)	28.73 – 34.06 (31.12 ± 1.33)
BD (%BL)	6.45 – 28.53 (19.50 ± 2.90)	25.76 – 39.25 (30.91 ± 1.53)	24.74 – 35.52 (29.15 ± 2.08)
PAL (%BL)	42.15 – 55.76 (49.20 ± 1.46)	51.87 – 67.19 (57.97 ± 1.87)	57.39 – 66.57 (62.57 ± 2.17)

Pigmentation. Larvae are lightly pigmented throughout development. No defined pigment pattern was observed in reared, newly-emerged yolk-sac larvae except for melanophores over the snout, and scattered along the trunk, tail and ventral surface of yolk, all of which are typical of embryos in late-stage eggs of this species (Fig. 2.2h, i). Pigment becomes more defined in late yolk-sac larvae. Body melanophores migrate downwards to the ventral surface of trunk and tail as far as myomere 20, and one prominent stellate melanophore remains dorsally on the tail at myomeres 16-18 (Fig. 2.3a-c). Most of the ventral pigment on the tail disappears early in the preflexion stage (post yolk-sac stage), leaving another prominent stellate melanophore directly opposite the dorsal melanophore; both melanophores persist throughout development but

contract from late flexion and remain at the posterior end of the anal- and dorsal-fin bases. Additional melanophores appear from mid-preflexion, including over the snout and tip of lower jaw, jaw angle, fore- to midbrain, operculum, pectoral-fin base and along the isthmus. Melanophores also form ventrally along the foregut and under the anus, and laterally along the tail midline between the stellate opposing melanophores described above. Internal pigment is evident under the forebrain, continuing posteriorly towards the hindgut. Pigmentation over the gut, including the anterior surface, persists and increases with development. Internal pigment is also present along the notochord between the stellate melanophores from the flexion stage, extending anteriorly and posteriorly with development. Pigmentation in the largest postflexion larva examined comprised small punctate melanophores scattered over most of the body, particularly over the gut and along the dorsal surface from head to end of caudal peduncle (Fig. 2.3f).

Formation of fins. Larvae emerge from eggs with tiny pectoral-fin buds, a small preanal finfold and a long body finfold from the nape around to the anus. The caudal-fin anlage is visible during preflexion from 4.8 mm, with all principal caudal fin rays (9+8) formed by 8 mm. Dorsal- and anal-fin anlagen appear simultaneously from 5.3 mm, with a distinct gap between the anal-fin origin and the anus which closes once the fin is formed. All anal-fin (III, 9-10) and dorsal-fin (XIII-XIV, 9-11) elements are formed by about 8 and 12 mm, respectively, with the spines in both fins forming last. Pelvic-fin buds appear by 6.5 mm during flexion, and all elements (I, 5) are present by 12 mm, by which stage all pectoral-fin rays are present. The sequence of fin formation is C, A, D, P_1 and P_2.

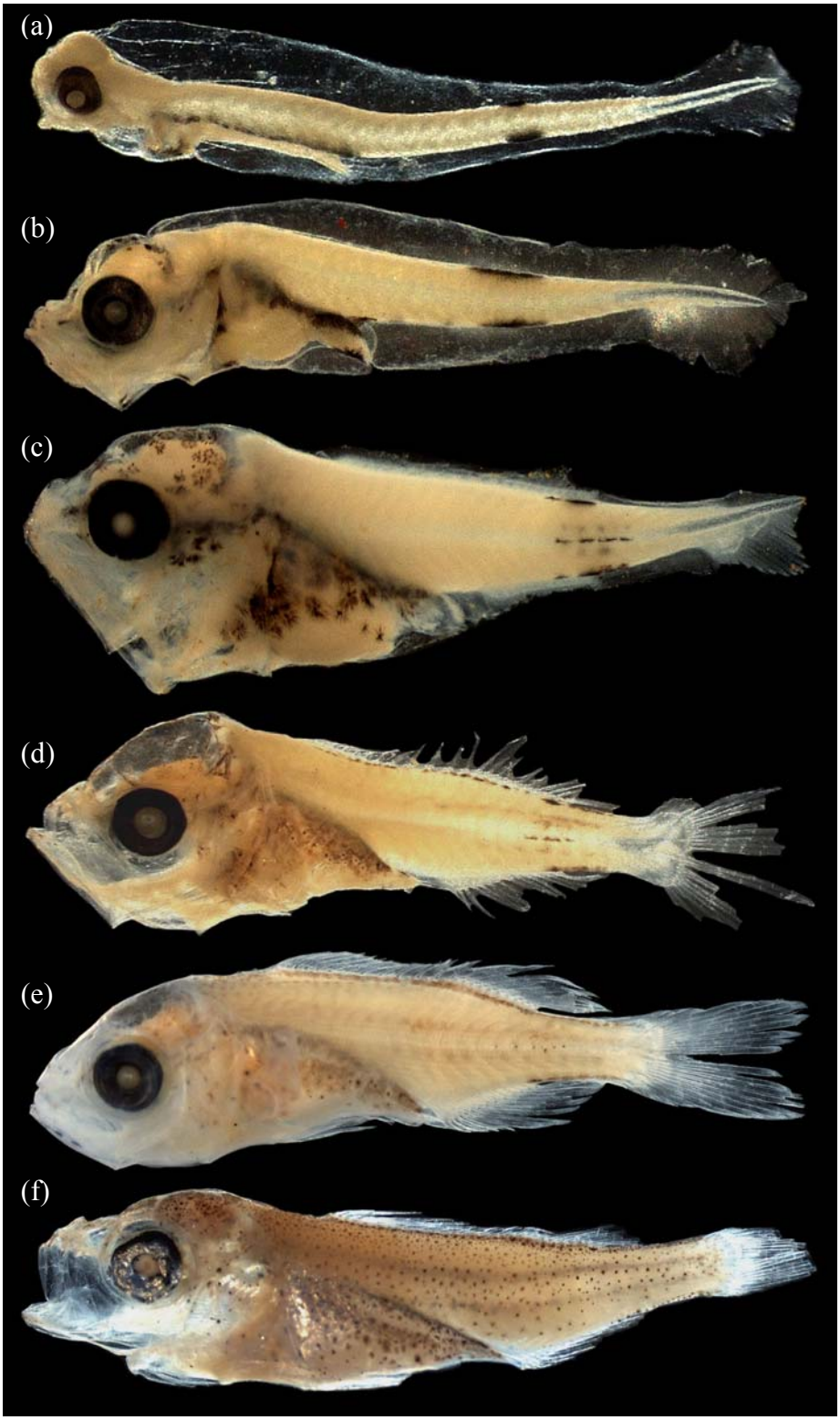

Fig. 2.3 Larval redbait stages from south-eastern Australia. (a) 3.3 mm early preflexion; note exhausted yolk sac; (b) 4.8 mm late preflexion; (c) 6.5 mm flexion; (d) 8.4 mm early postflexion; (e) 12.3 mm postflexion; (f) 17.5 mm late postflexion/transforming. Larvae in (a), (b) and (c) from eastern Tasmania in October 2005; (d) from 32 nm south of Portland (Vic) in January 1997 (depth: 50-75m); (e) from 32 nm south off Port MacDonnell (SA) in January 1997 (depth: 0-25m); (f) from south-eastern Tasmania in February 1999 (AMS I.39899-001). Photos by JPK.

2.3.4 Temperature-dependent incubation model

All redbait eggs reared at 16.5°C hatched by 54 hours post-fertilisation (Table 2.4). First hatchings at 13.1°C and 14.4°C occurred after 96 and 84 hours, respectively, while all eggs in the two baths had hatched after 102 and 90 hours, respectively. Yolk-sac larvae in the 13.1°C bath were kept live for another 60 hours (2.5 days) before rearing was terminated. Mean ages (hours) of reared redbait eggs were computed for all except those development stages for which data were unavailable, i.e. Stage I eggs at 14.4 and 16.5°C, and Stage VII eggs (pre-hatching) at 13.1 and 14.4°C (Table 2.4). Observed mean ages obtained for each egg development stage at the three rearing temperatures closely corresponded with model-predicted mean ages from the incubation model, with the discrepancy between values not exceeding 3.4 hours (Table 2.4). Observed and predicted mean ages (hours) of egg stages plotted against temperature showed a decrease in hatching time with increasing temperature, with eggs at the extreme modelled temperatures of 9 and 17°C expected to hatch after 163 and 41 hours, respectively (Fig. 2.4). Likewise, the duration of individual developmental stages decreased with increasing temperatures, with the transition periods between stages I and II, and stages II and III being the longest based on computed mean ages. The resulting temperature-dependent development model fitted to the egg incubation data obtained for redbait during this study was:

$$y_{i,t} = 35.911\ e^{-(0.155t + 0.262i)}\ i^{\,2.436}$$

All four model coefficients were significant, the temperature coefficient contributing the greatest to the overall model fit (Table 2.5).

Table 2.4 Observed (O) and predicted (P) mean age (hours) of rebait eggs by development stage. Observed values are based on incubation data obtained at mean temperatures of 13.1, 14.4 and 16.5°C; predicted values were computed for each temperature from incubation model. Times (hours) are also provided for first hatchings and when all eggs had hatched, based on occurrence of newly-emerged, yolk-sac larvae in each treatment. NA, not available.

	Mean rearing temperature (°C)					
Stage	**13.1**		**14.4**		**16.5**	
	O	P	O	P	O	P
I	0.75	3.63	-	2.97	-	2.14
II	13.40	15.11	11.93	12.35	11.05	8.92
III	33.67	31.22	25.61	25.52	21.83	18.43
IV	47.69	48.42	40.77	39.58	27.20	28.59
V	63.23	64.17	NA	52.46	36.00	37.89
VI	78.88	76.99	60.00	62.94	NA	45.46
VII	NA	86.25	NA	70.51	52.67	50.92
First hatchings	96		84		NA	
All hatched	102		90		54	

Table 2.5 Estimated coefficients and corresponding standard errors for the temperature-dependent development model for redbait eggs based on incubation data obtained during this study. Residual standard error was 2.216 on 13 degrees of freedom. Significance: ***<0.0001; **<0.001.

Parameter	**Estimate**	**Std. error**	***t* value**	***P***
Intercept (*a*)	35.911	8.410	4.270	0.0009 **
b	0.155	0.011	13.977	3.29e-09 ***
c	0.262	0.066	3.963	0.00162 **
d	2.436	0.293	8.315	1.46e-06 ***

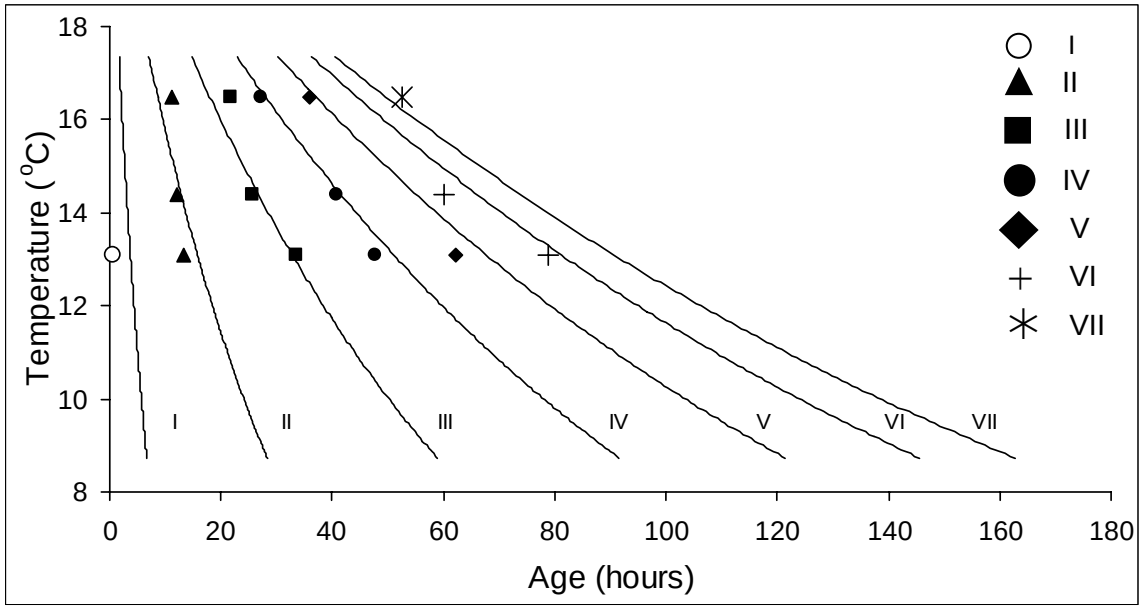

Fig. 2.4 Observed (symbols) and predicted (smooth exponential curves) mean ages of redbait eggs by development stage (I to VII) as a function of temperature (°C), based on incubation data obtained during artificial egg rearing carried out at sea in October 2006. Predicted values were computed from the traditional egg incubation model of Lo (1985).

2.4 DISCUSSION

2.4.1 Eggs

This paper provides the first descriptive account of the developmental stages of the pelagic eggs and larvae of redbait, a mid-water schooling percoid species common to shelf waters of temperate Australia. Descriptions are based on eggs extracted from adults trawled from known spawning areas off eastern Tasmania and successfully reared to the yolk-sac stage, and on eggs and larvae caught during ichthyoplankton surveys in October 2005 and 2006 along shelf waters of eastern to south-western Tasmania. Surveys were part of an integrated study aimed at providing future DEPM-based spawning biomass estimates of this species.

Identifications of field-caught eggs as belonging to redbait were validated using DNA techniques, a step which is becoming increasingly necessary when dealing with egg-based stock assessments (Taylor *et al*., 2002; Fox *et al*., 2005; Neira and Keane, 2008). In the case of redbait, the high level of agreement (80-100%) between the molecular and morphological identifications of eggs across all development stages collected throughout the survey area provides a high level of confidence in the use of morphology to correctly identify redbait eggs. The fact that around 20% of stage V-VI eggs were not genetically identified as redbait suggests either that some were misidentified and/or that insufficient DNA was available for PCR amplifications. However, given the advanced embryonic development and distinct pigment pattern of redbait eggs by those stages, it is more likely that these results represent false negatives arising from a lack of DNA. Assuming this to be the case, the success rate for morphological identification would have actually been >95%. Furthermore, given the likelihood of false negatives, the overall very high rate of agreement for early stage eggs, e.g. I-II prior to embryonic differentiation, is particularly significant and strongly supports the accuracy of morphological identification protocols.

The spherical eggs of redbait (1.00-1.05 mm) can be identified using a combination of morphological features, including smooth, transparent chorion, small perivitelline space and prominent, unsegmented yolk with a single oil globule that becomes lightly pigmented with development. In addition, diagnostic characters of mid- to late-stage eggs include two snout melanophores and pigment dorsally along the trunk and tail of the embryo. Redbait eggs are likely to be confused with those of other percoid fishes that spawn morphologically similar pelagic eggs within the same area and season, particularly those of *Latris lineata* (Latrididae) and *Trachurus declivis* (Carangidae). However, eggs of *L. lineata* are larger (1.26-1.44 mm; mean = 1.31-1.35 mm), embryos are more heavily pigmented and the single, pigmented oil globule lies at the centre of the unsegmented yolk both in late-stage eggs and newly-emerged, yolk-sac larvae (Furlani and Ruwald, 1999). Eggs of *T. declivis*, on the other hand, are slightly smaller (0.97-1.03 mm; mean = 0.998 mm) and possess a clearly segmented yolk (Robertson, 1975; Crossland, 1981).

2.4.2 Egg development

The development of redbait eggs could be separated into seven stages (I-VII) based on milestone changes in morphology during embryogenesis. Decision limits on the onset and ending of each stage were based on the presence of key structural features that were clearly recognisable in the artificially-reared eggs examined. Thus, prior to the embryo formation, stages I - III were defined by the extent of epiboly, as signified by the advancement of the germ ring (blastodisc), until the closure of the blastopore. Stages IV - VII were subsequently defined by the degree of embryo growth, including development of pigment and myomeres as well as tail separation from the yolk mass. Morphological changes during the development of redbait eggs were similar to those described for eggs of other small pelagic species such as *Engraulis mordax* (Moser and Ahlstrom, 1985), *Sardinops sagax* (Lo *et al*., 1996; White and Fletcher, 1998), *Scomber japonicus* (Watanabe, 1970) and *Scomber scombrus* (Mendiola *et al*., 2007), including the detachment of the embryo's tail from the yolk mass and its growth towards the head prior to hatching.

The seven egg stages defined for redbait during this study are comparable to the six stages described for the western European stock of *S. scombru*s from laboratory-reared eggs (Lockwood *et al*., 1981), and seven stages described for *Scomber australasicus* from south-eastern Australia (F.J. Neira, unpublished data) from field-caught eggs. However, they are fewer than those assigned to laboratory-reared eggs of *Sardina pilchardus* (10; Miranda *et al*., 1990), *E. mordax* (11; Moser and Ahlstrom, 1985), *S. sagax* (11-12; Lo *et al*., 1996; White and Fletcher, 1998), *S. scombrus* (13; Mendiola *et al*., 2007) and *S. japonicus* (15; Watanabe, 1970), and those allocated to *E. japonicus* (9; Kim and Lo, 2001) based on field-caught eggs from Korean waters. The greater number of egg stages defined for these species reflects increased stage differentiation mostly after the formation of the embryonic shield, i.e. from late stage III. Thus, the last four stages defined herein for redbait embryogenesis are equivalent to the final five stages in *E. japonicus*, seven in *S. scombrus*, and eight both in *E. mordax* and *S. sagax* (Moser and Ahlstrom, 1985; Lo *et al*., 1996; Kim and Lo, 2001; Mendiola *et al*., 2007).

While discrepancies in the number of stages allocated to eggs of pelagic fishes may well reflect intrinsic morphological differences between families, staging remains a highly arbitrary process which depends on the quality of the reared series available as well as microscope optics employed to define stages. For redbait, the allocation of seven discrete developmental stages based on a reared series adequately described changes during embryogenesis, especially the last four stages, with each stage being sufficiently distinct to be employed in the staging of field-caught eggs. However, increased staging resolution during early to mid embryogenesis, i.e. prior to stage IV, may be necessary to reduce stage-to-age variance (Stratoudakis *et al*., 2006).

2.4.3 Larvae

Larval redbait possess all the main percoid features, including an elongate to moderate body, a large mouth with a noticeably angled lower jaw, small preopercular head spines, a coiled, triangular-shaped gut with a gap between anus and anal-fin origin, and 24-25 myomeres. They are also lightly pigmented, and have two distinct pigment patches opposite to each other posteriorly on the tail which are diagnostic. After hatching, yolk-sac larvae can be identified by their prominent yolk mass with a single, pigmented oil globule located at the rear of the yolk. Development of Emmelichthyidae has been described in detail based on larvae of two of the three genera currently in the family, namely *Emmelichthys* and *Erythrocles* (Konishi, 2000). The development of redbait follows closely that of *Emmelichthys struhsakeri* larvae from Japan, both in terms of size at notochord flexion (from ca. 5mm), and sequence of head spine and fin formation. In fact, except for a more extensive snout and head pigment in redbait from the flexion stage, larvae of the two species are very similar in all other features, including the distinct opposite pigment patches which remain at the distal end of the dorsal- and anal-fin bases from the mid-flexion stage (Konishi, 1988, 2000).

Morphologically, larval redbait are likely to be confused with those of some species of families such as Carangidae and Pomacentridae, which are also known to occur along south-eastern Australia during spring (Gomon *et al*., 1994; Neira *et al*., 1998). However, most larval carangids possess a distinct supraoccipital crest, more extensive head spination with longer posterior preopercular spines and fewer dorsal-fin spines. In addition, carangids become deeper earlier during development and are usually more heavily pigmented (Trnski, 1998; Leis and Carson-Ewart, 2000). Larvae of the pomacentrid *Chromis hypsilepis* have a very similar pigment pattern posteriorly on the tail, with two large opposing melanophores and pigment along the lateral midline between the two (F.J. Neira, *unpublished data*). However, they differ from larval redbait in that *Chromis* possess two anal-fin spines and 26 myomeres (Watson, 1996).

2.4.4 Incubation model

This study constitutes the first attempt at rearing redbait eggs, and possibly the first for a member of the Emmelicthyidae. The deterministic stage-to-age model fitted to estimate mean age of redbait eggs by developmental stage (Lo, 1985) appears to adequately explain age variability as a function of stage and temperature. The same function has been applied to estimate mean egg ages of *S. pilchardus* (Miranda *et al*., 1990), *S. sagax* (Lo *et al*., 1996) and *E. japonicus* (Kim and Lo, 2001), while a stochastic version of the model was tested to age eggs of *S. pilchardus* (Bernal *et al*., 2001). However, outputs would need to be interpreted with caution given a number of uncertainties associated with model assumptions and available incubation data. For example, the traditional deterministic function assumes a distinct spawning synchronicity, i.e. the instantaneous release of eggs within a fixed peak time, and thus neglects the variance associated with egg aging that would otherwise reflect the typical release of eggs over a given time period (Bernal *et al*., 2001). Egg rearing trials, on the other hand, were conducted under conditions not as ideal as those in laboratories equipped with suitable temperature-controlled incubating rooms and water baths, thus

yielding an incomplete development data set based on a narrow temperature range (13.1-16.5°C). Ideally, a more extensive egg incubation dataset over a wider range of temperatures, would permit the alternative ageing option of fitting a continuation-ratio logit model (ICES, 2004; Stratoudakis *et al*., 2006) which considers the data as a multinomial distribution, i.e. it models the natural probability of an egg being at a specific stage given certain age and temperature.

Despite the uncertainties and suboptimal rearing conditions, model outputs obtained for redbait eggs paralleled those reported for laboratory-reared eggs of *E. mordax*, *E. japonicus, S. pilchardus*, *S. sagax* and *S. scombrus* (Lo, 1985; Miranda *et al*., 1990; Lo *et al*., 1996; Kim and Lo, 2001; Mendiola *et al*., 2007), whereby hatching time decreased with increasing temperature as did transition periods between stages, particularly during early embryogenesis. In terms of embryogenesis duration, redbait appear to develop at a slower rate than those of some clupeoids at 12-15°C, i.e. the mean temperature range of the grounds where this species spawns off eastern Tasmania (100-140m; F.J. Neira, *unpublished data*). For example, all redbait eggs hatched after 102 hours at 13.1°C, whereas hatching of *E. mordax*, *S. sagax* and *S. pilchardus*, based on mean ages at their last egg stages (XI, XI and X, respectively), occurred soon after 75, 84 and 91 hours at 13°C, respectively (Lo, 1985; Miranda *et al*., 1990; Lo *et al*., 1996). By contrast, redbait eggs develop faster than reared pelagic eggs of *S. scombrus*, which are fully hatched after 125-150 hours at 13.2-13.4°C (Lockwood *et al.*, 1981; Mendiola *et al*., 2007).

The mean age data obtained here for redbait eggs are sufficiently robust to be employed in the assignation of ages of staged field-caught eggs of this mid-water species. These data are required prior to computing daily egg production (*P*) via an exponential decay model for the purposes of providing initial estimates of spawning biomass with the DEPM (Picquelle and Stauffer, 1985; Hunter and Lo, 1997; Stratoudakis *et al*., 2006). However, improvements to the stage-to-age model employed during this study would need additional incubation trials under a wider range of temperatures to augment egg aging precision. Such trials should also include the entire yolk-sac stage, as aging of field-caught yolk-sac larvae is considered to increase precision of daily egg production estimates (Lo *et al*., 1996; Hunter and Lo, 1997). This would be particularly advantageous in the case of redbait, given the relatively long duration of the yolk-sac stage (i.e. 2.5 days at 13.1°C) as determined from the incubation trials.

In summary, the development of the pelagic eggs and larvae of redbait is described here for the first time based on reared eggs and field-collected material. Oocytes from adults trawled from spawning grounds off eastern Tasmania were fertilized and reared to the yolk-sac stage at a range of temperatures, and the data employed to build a temperature-dependent egg incubation model as required by the daily egg production method (DEPM) to estimate spawning biomass. Embryogenesis was divided into seven stages based on extent of epiboly until blastopore closure (stages I-III) and embryo growth (stages IV-VII). Morphological identification of eggs collected during surveys off Tasmania was validated using quantitative PCR amplification of the mtDNA d-loop gene region unique to redbait, producing an 80-100% agreement for all seven stages. All eggs hatched after 96, 84 and 54 hours at mean temperatures of 13.1, 14.4 and

16.5^{o}C, respectively. Variability in mean egg ages (y) in function of temperature (t) and stage was adequately described by the standard deterministic stage-to-age model of Lo (1985). Developmental changes and model outputs paralleled those reported for laboratory-reared eggs of known clupeoids and scombrids, whereby hatching time and transition periods between stages decreased with increasing temperatures. The incubation model is suitable to assign ages to staged field-caught redbait eggs, although improvements can be made to maximise its application to DEPM-based spawning biomass estimates for the species.

CHAPTER 3: SPAWNING HABITAT AND DAILY EGG PRODUCTION OF REDBAIT ALONG SHELF WATERS OF SOUTH-EASTERN AUSTRALIA

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

Objective 3: To estimate the location and extent of spawning areas of redbait on the east coast of Tasmania, including a quantification of the levels of egg production of this species.

Redbait spawning habitat is described from egg, larval and environmental data collected in October 2005 and 2006 over shelf waters between north-eastern Bass Strait and the lower south-west of Tasmania (Tas). Egg data were further analysed to provide size of spawning areas off eastern Tas, while daily egg abundance-at-age data were employed to compute mean daily egg production (P_0) and instantaneous mortality (Z) estimates in these areas. Eggs occurred along the entire area sampled in 2005 (15,650 km^2; 38.8-43.5^{o}S). By contrast, 96% of the eggs caught in the much larger area sampled in 2006 (21,351 km^2) came from the shelf off eastern Tas (40.5-43.5^{o}S) while very few occurred <43.5^{o}S along the southern to south-west coast (145.5-147.7^{o}E). Distribution and abundance of the 10,393 eggs and 378 larvae of redbait caught off Tas during the known October spawning peak indicate that spawning takes place mostly along a 5 nm corridor over the shelf break, in areas 125-35 m deep, and in average mid-water temperatures of 13.5-14^{o}C. This observation is supported by the significantly greater mean abundance of day-1 eggs at shelf break than at either shoreward or offshore stations, and by quotient analyses on day-1 egg abundances pooled across these shelf regions. Estimated spawning areas (% of total survey area) were 13,220 km^2 (84.5) in 2005 and 8,695 km^2 (40.7) in 2006. Both the 2005 and 2006 egg mortality curves followed the typical exponential decay model described for eggs of other small pelagic fishes. Mean P_0 and Z were computed for two data scenarios by fitting a least squares non-linear regression (NLS) model and a generalised linear model (GLM) with a negative binomial error distribution, with the latter technique better describing the data based on model diagnostics. Excluding eggs assigned ages $\leq$4 hours and $\geq$98% of incubation time (scenario 2), GLM-derived P_0 (eggs/0.05m^2 day^{-1}) was estimated as 4.04 in both 2005 (CV 0.14) and 2006 (CV 0.19), with Z of 0.53 (CV 0.20) and 0.39 (CV 0.57) for 2005 and 2006, respectively. The finding of identical mean P_0 estimates for the two consecutive years is indicative of a highly consistent spawning effort across the surveyed area, and may also reflect the fact that no apparent differences in environmental conditions were detected between surveys. Total egg production per spawning area (eggs x 10^{12}) was greater in 2005 (1.26) than 2006 (1.05), which was attributed to the larger spawning area in 2005. Combined, these findings indicate that redbait is a suitable species for the application of the DEPM to estimate spawning biomass.

3.1 INTRODUCTION

Most of the 15 known species currently placed in the family Emmelichthyidae support limited commercial fisheries through their geographical range, with catches used predominantly for human consumption, bait and/or fish meal. They are taken by bottom, demersal and mid-water trawling in Europe (Russia, Georgia and Ukraine), South Africa, Australia and New Zealand, either as primary target or by-catch of other offshore trawl fisheries (Paul, 1997; Anon, 2001; Welsford and Lyle, 2003; Bulman *et al*., 2008). Of the two emmelichthyids found in temperate Australia, redbait have been trawled in increasing commercial quantities off eastern and south-western Tasmania since 2002, where captures of around 7,000 t p.a. are processed mainly to feed farmed tuna (Welsford and Lyle, 2003; McLaughlin, 2006). This small (to 36 cm TL) mid-water schooling species occurs in shelf waters of temperate Australia <30^{o}S, as well as in New Zealand, South Africa and Chile, including oceanic islands along the same latitudes (Heemstra and Randall, 1977; Last *et al*., 1983; Gomon *et al*., 1994; Hoese *et al*., 2007).

As an established fishery-independent technique to estimate stock levels of pelagic fishes, the daily egg production method (DEPM) was deemed suitable for redbait since several attributes of its reproductive biology, including the release of pelagic eggs in batches (Chapter 1), fall within those typically exhibited by DEPM-assessed small pelagic species (Stratoudakis *et al*., 2006). Before such method could be applied, however, it was critical to define the spawning habitat as well as having sound information of timing and geographical extent of spawning. In the case of small pelagic fishes, the spatio-temporal characterisation of spawning habitats through the use of hydrography and shelf bathymetry is becoming increasingly important to fishery science, particularly in the context of biomass assessment and subsequent predictions of recruitment success and stock health (e.g. Checkley *et al*., 1999; van der Lingen *et al*., 2001, 2005; Ibaibarriaga *et al*., 2007; Neira and Keane, 2008).

In this chapter we describe the spawning habitat characteristics of redbait based on eggs and larvae caught primarily during extensive surveys carried out along shelf waters of eastern to south-western Tasmania in October 2005 and 2006. Input data for this paper follows from concurrent identification and aging protocols developed for eggs of this species in Chapter 2 (Neira *et al*., 2008). Supplementary ichthyoplankton data from southern New South Wales (NSW) in October 2002 and 2003 are employed to examine aspects of the geographical extent of the redbait spawning stock in south-eastern Australia. Cross- and along-shelf egg and larval distributions off Tasmania are examined in terms of environmental conditions, and results discussed in relation to linkages with regional oceanography, including water masses present during each survey. Overall results are discussed in terms of the suitability of DEPM to estimate biomass of this mid-water, shelf-associated species.

3.2 MATERIALS AND METHODS

3.2.1 Study area and surveys

Ichthyoplankton surveys were carried out during October 2005 and 2006, corresponding to the peak spring spawning season of redbait off eastern Tasmania (Chapter 1). Sampling was conducted over the continental shelf region between north-east of Flinders Is. in Bass Strait and south-east of the Tasman Peninsula (2005), and from Cape Barren Is. around to Port Davey (2006) in south-western Tasmania (Figs 3.1, 3.2). The rationale for extending the sampling coverage in 2006 to south-western Tasmania was to sample as much of the spawning area of redbait as feasible, based on the distribution of eggs and larvae from the 2005 survey, and the presence of spawning females in that region (Chapter 1). In all, 201 plankton samples were collected from 198 stations across the two surveys (Table 3.1).

The 2005 survey comprised a sampling grid containing 2-4 stations along 29 transects (T; T1-T29) perpendicular to the coastline and 10 nautical miles (nm) apart (Fig. 3.2; Appendix 4). Stations were positioned at the shelf break (200 m contour) and then every 5 nm to the shoreline except along T3, T5, T7 and T9, where stations were also positioned 5 nm offshore from the break (Fig. 3.2). In all, 94 plankton samples from 91 stations covering an area of ~ 4,563 nm^2 (15,650 km^2) were collected during this survey (Table 3.1).

The 2006 survey comprised a grid containing 4-5 stations along 22 transects (T1-T22) also perpendicular to the coastline but 15 nm apart (Fig. 3.2; Appendix 5). Sampling effort was concentrated mostly along a narrow 15 nm stretch that followed the shelf break, with stations located 7.5 and 2.5 nm inshore from the break, over the break, and 2.5 and 7.5 nm offshore from the break. In all, 107 samples from 107 stations covering a total area of ~ 6,225 nm^2 (21,351 km^2) were obtained during this survey. Since just over 96% of the redbait eggs collected during this survey came from stations across the first 13 transects, i.e. Cape Barren Is. (T1) to south-east of the Tasman Peninsula (T13), the region was deemed as encompassing the 2006 spawning area for the purpose of describing daily egg production (section 3.2.6). This region is hereafter referred to as eastern Tasmania, while the remaining surveyed region, which included 43 stations across T14 to T22 along the lower south-east to south-west, is referred to as southern Tasmania (Fig. 3.2).

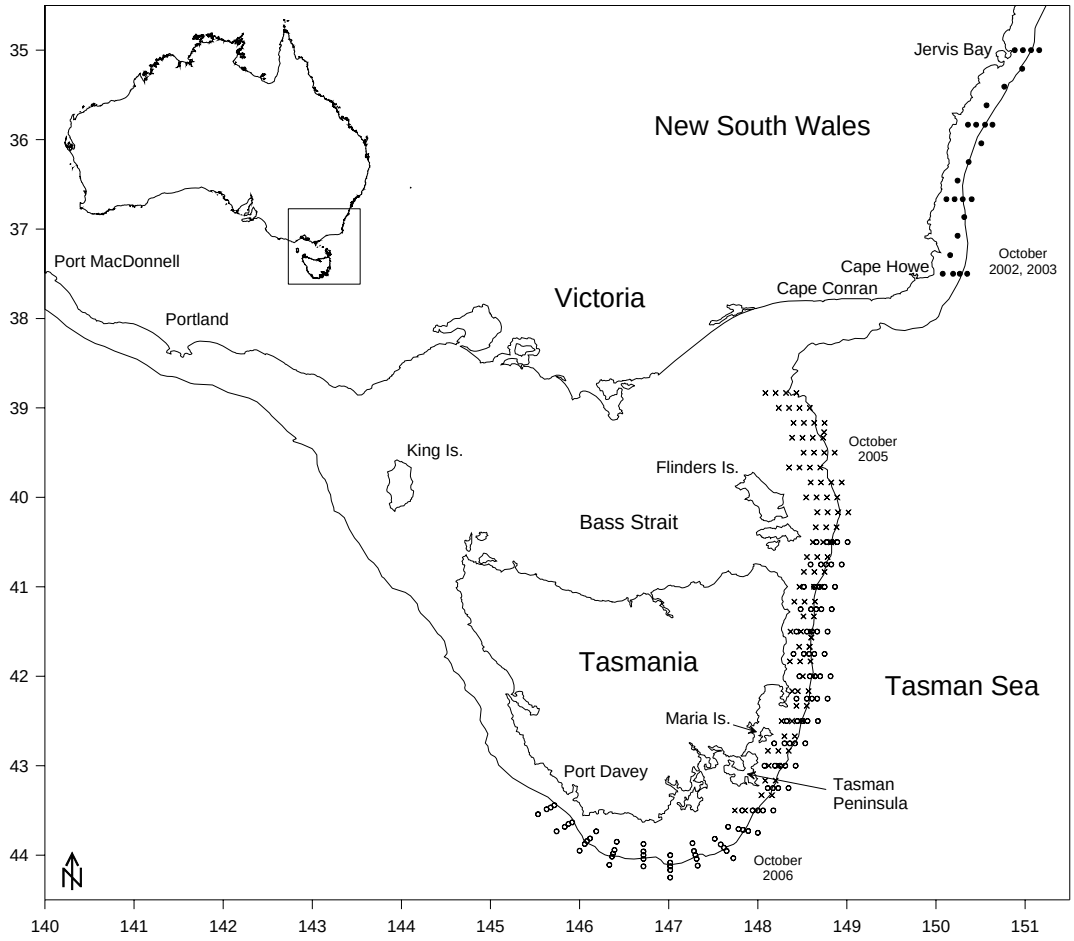

Fig. 3.1 Map of south-eastern Australia showing source of redbait eggs and larvae examined for this study. Symbols correspond to stations sampled during October 2002 and 2003 along southern New South Wales (dark circles), and October 2005 (crosses) and October 2006 (open circles) around north-eastern to south-western Tasmania (refer to Table 3.1 for localities of additional material). Line bordering coastline in this and all figures showing a map of south-eastern Australia including Tasmania, corresponds to the approximate position of the 200 m depth contour.

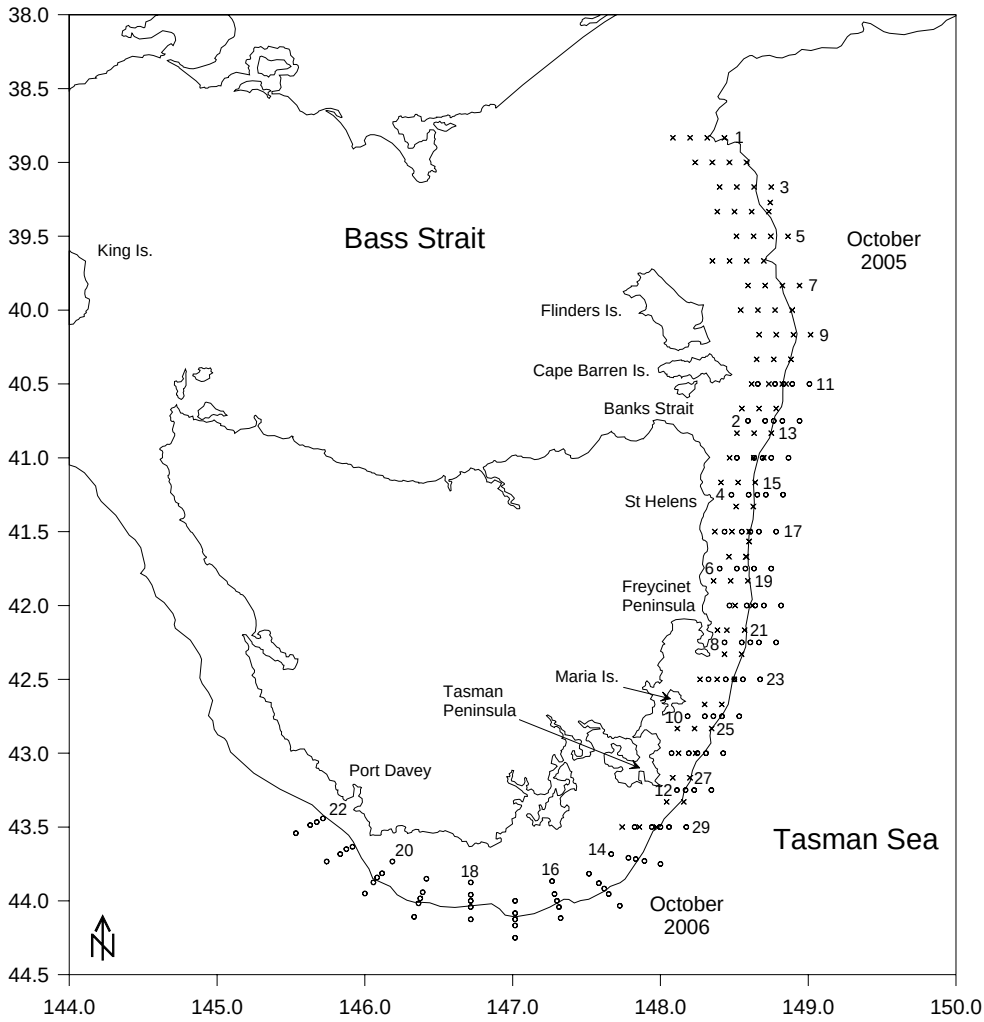

Fig. 3.2 Map of Tasmania showing transects (numbered) and stations sampled during the redbait egg and larval surveys conducted in October 2005 and 2006. For clarity, odd and even numbers indicate 2005 (1-29) and 2006 (2-22) transects, respectively (refer to Appendices 4 and 5 for exact geographical positions of stations).

Table 3.1 Details of surveys undertaken to collect redbait eggs and larvae along shelf waters off Tasmania in October 2005 and 2006. Details of sources of supplementary material examined for this study are also provided. Distance from shore (nm) and capture depth (m) have been provided for redbait larvae caught during the 1997 summer (F.J. Neira, *unpublished data*).

Abbreviations: AMS, Australian Museum Fish Catalogue number; NE, north-east; NSW, New South Wales; SA, South Australia; SE, south-east; T, transect; Tas, Tasmania; Vic, Victoria. Refer to map Fig. 3.1 for localities.

Survey date	**Survey area**	**Transects (no. stations)**	**Sample type (no. samples)**	
12-17 Oct 2005	NE Bass Strait to SE Tas	1-29 (91)	Vertical (94)	
10-14 Oct 2006	Eastern Tas (T1-T12)	1-12 (59)	Vertical (59)	
30-31 Oct 2006	Southern Tas (T13-T22)	13-22 (48)	Vertical (48)	
Supplementary material				
Survey date	**Shelf region/locality**	**Sample type**	**Eggs**	**Larvae (depth, m)**
Oct 1984	Eastern Tas (AMS I. 39851-1001, -002)	Oblique		+
Nov 1984	Eastern Tas (AMS I. 39853-001)	Oblique		+
Feb 1985	Eastern Tas (Maria Is) (AMS I. 39852-001, 002)	Oblique		+
Nov – Dec 1996	Eastern Bass Strait – southern NSW	Surface		+
Jan – Feb 1997	Eastern SA Port MacDonnell (32 nm offshore)	Vertically stratified		+ (0-25)
Jan – Feb 1997	Western Vic Portland (32 nm offshore)	Vertically stratified		+ (50-75)
Mar 1997	Eastern Tas	Oblique		+
Nov – Dec 1997	King Island	Surface		+
Dec 1997	Western Vic Portland (8 nm offshore)	Vertically stratified		+ (50-75)
Dec 1997	Western Vic Portland (16 nm offshore)	Vertically stratified		+ (0-25)
Dec 1997	Northern Bass Strait (Eastern Vic) Cape Conran (32 nm offshore)	Vertically stratified		+ (25-50)
Feb 1999	Eastern Tas (AMS I. 39899-001)	Oblique		+
Oct 2002	Southern NSW	Vertical	+	+
Oct 2003	Southern NSW	Vertical	+	+

3.2.2 Field and laboratory procedures

Plankton samples were taken continuously day and night. Eggs and larvae in 2005 were caught using a bongo sampler consisting of 3 m long, 0.6 m diameter plankton nets of 300 and 500 µm mesh, encased within a custom-built, weighted stainless steel frame to facilitate vertical drops. Samples in 2006 were taken with a modified PAIROVET sampler (bongo version of CalVET sampler; Smith *et al*., 1985) consisting of 1.5 m long, 0.25 m diameter plankton nets of 300 µm mesh, placed inside a similar but smaller weighted frame. The mouth of each net was fitted with a mechanical General Oceanic flowmeter to estimate volume (m^3) of water filtered.

At each station the sampler was lowered vertically to within ~5 m of the seabed or to a maximum depth of 190 m and immediately brought back on board. Sampling depth was determined using the onboard echosounder. Nets were thoroughly washed soon after the sampler was on deck, and samples from the codends combined and fixed either in 98% ethanol (2005) or 10% formaldehyde-seawater (2006); redbait eggs fixed in ethanol were subjected to mtDNA analyses to confirm species identifications (Chapter 2).

Vertical data on conductivity, temperature and depth were obtained simultaneously with each plankton sample using a Conductivity-Temperature-Depth (CTD) profiler fitted to the sampler's frame. Temperature data were plotted by depth for the inshore- and offshore-most stations of selected transects in October 2005 (T1, T11 and T29) and October 2006 (T1, T13 and T22) to determine presence and depth of thermal stratification. Temperatures and salinities at the surface (average of first 10 m) and mid-water (median to 100 m or to maximum depth if <100 m) were calculated for each station, and the latter data employed to construct a bubble plot of egg abundances for the 2005 survey; conductivity data from all stations in 2006 were omitted as some values recorded by the profiler were deemed unrealistically high and thus likely to be erroneous.

Composite, high resolution sea-surface temperature (SST) images of eastern Tasmania (NOAA AVHRR satellite) were obtained for the survey periods in 2005 (6-11 October) and 2006 (27 October-1 November). Images were selected from 5-day averages centred on the sampling days, and processed for cloud cover (CSIRO Marine & Atmospheric Research, Hobart). Altimetric sea-level and current velocity images of 15 October 2005 and 2006 were examined to obtain data on prevailing surface currents during the sampling periods (http://www.marine.csiro.au/remotesensing/oceancurrents).

Terminology pertaining to eggs and larvae follows Neira *et al*. (1998, 2008). All eggs and larvae were removed from samples under a dissecting scope, and stored in 70-98% ethanol for analyses. Redbait eggs were identified and sorted by developmental stage (I to VII; Chapter 2), while larvae were separated into preflexion (including yolk-sac larvae), flexion and postflexion stages.

3.2.3 Supplementary data

Redbait eggs and larvae from surveys carried out elsewhere in south-eastern Australia were also examined for this study to provide additional information on temporal and spatial distribution (Table 3.1). These included larvae from vertically-stratified samples collected with a 1 m^2 mouth opening-closing BIONESS sampler at locations across northern Bass Strait, larvae archived in museums and research institutions, and eggs and larvae caught along shelf waters between Jervis Bay and Cape Howe in southern New South Wales (NSW) in October 2002 and 2003 (Figs 3.1, 3.2). The latter were caught with the same bongo gear used off eastern Tasmania in 2005, at stations across four transects perpendicular to the coastline and 50 nm apart, as well as along-shelf stations between transects. Stations during those surveys were positioned 10 and 5 nm inshore from the shelf break, at the break and 5 nm offshore from the break, with vertical samples taken from within ~5 m of the seabed, or from a maximum depth of 200 m. Sampling depth was regulated using a Scanmar depth sensor fitted to the sampler frame. All samples were fixed in 98% ethanol, and all redbait eggs and larvae removed for subsequent analyses.

3.2.4 Treatment of egg and larval data

Total numbers of redbait eggs and larvae were standardised to abundance per surface area (numbers/m^2) based on water volume filtered and depth of net drop. Each redbait egg caught in the 2005 and 2006 surveys was assigned a specific age (hours) using the temperature-dependent egg incubation model described in Chapter 2. Overall egg and larval abundances for the surveys off Tasmania (2005, 2006) and southern NSW (2002, 2003), as well as egg abundances by age (days) for the former surveys, were plotted using SURFER®. Statistical analyses were performed using STATISTICA®. The 2005 and 2006 egg and larval abundances were compared statistically in terms of shelf region, following the classification of each station as shoreward ($\geq$5 nm inshore from shelf break), shelf break (2.5 nm either side of the break, including at the break) or offshore ($\geq$5 nm offshore from break). Main effects ANOVA (unequal sample size) was performed on the pooled data to determine whether mean egg (all and day-1 eggs) and larval abundances differed significantly by shelf region and survey. All data were log-transformed ($\log_{10}$ [n+1]) to account for heterogeneity of variance following Cochran's test. When factors were found to be significant, the Bonferroni comparison test was applied to ascertain which levels were different (Quinn and Keough, 2002). Analyses employed all data collected in 2005 but only data from eastern Tasmania in 2006, which was identified as the main spawning area for that survey (refer to next section for details). All percentage values are based on standardised abundances unless stated otherwise.

Quotient analyses (van der Lingen *et al*., 2001; Checkley, 2005) were performed on the abundance of day-1 eggs ($\leq$24 hours) from the 2005 and 2006 surveys to describe selection of spawning habitat in terms of bathymetric depth (100 m intervals), region of continental shelf (shoreward, shelf break and offshore) and mid-water temperatures (0.5^{o}C classes). For these analyses, day-1 egg abundances within each depth interval, shelf region and temperature class were expressed as a percentage of total (standardised)

abundance, divided by the percentage frequency of occurrence under each shelf region and temperature class, respectively. Quotients >1 indicate positive spawning location, i.e. favoured region and temperature range. As with ANOVA, these analyses employed all data from 2005 but only data from eastern Tasmania in 2006. Egg and larval abundances by temperatures and salinity (mid-water values) were constructed for the 2005 survey to ascertain the specific temperature/salinity (T/S) range in which eggs and larvae occurred.

3.2.5 Estimates of spawning area

Spawning areas in 2005 and 2006 (i.e. positive area in nm^2 and km^2) were estimated using ArcView GIS 3.2. For each survey a 5 nm diameter circular area was drawn around each sampling station, based on the distance between stations across each transect, and each assumed to have the same weighting. A closed polygon was then drawn over the entire survey area encompassing all positive stations (i.e. with redbait eggs) as well as negative stations (i.e. no redbait eggs) embedded between positive stations, and considered as the main spawning area. All negative stations outside the positive area were omitted from spawning area estimates. The estimated 2005 spawning area encompassed all but 13 negative stations (3,854 nm^2; 13,220 km^2), while the estimated 2006 spawning area along eastern Tasmania (2,535 nm^2; 8,695 km^2) included all but 17 negative stations (Fig. 3.2).

3.2.6 Daily egg production and mortality

Estimates of mean daily egg production at spawning time zero (P_0; eggs/0.05m^2 day^{-1}) and daily instantaneous mortality rate (Z; day^{-1}) of redbait eggs were computed using an exponential decay model based on egg abundance-at-age data (Picquelle and Stauffer, 1985). Model assumes parameters to be independent observations from a population with a common P_0 and Z (Stratoudakis *et al*., 2006). Datasets for these runs comprised egg counts grouped by daily cohorts per station and standardized to area (response variable in numbers/m^2), with egg ages (days) assigned with the existing temperature-dependent egg incubation model (Chapter 2). The computing of egg ages by stage employed mid-water temperatures of each station and local time (hour) of collection. For the purpose of aging eggs, peak spawning time for redbait was assumed to be 21:00 h based on adult reproductive data, noting that spawning was more or less completed by midnight, inferring that the spawning peak had occurred earlier (Chapter 1).

Two functions were fitted to the daily egg abundance-at-age data, namely the traditional least squares non-linear regression (NLS) model (Lo, 1996), and a generalized linear model (GLM) using negative binomial error distribution (ICES, 2004; Cubillos *et al*., 2007). The NLS model, 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. Coding functions for the two models are available in *Ichthyoanalysis*, a package of the free

software *R* (www.r-project.org) specifically dedicated to the analysis of ichthyoplankton data and application to DEPM (http://sourceforge.net/projects/ichthyoanalysis). Together with the required parameters, specific model diagnostic were also obtained from this *R* package to examine model fit (Appendix 6). Abundances of daily egg cohorts per station (eggs/m^2) plotted against mean ages (days) for 2005 and 2006 correspond to *R* outputs and are provided only for GLM fits.

Egg abundance data employed in model runs included all positive stations as well as negative stations embedded within spawning area (see section 3.2.5 above). Data for each station included all egg stages (I to VII) even if catches were not recorded for a particular stage(s) in a given station, i.e. all zeros need to be considered in the analyses (M. Bernal, Instituto Español de Oceanografía, CACYTMAR, Spain and G. Claramunt, Universidad Arturo Prat, Chile, *pers. comm.*). For comparative purposes, estimates of mean weighted P_0 and Z for the 2005 and 2006 spawning areas were provided for a dataset incorporating all eggs, and a dataset that omitted eggs assigned $\leq$4 hours old and those with $\geq$98% probability of being hatched at the temperature recorded for the station (mid-water values). Exclusion of eggs $\leq$4 hours old follows standard DEPM practice applied to anchovy (Hunter and Lo, 1997; Claramunt *et al*., 2007; Cubillos *et al*., 2007), given the lower probability of encountering small highly concentrated patches of newly-spawned eggs. Exclusion of eggs assigned $\geq$98% of incubation time follows current work with anchovy in Chile (Claramunt *et al*., 2007).

In the case of the NLS, the intercept constitutes the unweighted mean P_0, while the CV is calculated by dividing the standard error of the intercept by P_0. With GLM, the exponent of the intercept corresponds to the unweighted mean P_0, while the standard error is equivalent to the CV (Stratoudakis *et al*., 2006). The weighted mean P_0 estimate (eggs/0.05m^2 day^{-1}) is then obtained by multiplying the unweighted value by the spawning (positive)/total survey area ratio. The same applies to the weighted CV of the P_0 estimates. For both models, the CVs for mortality (Z; slope) are calculated by dividing the standard error by the absolute Z value.

3.3 RESULTS

3.3.1 Environmental conditions

Composite SST imagery of the eastern shelf show Tasman Sea water (12-14^{o}C) along the inner shelf, and the south-flowing East Australian Current (EAC; 15-16^{o}C) encroaching along the outer shelf, with a longitudinal front between these two masses clearly defined along the shelf break in both 2005 and 2006 (Fig. 3.3). Vertical temperature profiles from CTD-derived data showed this front being more evident in 2005 than in 2006, particularly over mid-eastern Bass Strait where offshore waters were up to 1.5^{o}C warmer that those inshore due to EAC influence (Fig. 3.4). Furthermore, inshore waters were vertically well mixed throughout most of the 2005 survey area except towards the southern end, as indicated by the breakdown in thermal structure detected from the surface to 40 m off T29 (43.5^{o}S) in the lower south-east (Fig. 3.4C). By contrast, inshore waters at the same latitude in 2006 (T13) were well mixed, while offshore waters exhibited signs of thermal stratification at a depth of 20-80 m (Fig. 3.4E).

Average surface and mid-water temperatures along the survey area in October 2005 fell within the same range of those recorded off eastern Tasmania in October 2006, i.e. 12.16-14.5^{o}C (Table 3.2). By contrast, average temperatures along southern Tasmania in 2006 were lower than those along the eastern shelf, i.e. 11.73-12.12^{o}C. The cooler water detected off southern Tasmania in 2006 corresponds to upwelled subantarctic water resulting from the prevailing easterly winds, following a receding warm Zeehan Current which floods the entire western shelf during June-August (Ridgway, 2007a). Surface and mid-water salinities off eastern Tasmania during 2005 averaged 35.5 off mid Bass Strait, and 35.0-35.2 to the south-east of the Tasman Peninsula (Table 3.2). Altimetric sea-level images of south-eastern Australia obtained for around the middle of the sampling period in 2005 and 2006 showed a prevailing 0.5 knot (0.25 m s^{-1}) south-bound surface current flowing over the continental shelf along the length of eastern Tasmania (Fig. 3.5).

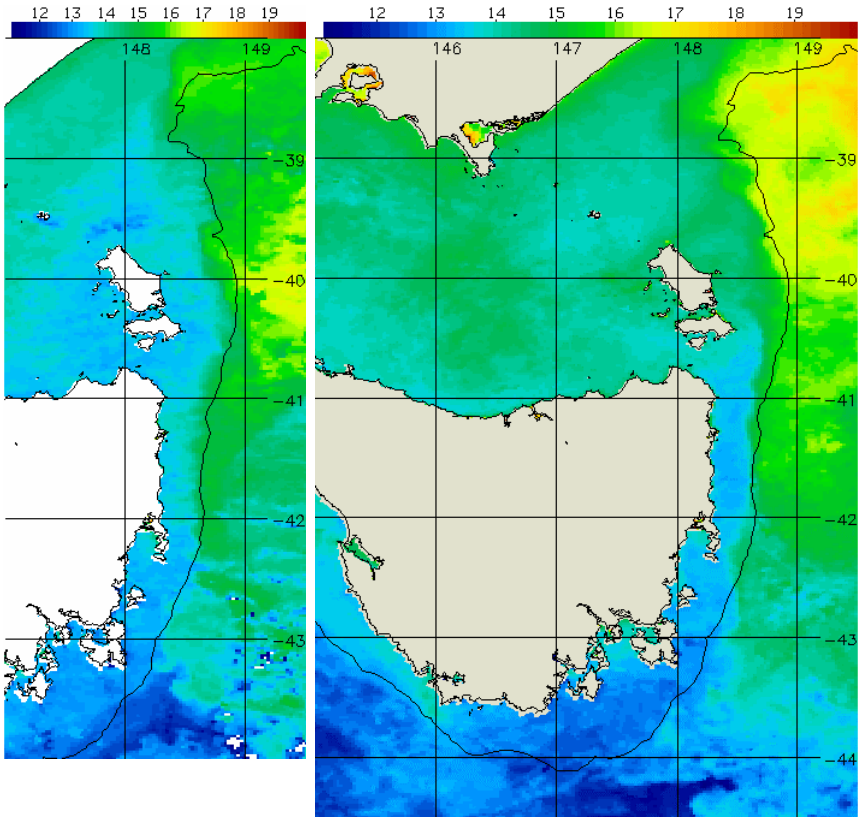

Fig. 3.3 Sea surface temperature (SST) images (composite 5-day averages) along eastern Tasmania for the period 6-11 October 2005 (left) and 27 October – 1 November 2006 (right), corresponding to the dates of the two ichthyoplankton surveys.

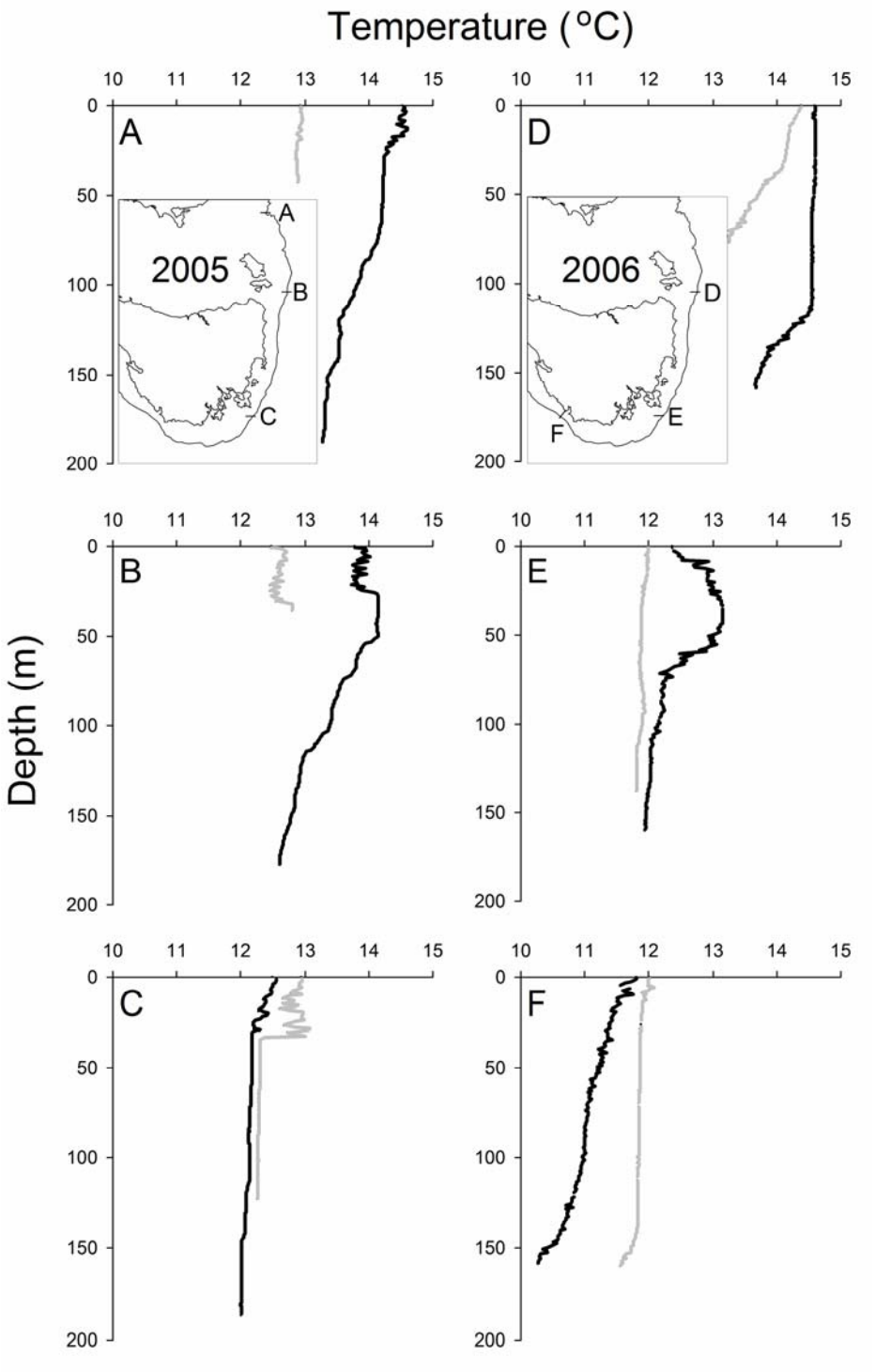

Fig. 3.4 Vertical temperature profiles at inshore-most (grey lines) and offshore-most (black line) stations of selected transects along eastern and southern Tasmania in October 2005 and 2006. Positions of transects in 2005 (A, T1; B, T11; C, T29) and 2006 (D, T1; E, T13; F, T22) are indicated in maps embedded in top plots.

Table 3.2 Summary of temperature and salinity conditions along shelf waters of north-eastern to south-western Tasmania during the 2005 and 2006 ichthyoplankton surveys.

Surface values correspond to 10 m averages; mid-water values correspond to averages of medians to a depth of 100 m, or to maximum depth if <100 m. Maximum (Max.), minimum (Min.) and average (Av.) values provided for the offshore region include all stations at or offshore of the shelf break; inshore includes stations closest to shoreline. A dash indicates data not available.

Survey date	Region	Transect (no. stations)	Latitude (°S)		Temperature		Salinity	
					Surface	Mid-water	Surface	Mid-water
2005	Whole	1 (4)	38.8	Av.	13.36	13.40	35.49	35.48
		29 (3)	43.5	Av.	12.60	12.80	35.04	35.15
		1 – 29 (91) Inshore		Max.	13.97	13.51	35.50	35.54
				Min.	12.37	12.21	34.69	35.08
				Av.	**13.09**	**12.85**	**35.17**	**35.25**
		1 – 29 (91) Offshore		Max.	15.32	15.05	35.63	35.57
				Min.	12.16	11.96	34.85	35.07
				Av.	**13.74**	**13.43**	**35.26**	**35.32**
2006	Whole	1 (5)	40.5	Av.	14.50	14.30	-	-
		13 (5)	43.5	Av.	12.16	12.12	-	-
		22 (4)	43.5	Av.	11.76	11.73	-	-
	Eastern Tasmania	1 – 13 (64) Inshore		Max.	12.89	12.91	-	-
				Min.	11.97	11.90	-	-
				Av.	**12.25**	**12.15**	-	-
		1 – 13 (64) Offshore		Max.	14.61	14.62	-	-
				Min.	12.34	12.03	-	-
				Av.	**13.66**	**13.62**	-	-
	Southern Tasmania	14 – 22 (43) Inshore		Max.	12.50	12.18	-	-
				Min.	11.52	11.46	-	-
				Av.	**12.08**	**11.88**	-	-
		14 – 22 (43) Offshore		Max.	13.04	12.97	-	-
				Min.	10.94	10.59	-	-
				Av.	**11.92**	**11.71**	-	-

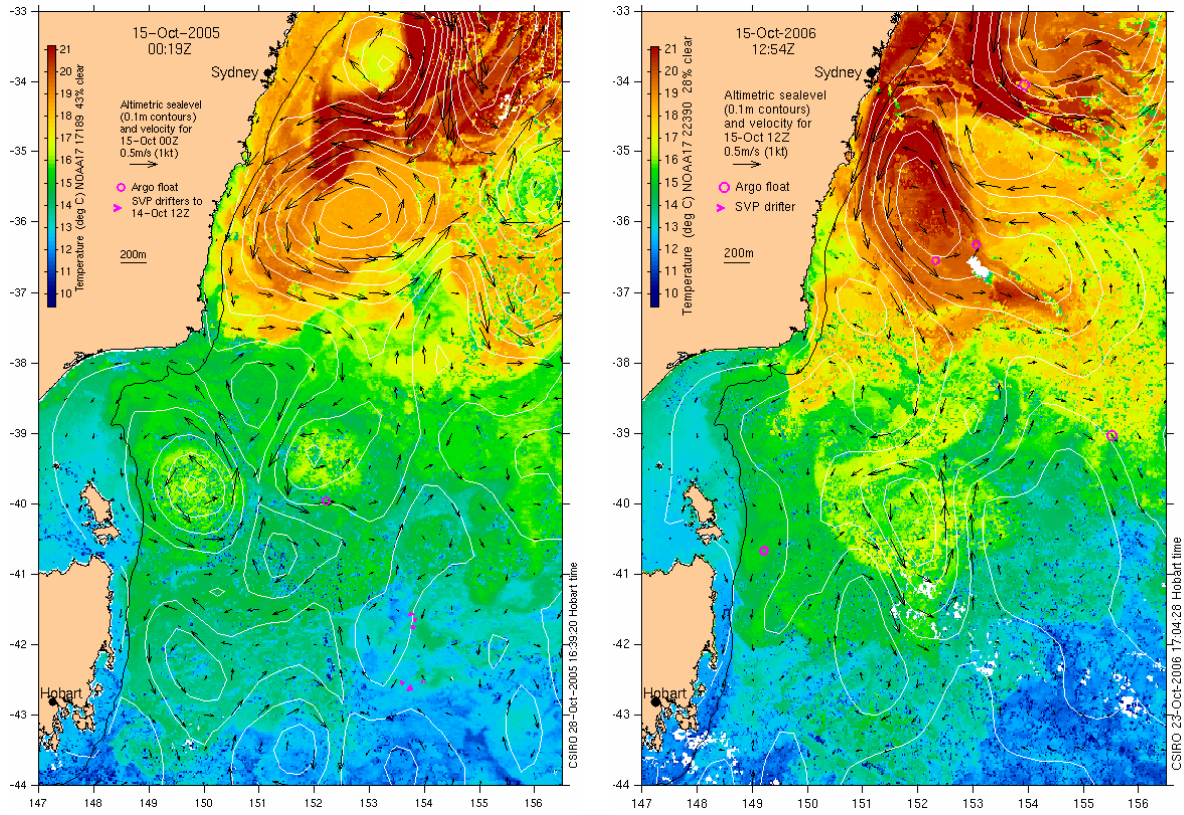

Fig. 3.5 Altimetric sea-level and current velocity images of 15 October 2005 (left) and 2006 (right) superimposed over sea surface temperatures in south-eastern Australia.

3.3.2 Distribution and abundance of eggs and larvae

A total of 9,280 and 1,113 redbait eggs were caught along shelf waters off Tasmania during the 2005 and 2006 surveys, respectively (Table 3.3). Of all stations sampled during the surveys, eggs occurred in 85% of stations sampled in 2005 and 59% of stations sampled in 2006. Redbait larvae totalled 311 in 2005 and 67 in 2006.

Redbait eggs occurred along the entire shelf area in 2005 (Fig. 3.6). By contrast, 96.2% of eggs in 2006 originated from eastern Tasmania while the remaining 3.8% came from southern Tasmania. Unlike eggs, redbait larvae in 2005 were confined mostly to the area between transects 16 (41.1°S) off St Helens and 27 (43.2°S) off the Tasman Peninsula, while nearly 80% of larvae in 2006 were caught along eastern Tasmania, almost matching the distribution of eggs for that survey (Fig. 3.6).

The greatest abundance of redbait eggs in 2005 (1,953 eggs/m^2) was obtained at the shelf break station of transect 11 (40.5°S) off Cape Barren Island. Greatest abundance during 2006 (2,644 eggs/m^2) was recorded at a station 2.5 nm inshore from the shelf break along T5 (41.5°S), i.e. further south than in 2005 (Table 3.4; Fig. 3.6).

Greatest larval abundances in 2005 (67 larvae/m^2) and 2006 (62 larvae/m^2) were obtained off the north-east coast between St Helens and Freycinet Peninsula, in waters between the shelf break and 5 nm inshore from the break (Table 3.4). Nearly 99% of the 270 redbait larvae caught in 2005 were at the preflexion stage, measuring 2.4-7.2 mm in body length (Fig. 3.7).

Redbait egg abundances varied significantly with shelf region across the two surveys combined (F=24.87; P<0.0001), with eggs being more abundant at shelf break stations (2.5 nm either side of break) than at those ≥5 nm either inshore or offshore from the break. By contrast, redbait larval abundances did not vary significantly by shelf region, with larvae showing a broader cross-shelf distribution (Fig. 3.8).

Plankton samples taken in shelf waters off southern NSW in October 2002 and 2003 produced 962 eggs and 71 larvae of redbait (Fig. 3.1; Table 3.3). Eggs occurred along the region of southern NSW between Jervis Bay Cape Howe, but were significantly more abundant during 2003, with the highest abundance (651 eggs/m^2) obtained at a station 5 nm inshore from the shelf break in water depth of 125 m (36.7^o S; Figs 3.1, 3.9).

Additional records of redbait larvae are available for waters off eastern Tasmania between October and March, as far south as Maria Is. (Table 3.1). Elsewhere in south-eastern Australia redbait larvae have been caught in December and January along northern Bass Strait, in waters 8 to 32 nm from the coast and between surface and 75 m (Table 3.1; Fig. 3.1). Records show these larvae occurring as far west as Port MacDonnell in eastern South Australia, as well as off Portland and Cape Conran in western and eastern Victoria, respectively (F.J. Neira, *unpublished data*).

Table 3.3 Survey details and total number (unadjusted) of redbait eggs and larvae caught in shelf waters along north-eastern to south-western Tasmania in October 2005 and 2006. Details from supplementary surveys of shelf waters along southern New South Wales in October 2002 and 2003 are also provided.

Abbreviations: E, eastern; NE, north-eastern; NSW, New South Wales; S, southern; SE, south-eastern; SW, south-western; T, transect; Tas, Tasmania.

Survey date	Area (transects; no. stations)	Samples	Eggs (% positive stations)	Larvae (% positive stations)
Oct 2005	NE Bass Strait to SE Tas (T1 – T29; 91)	94	9,280 (85)	311 (57)
Oct 2006	Eastern Tas (T1 – T13; 64)	64	1,069 (59)	53 (39)
Oct 2006	Southern Tas (T14 – T22; 43)	43	44 (39)	14 (19)
Supplementary data				
Oct 2002	Southern NSW	23	60 (43)	10 (22)
Oct 2003	Southern NSW	15	902 (53)	51 (80)

Table 3.4 Summary statistics of redbait eggs and larvae caught during surveys along shelf waters of north-eastern to south-western Tasmania in October 2005 and 2006 (refer to Materials and Methods for estimation of total survey area and spawning area for each survey). "All" correspond to greatest abundance of eggs and larvae across entire survey area; egg abundance data have also been provided in terms of age (days old). Greatest egg and larval abundances (numbers per m^2) obtained during each survey are provided for each record together with latitude (^{0}S) of transect and distance from shelf break (nm) where these were obtained, e.g. -5, 5 nm shoreward from shelf break; 0 nm, shelf break.

Abbreviations: NA, not applicable; NE, north-east; SE, south-east; T, transect.

Survey date	Survey area	Total survey area/spawning area (km^2)	Greatest abundance (numbers/m^2) (Latitude ^{0}S, distance from shelf break in nm)					
			All eggs	All larvae	Eggs			
					Day 1	Day 2	Day 3	Day 4
Oct 2005	NE Bass Strait to SE Tas	15,650 / 13,220	1,953 (40.5, 0)	67 (42.0, -5)	1,831 (40.7, 0)	513 (39.8, 0)	158 (42.7, 5)	153 (43.3, -5)
Oct 2006	Eastern Tasmania (T1-T13)	12,924 / 8,695	2,644 (41.5, -2.5)	62 (41.2, 0)	2,272 (41.5, -2.5)	425 (41.2, 0)	543 (41.2, 5)	621 (40.5, 0)
	Southern Tasmania (T14-T22)	7,728 / NA	53 (43.4, -2.5)	54 (43.9, -2.5)	22 (43.7, -7.5)	11 (44.1, -2.5)	39 (43.7, -7.5)	32 (43.9, -7.5)

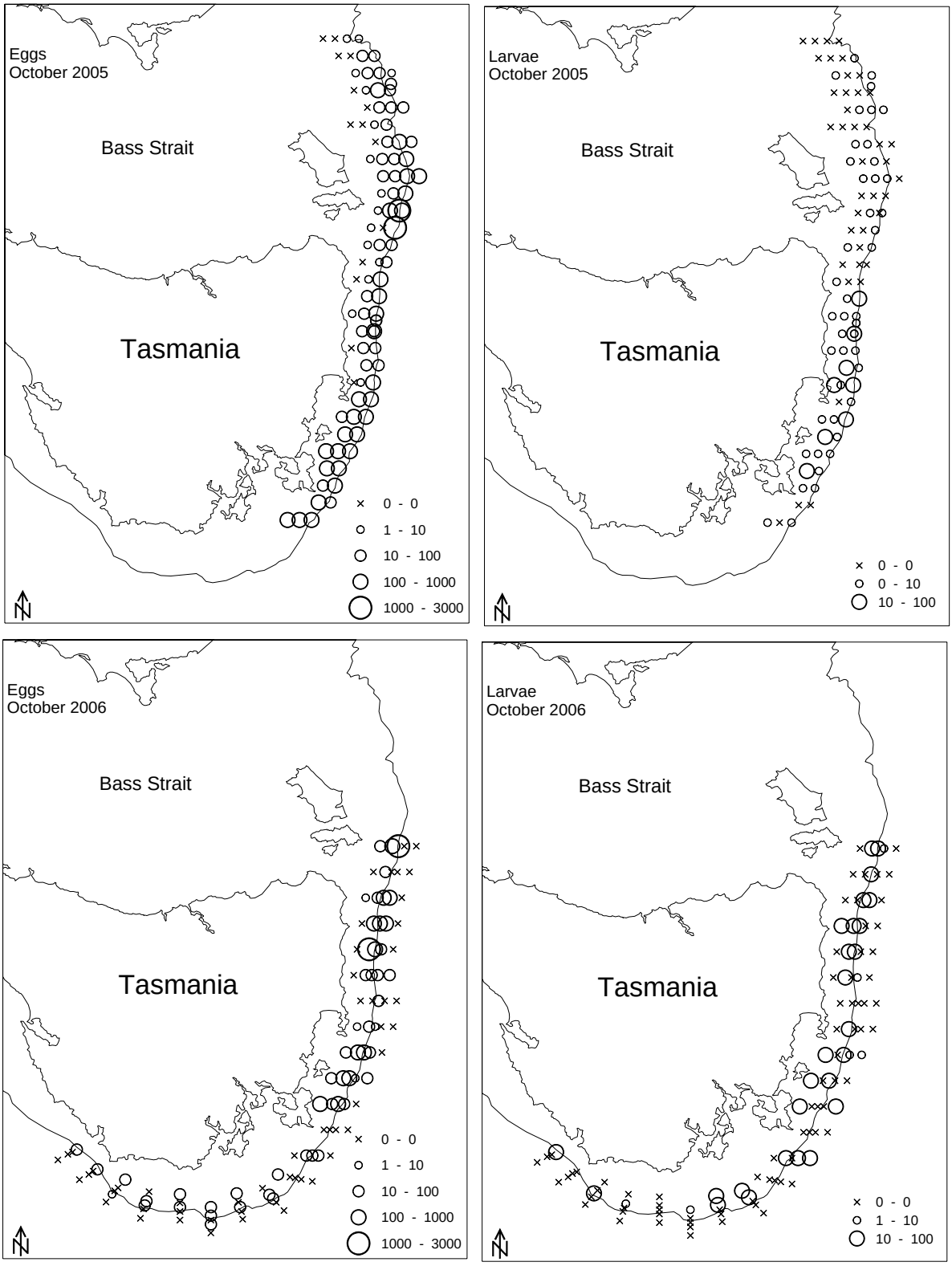

Fig. 3.6 Distribution of redbait eggs and larvae (numbers/m^2) around north-eastern to south-western Tasmania during October 2005 (top) and October 2006 (bottom).

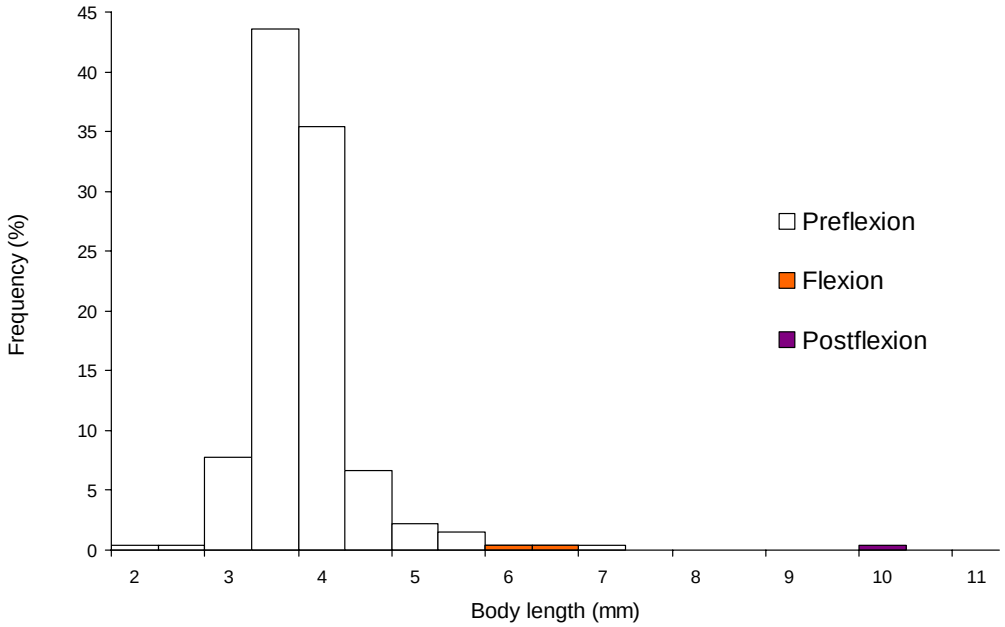

Fig. 3.7 Body length distribution of redbait larvae caught in shelf waters of eastern Tasmania during October 2005.

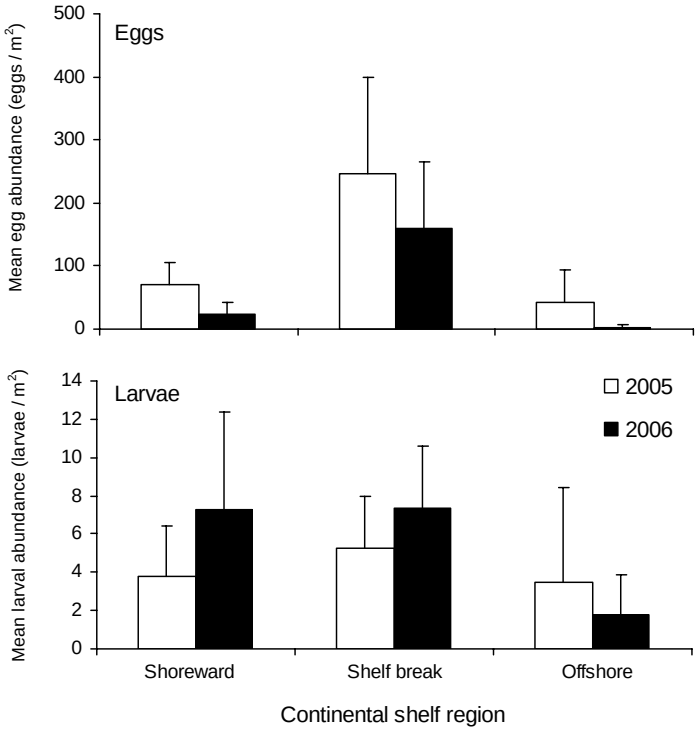

Fig. 3.8 Cross-shelf distribution of eggs (top) and larvae (bottom) of redbait (mean numbers/m^2 + 95% C.I.) in shelf waters of north-eastern to south-eastern Tasmania in October 2005 and 2006. Data from each sampling station were pooled into one of three regions (x-axis): shoreward (≥5 nm inshore from shelf break); shelf break (2.5 nm either side of break, including at break); and offshore (≥5 nm offshore from break). Data for 2006 correspond to the eastern Tasmania sub-area (refer to Materials and methods for details).

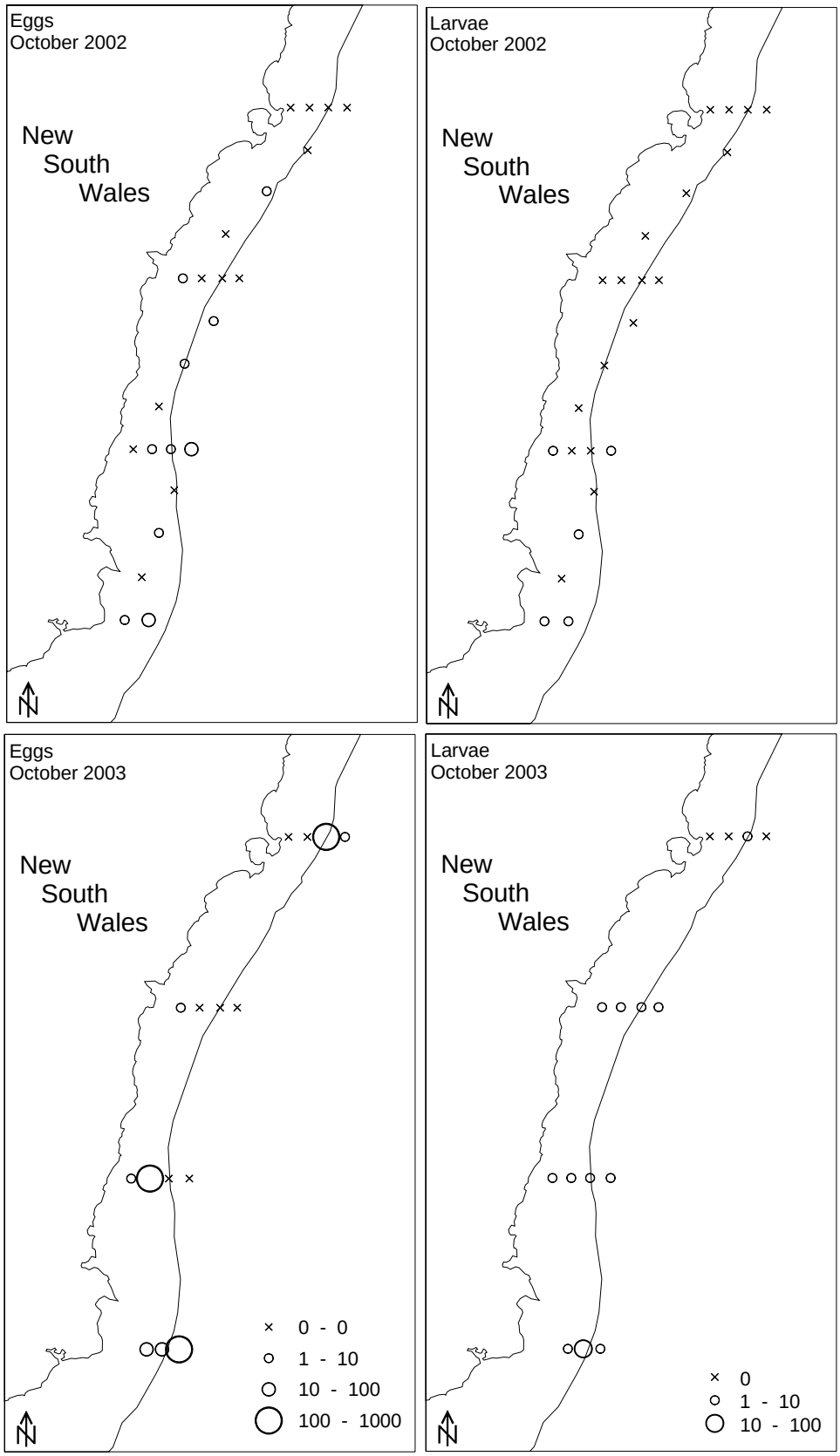

Fig. 3.9 Distribution of redbait eggs and larvae (numbers/m^2) along southern New South Wales during October 2002 (top) and October 2003 (bottom).

3.3.3 Egg age distributions

Redbait eggs collected in 2005 and 2006 ranged between one and five days old (Fig. 3.10). Average abundances (numbers/m^2) ranged between 97-175 day-1 eggs and 18-34 day-5 eggs, with abundances during both surveys steadily declining toward late-stage eggs (Table 3.4). No discernible pattern of long-shelf distribution could be observed for day-1 to day-4 eggs in 2005. However, it appeared that day-1 and day-2 eggs grouped more distinctly along the shelf break region between Flinders Is. and Banks Strait compared to day-3 and day-4 eggs (Fig. 3.11). In 2006 day-2 and day-3 eggs were more frequent off the north-eastern tip of Tasmania including Banks Strait, whereas day-1 and day-4 eggs were broadly distributed throughout eastern Tasmania (Fig. 3.12).

Mean abundances of day-1 to day-4 redbait eggs exhibited a distinct cross-shelf pattern during each survey, with abundances declining markedly from the shelf break region both shoreward and offshore (Fig. 3.13). Analysis of the data pooled for both surveys indicated that day-1 eggs were significantly more abundant at shelf break stations than at those $\geq$5 nm either inshore or offshore from the break (F=11.911; P<0.0001). Quotients of day-1 egg abundances by shelf region pooled across 2005 and 2006 reached ~2 for stations close to and at the shelf break region, dropping to <0.5 for stations in the shoreward and offshore regions (Fig. 3.14). In terms of depth, quotients of day-1 egg abundances pooled across years peaked at stations in waters 225-325 m deep along the shelf break region (Fig. 3.14).

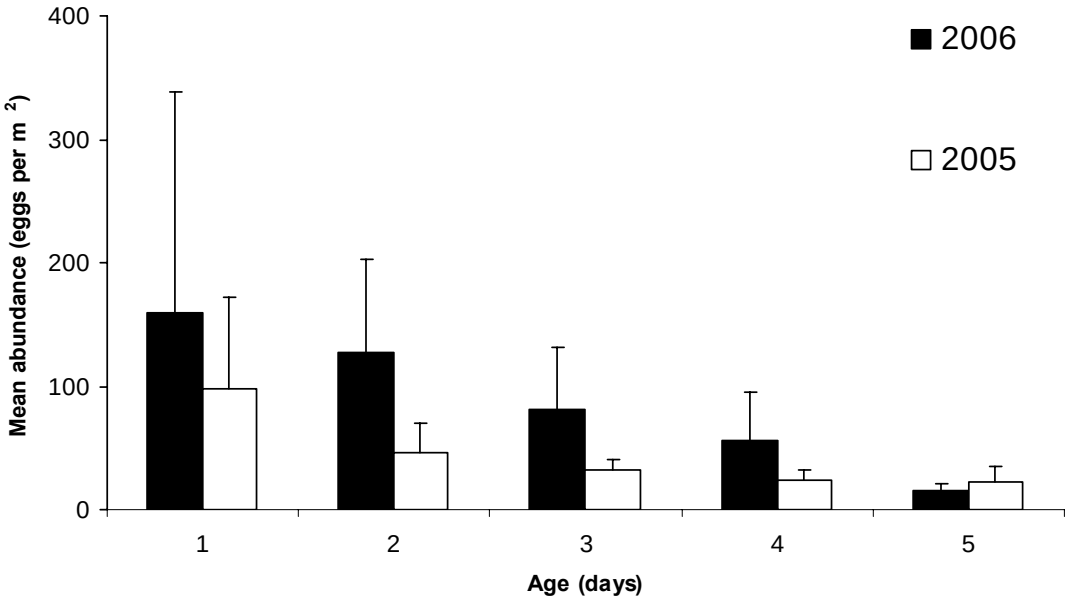

Fig. 3.10 Mean abundance (+95% C.I.) of redbait eggs (numbers/m^2) by age (days old) in shelf waters of north-eastern to south-western Tasmania during October 2005 and October 2006 (all transects included).

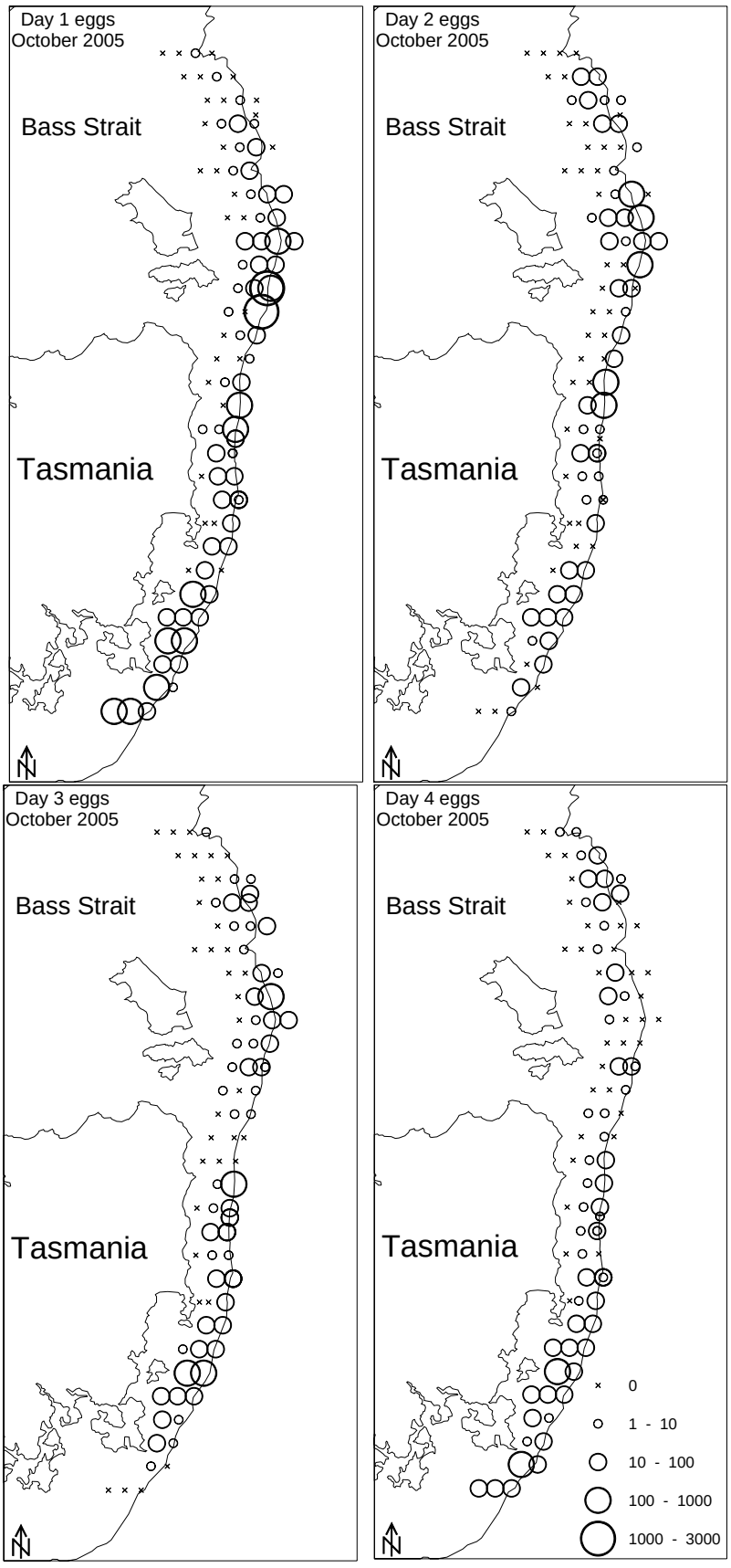

Fig. 3.11 Distribution of redbait eggs (numbers/m^2) by age (days – weighted ages) along north-eastern to south-western Tasmania during October 2005. Day-5 eggs have been omitted.

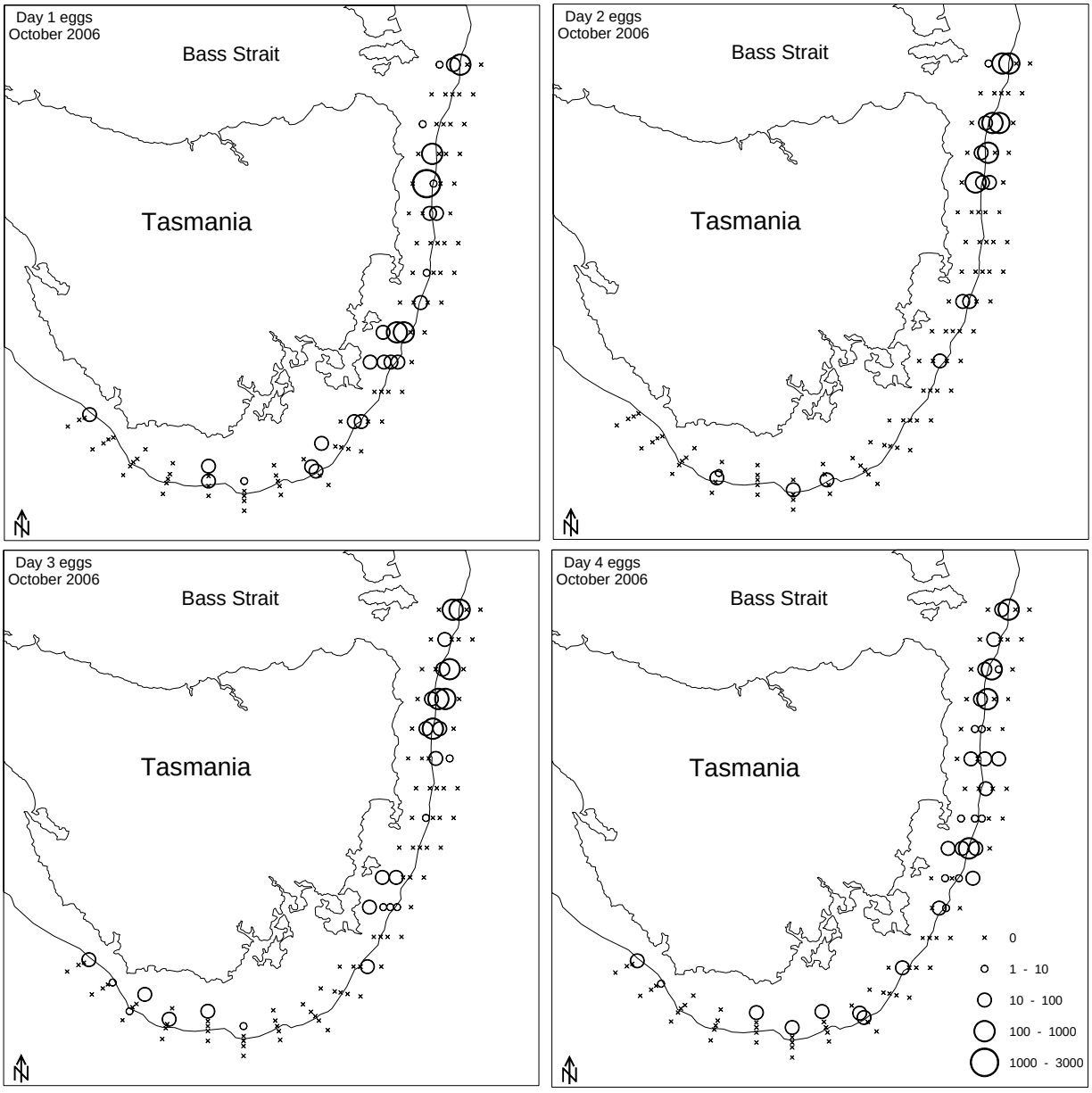

Fig. 3.12 Distribution of redbait eggs (numbers/m^2) by age (days – weighted ages) along north-eastern to south-western Tasmania during October 2006.

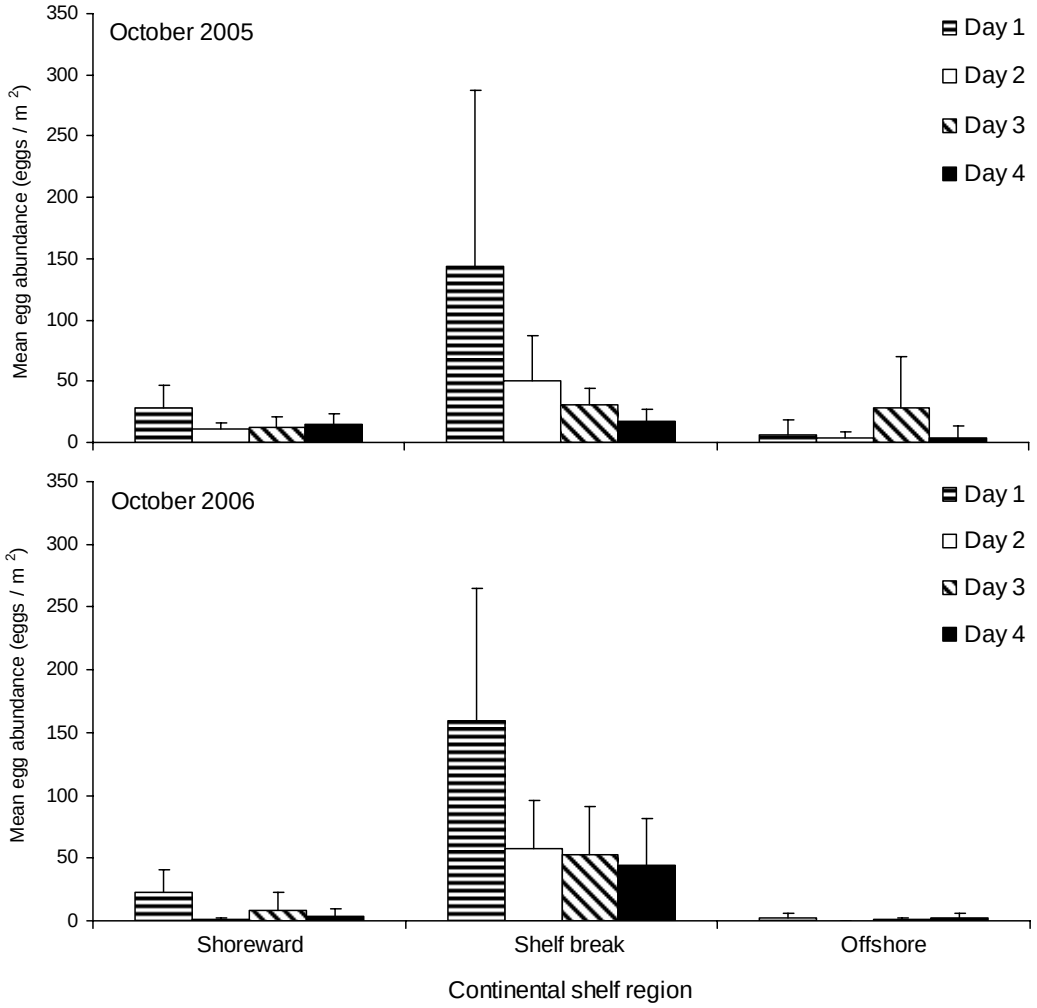

Fig. 3.13 Cross-shelf distribution of day-1 to day-4 eggs of redbait (mean numbers/m^2 + 95% C.I.) in shelf waters of north-eastern to south-eastern Tasmania in October 2005 (top) and 2006 (bottom). Data from each sampling station were pooled into one of three regions (x-axis): shoreward ($\geq$5 nm inshore from shelf break); shelf break (2.5 nm either side of break, including at break); and offshore ($\geq$5 nm offshore from break). Data for 2006 correspond to eastern Tasmania.

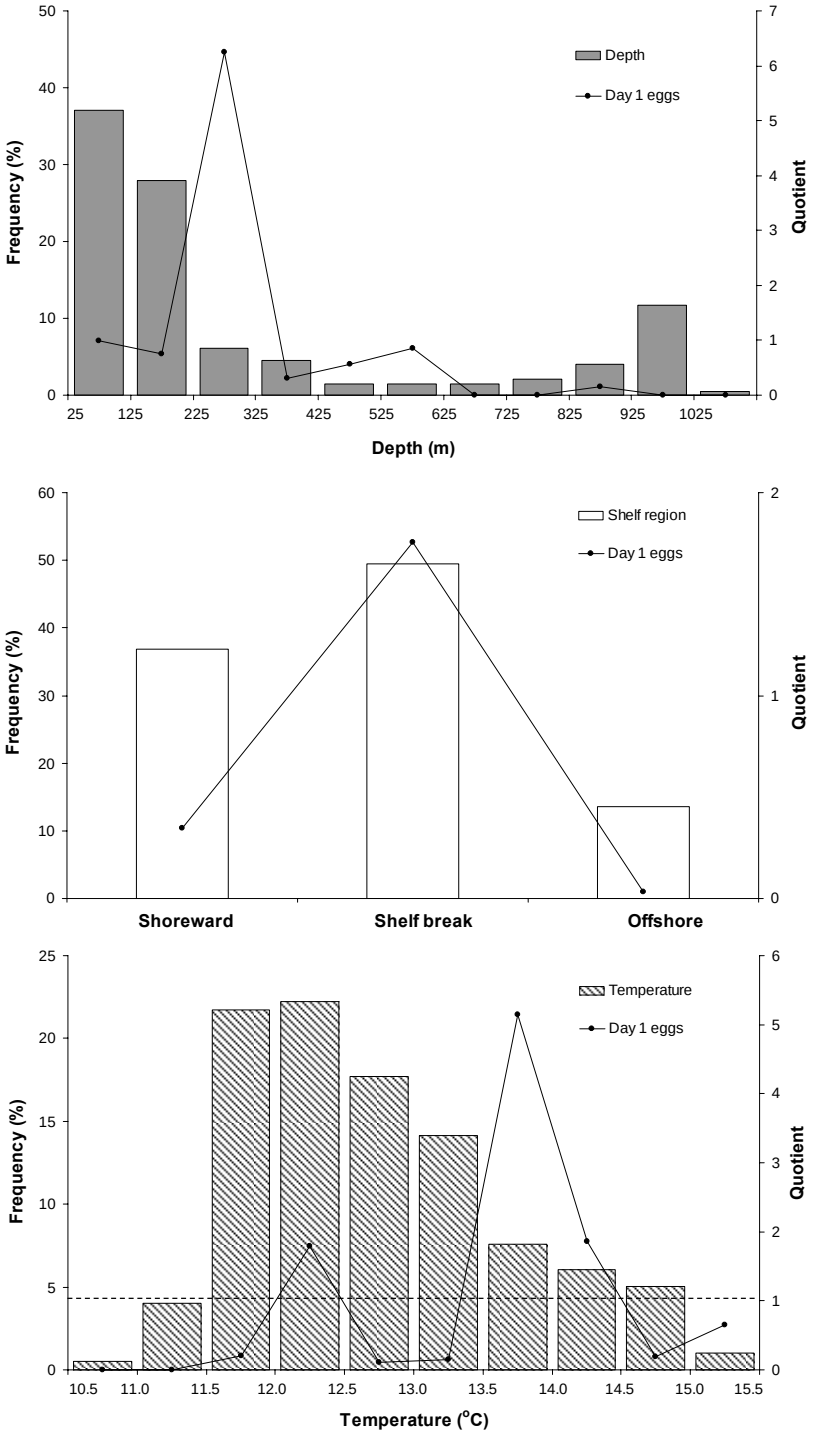

Fig. 3.14 Quotients of abundances of day-1 eggs of redbait (numbers/m^2) by depth (100 m intervals), shelf region and mean temperature (0.5°C classes), based on data from shelf waters of north-eastern to south-western Tasmania in October 2005 and 2006. Data from each station in middle plot were pooled into one of three regions, namely shoreward (≥5 nm inshore from shelf break), shelf break (2.5 nm either side of break, including at break), and offshore (≥5 nm offshore from break). Bars along x-axis in plots correspond to percentage frequencies of sampling occurrences of depth intervals, shelf regions and temperatures, and include all stations across the two years. Temperature corresponds to mid-water values at each station. Positive temperature selection (quotient values >1) is indicted by broken line in bottom plot.

3.3.4 Association with environmental variables

Day-1 eggs of redbait caught during 2005 originated from stations with mid-water temperatures of 12.0–15.2°C and mid-water salinities of 35.0-35.5 (Table 3.2; Fig. 3.15). Quotients of day-1 egg abundances by 0.5°C temperature classes were bi-modal across the two years, comprising a small peak at 12.0-12.5°C and a major peak at 13.5-14.0°C (Fig. 3.14). Redbait eggs and larvae sampled off southern NSW during 2002 and 2003 originated from stations with mid-water temperatures of 14.1-17.0°C, with the greatest abundance obtained in 14.4°C.

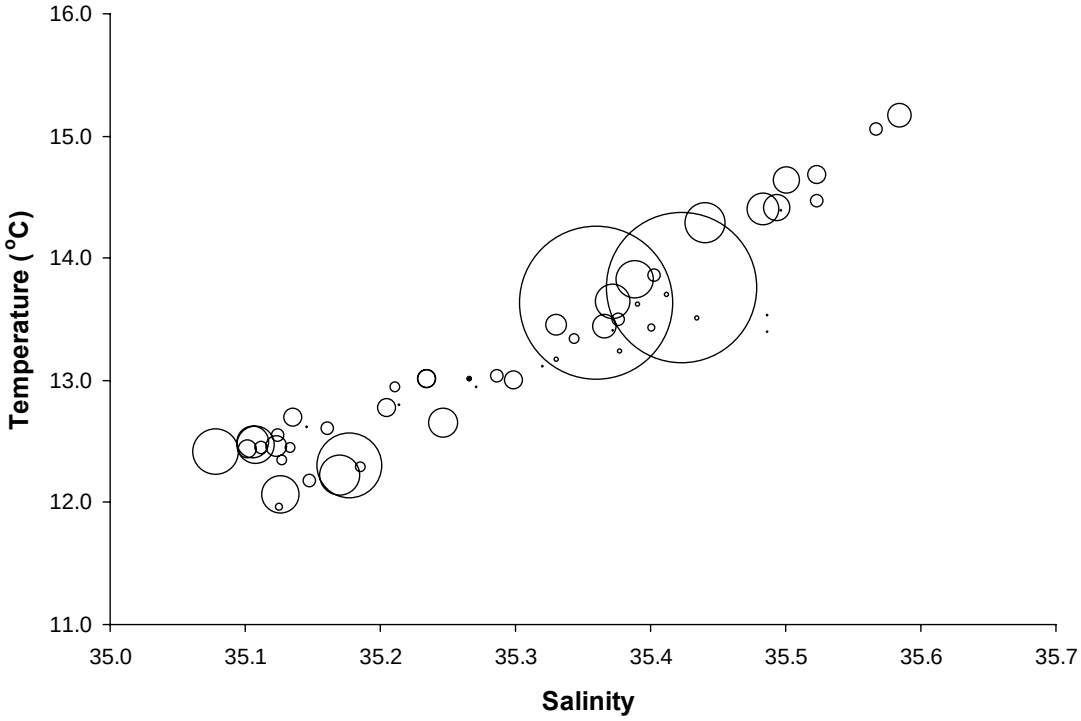

Fig. 3.15 Abundances of day-1 redbait eggs obtained at different combinations of temperatures and salinities (mid-water values at each station) along eastern Tasmania in October 2005. Bubbles sizes are proportional to eggs abundances (numbers/m^2).

3.3.5 Spawning area and daily egg production

The spawning area of redbait along eastern Tasmania in 2005 was estimated in 13,220 km^2 (Fig. 3.16), accounting for 84.5% of the total area sampled during that survey. The whole area sampled during October 2006 covered 21,351 km^2. However, the spawning area for that year was estimated in 8,695 km^2 (Fig. 3.17), which comprised 67.3% of the eastern Tasmania area sampled (Table 3.4).

Weighted mean daily egg production (P_0; eggs/0.05m^2 day^{-1}), egg mortality (Z) and total egg production (eggs x 10^{12}) estimates were generally consistent in magnitude between years, models used and data scenarios apart from the 2005 NLS estimates based on omitted extreme data (Table 3.5). Excluding the latter, estimated P_0 values for all other runs varied between 3.08 and 4.60, with those for GLM excluding extreme egg cohorts estimated at 4.04 in years. Egg mortality estimates ranged between 0.23 and 0.52, while total egg production estimates per spawning area ranged between 0.88 and 1.20 (Table 3.5). CVs for GLM derived estimates were, however, consistently lower than those for the NLS approach.

Table 3.5 Daily egg production (P_0) by area (eggs/0.05m^2 day^{-1}) for redbait estimated from data obtained during the October 2005 and 2006 egg surveys along shelf waters off eastern Tasmania. Estimates are provided for two models (NLS and GLM) under two different data scenarios, namely all data and no extremes, the latter referring to the exclusion of extreme egg cohorts, i.e. eggs aged ≤4 hours and ≥98% of incubation time (refer to Materials and Methods for details).

	October 2005		**October 2006**	
Estimated spawning area (km^2)	13,220		8,695	
Number stations included	84		47	
Data scenarios	All data	No extremes	All data	No extremes
Data points (including zeros)	332	280	209	175
Model 1: least squares non-linear regression (NLS)				
Intercept (P_0)	90.89	157.75	101.40	113.22
Std Error of P_0	24.29	46.23	37.2	46.26
Age (Z)	-0.52	-1.01	-0.34	-0.36
Std error (Z)	0.22	0.38	0.22	0.27
CV(Z - R output table)	0.43	0.37	0.65	0.73
Weighted egg production (eggs/0.05 m^2 day^{-1})	**3.83**	**6.66**	**3.41**	**3.81**
CV - weighted value (%)	29.2	32.1	45.2	50.3
Total egg production (positive area * 10^{12})	**1.20**	**2.08**	**0.88**	**0.98**
Model 2: GLM - negative binomial distribution				
Intercept (P_0)	4.29	4.56	4.92	4.79
Std Error of P_0	0.11	0.12	0.14	0.16
Age (Z)	-0.37	-0.53	-0.50	-0.39
Std error (Z)	0.05	0.06	0.05	0.07
CV(Z - R output table)	0.24	0.20	0.34	0.57
AIC	2,640	2,272	1,270	1,085
Weighted egg production (eggs/0.05 m^2 day^{-1})	**3.08**	**4.04**	**4.60**	**4.04**
CV - weighted value (%)	11.9	13.7	17.0	19.2
Total egg production (positive area * 10^{12})	**0.96**	**1.26**	**1.19**	**1.05**

Egg abundances plotted against their respective ages (days) for 2005 and 2006 (extreme data omitted) followed the typical exponential decay model described for eggs of small pelagic fishes, with fitted mortality curves gradually sloping down from young to old cohorts (Fig. 3.18).

Four model diagnostic plots were obtained for each run carried out to estimate mean P_0 using GLM. The plot of residuals vs. predicted values that excluded extreme egg cohorts showed randomly scattered values with a slight negative trend. In addition, the standard deviance residuals vs. theoretical quantiles plot showed a reasonably linear plot with some degree of under-dispersion both in 2005 (dispersion parameter = 0.2925; AIC = 2,272) and 2006 (dispersion parameter =0.0884; AIC =1,085) (Table 3.5), i.e. less variability in the data than that predicted by the model (Appendix 6).

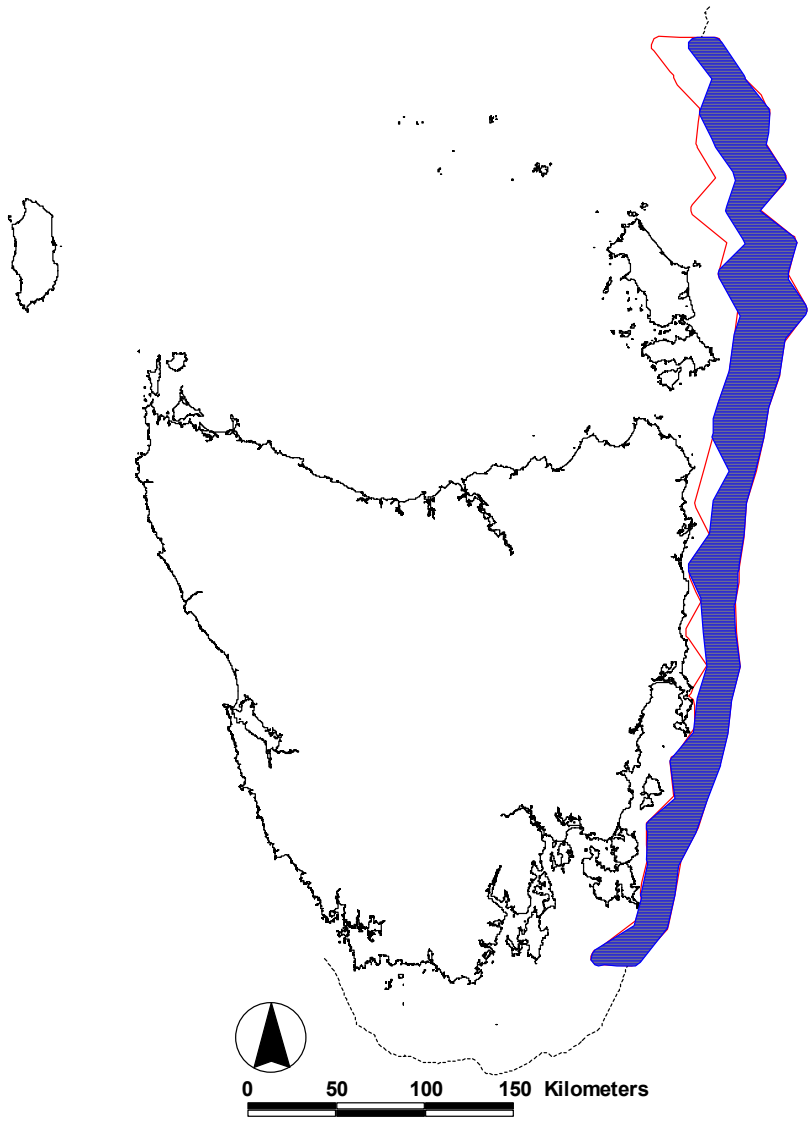

Fig. 3.16 Total area sampled for redbait eggs and larvae during the October 2005 survey (red outline – 15,650 km^2). Estimated spawning area (13,220 km^2) is indicated by the shaded blue area.

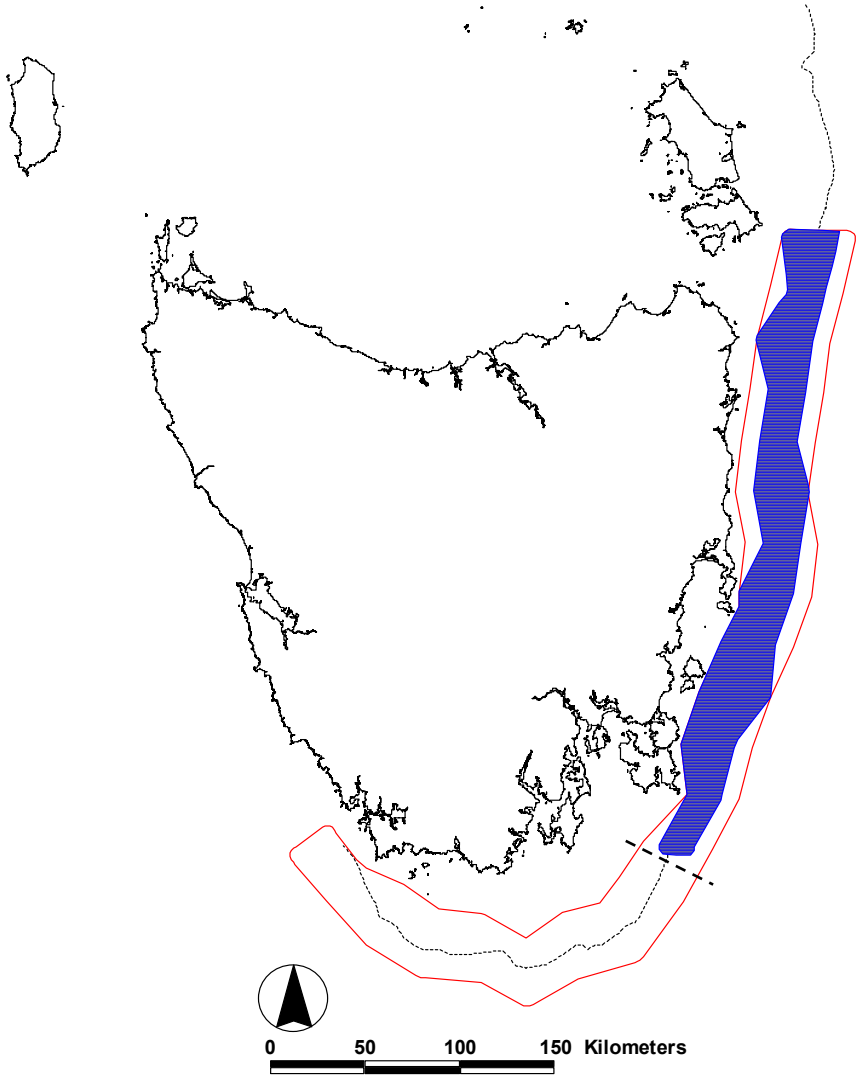

Fig. 3.17 Total area sampled for redbait eggs and larvae during the October 2006 survey (red outline – 21,351 km^2). Estimated spawning area (8,695 km^2) within eastern Tasmania (12,924 km^2; dashed line shows southern limit) is indicated by the shaded blue area.

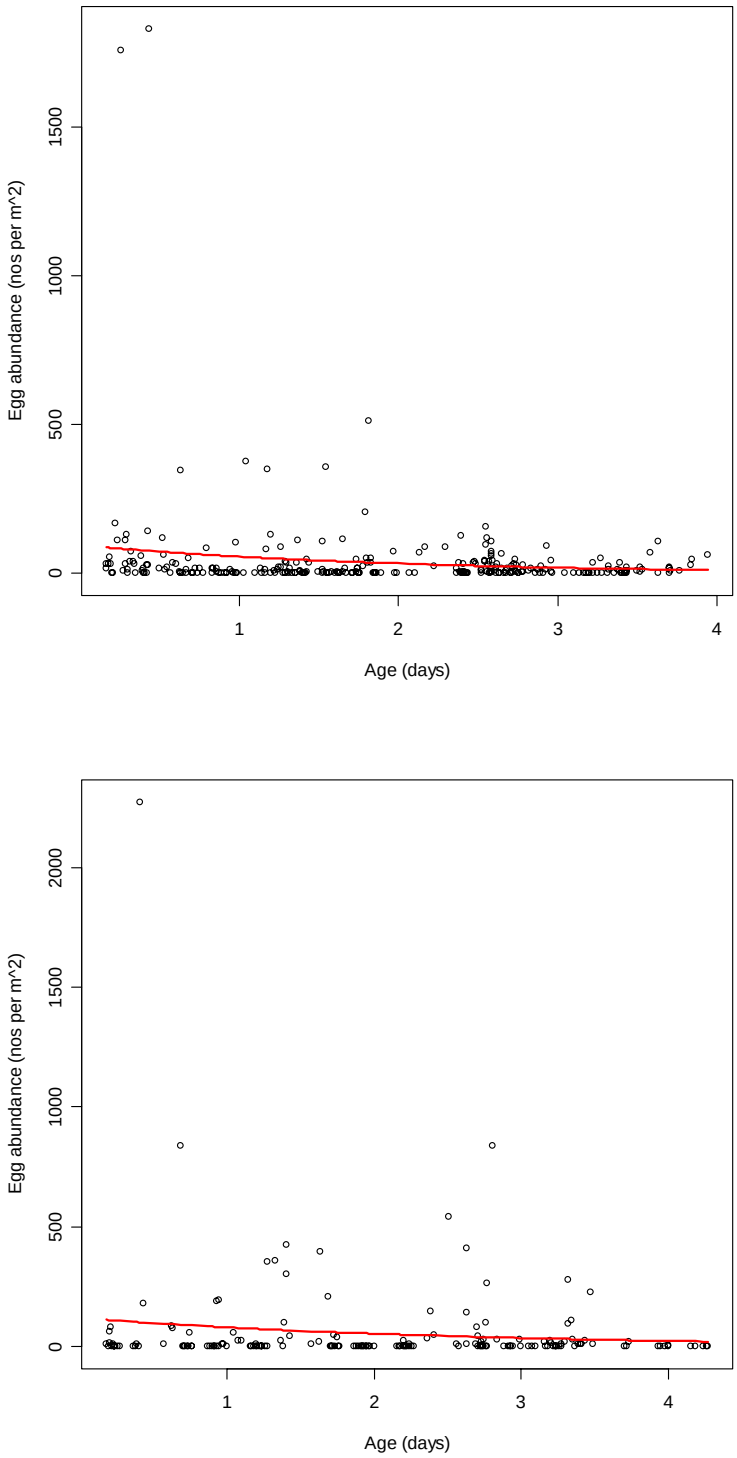

Fig. 3.18 Redbait egg mortality model for the 2005 (top) and 2006 (bottom) surveys off north-eastern to south-western Tasmania (extreme data omitted). Curve in each plot corresponds to the superimposed fitted mortality curve derived from GLM with negative binomial error distribution

3.4 DISCUSSION

3.4.1 Spawning season

This study constitutes the first descriptive account of the distribution and abundance of eggs and larvae of redbait in temperate Australia. Moreover, as far as we know it also constitutes the first and most comprehensive for a representative of the Emmelichthyidae worldwide, a fact which precludes detailed comparisons with other emmelichthyids in terms of spawning season and habitat.

Ichthyoplankton surveys undertaken during this study indicate that redbait in south-eastern Australia spawns predominantly during October. This finding is consistent with gonad development in fish off Tasmania that indicates spawning occurs mainly between September and November (Chapter 1). Furthermore, the timing also matches the presence of redbait eggs and larvae off southern NSW as far north as 35.0^{o}S (Jervis Bay), implying that spawning may occur more or less simultaneously throughout south-eastern Australia during spring. However, it is also possible that some spawning could extend well into February/March, based on records of preflexion redbait larvae caught between November and March along waters of northern Bass Strait, and off eastern Tasmania as far south as 42.7^{o}S (Chapter 2; Neira *et al*., 2008).

The temporally discrete spring spawning period of redbait coincides with the outburst of phytoplankton biomass, and hence abundant larval food, known to occur on average from mid-September to the end of November along eastern Tasmania (Harris *et al*., 1987). While no chlorophyll or productivity data were obtained during this study, the October SST images coupled with the CTD-derived vertical temperature data showed conditions that were consistent with those typically found during the spring productivity outburst, i.e. the shelf region bathed by warm, high salinity EAC-derived water mixed with cooler, fresh subantarctic water (Harris *et al*., 1987; Ridgway, 2007a). Furthermore, this nutrient-rich subantarctic water often remains well-mixed to about 300 m until the onset of thermal stratification in late spring/early summer, which denotes the end of the productivity outburst (Harris *et al*., 1987, 1992). In our surveys, a breakdown in the vertical thermal structure inshore was evident at least as far south as 43.5^{o}S (T29) in 2005, whereas signs of thermal stratification were evident at the same latitude (T13) in 2006. Since egg abundances declined significantly southwards of 43.5^{o}S and around southern Tasmania in 2006, it is likely that the spatial extent of spawning of redbait may be linked to some degree to the duration of the spring productivity outburst.

3.4.2 Extent of spawning area

Data on spatial distribution and abundance of eggs and larvae from this study indicate that redbait in south-eastern Australia spawns between 35.0^{o}S off central NSW and 43.5^{o}S off the lower south-east coast of Tasmania. This conclusion is based on three

main findings from this study, namely (1) eggs and larvae first appeared as far north as 35.0°S in October 2002 and 2003, occurring through to the southern limit of the survey area at 37.5°S; (2) a northern spawning boundary was not detected in the 2005 and 2005 surveys off Tasmania; and (3) there was a sharp decline in spawning activity south and westwards of 43.5°S in 2006, noting that day-1 eggs had occurred at the southern-most sampling location (T29) in 2005.

Based on oceanographic data, and quotient analyses on abundances of day-1 eggs, a likely explanation for a southern spawning area limit in the vicinity of 43.5°S would be a temperature front defined by the interface between EAC-derived water and subantarctic waters which are known to be present off that region at that time (Harris *et al*., 1987; Ridgway, 2007a). Such a boundary would thus be consistent with the fact that >96% of eggs in 2006 came from waters of 12.0-14.5°C compared to the significantly lower egg abundances from waters <12.0°C off southern Tasmania. The observation that the region off 43.5°S may represent the southern limit of redbait spawning off eastern Tasmania warrants further investigation, particularly since this region is where the western boundary, poleward Zeehan Current (ZC) meets the EAC, forming a well-defined front which is present between June and September (Ridgway, 2007a,b). Moreover, the extent to which this front is linked to the spring productivity outburst off eastern Tasmania remains to be assessed, noting that productivity associated with the ZC off the west coast of Tasmania is markedly lower than off the east coast (Harris *et al*., 1987).

3.4.3 Preferred spawning habitat

The spatial distribution of eggs and larvae indicates that redbait spawns primarily along a narrow 2.5 nm corridor either side of the continental shelf break, where seafloor depths are mostly 125-325 m. This observation is based on the significantly higher average egg abundances, including day-1 eggs, from stations close to and at the shelf break compared to catches from stations >5 nm either side of the break. The finding of the shelf break as preferred spawning region also matches echosounder-based observations of redbait aggregations in the latter region during spawning time (J.M. Lyle, *personal observation*). In this context, it is perhaps relevant that the shelf-break region off eastern Tasmania has also been described as a key spawning area for *Trachurus declivis* (Carangidae), although this pelagic species spawns predominantly during summer (Jordan *et al*., 1995).

Most redbait larvae caught during 2005 and 2006 were at the preflexion stage, i.e. close to newly-hatched. Unlike eggs, however, larvae were more evenly dispersed across the shelf, with abundances showing no significant differences among shoreward, shelf break and offshore areas. This finding implies the existence of dispersal mechanisms (e.g. wind-driven surface currents) that may affect late-stage eggs (>4 days old) as well as young larvae to a greater extent than early-stage eggs. Such dispersal scenario, moreover, would make sense if spawning occurs deep in the water column (e.g. shelf break), with eggs becoming increasingly buoyant with development. While no evidence of egg transport could be detected with the available data, the fact that >80%

of the larvae in 2005 came from the southern half of the survey area, i.e. further south of where the greatest abundances of eggs were obtained, suggests some level of southwards advection consistent with the prevailing south-bound surface flow present over the shelf region at that time. Since a similar south-bound shelf flow was present along the shelf in October 2006, it is likewise plausible that a proportion of the redbait larvae caught along the northern half of the survey area at that time may have originated from eggs spawned and hatched further north and subsequently advected southwards.

3.4.4 Egg production

Egg mortality curves obtained for redbait followed the typical exponential decay model described for eggs of other small pelagic fishes (e.g. Hewitt, 1985; Lo & Macewicz, 2004; Stratoudakis *et al*., 2006). Estimated mean daily egg production at time zero (P_0) using NLS and GLM approaches showed overall consistency in magnitude within each year and for the two data scenarios tested, apart from the high estimate obtained in 2005 from the NLS model with extreme data excluded. Overall, however, the GLM provided better fits to the data, resulting in substantially lower CVs when compared with the NLS approach. The current trend in the application of the DEPM is to depart from the traditional NLS model and employ GLM techniques since they better describe egg abundance data of small pelagic fishes (Stratoudakis *et al*., 2006; Claramunt *et al*., 2007; Cubillos *et al*., 2007). In this context, AICs for the data scenarios examined under the GLM approach indicated that exclusion of extreme egg cohorts, i.e. eggs assigned ages $\leq$4 hours and $\geq$98% of incubation time, produced a more parsimonious result. Such an approach is currently applied to anchovy and sardine in Chile (Claramunt *et al*., 2007; Cubillos *et al*., 2007), while excluding extreme egg data follows standard practices adopted to DEPM analysis of these clupeoids in California (Hunter and Lo, 1997), Europe (ICES, 2004) and Chile (Claramunt *et al*., 2007).

Daily egg production estimates for eastern Tasmania based on the GLM excluding extreme egg cohorts were essentially the same in 2005 and 2006, i.e. 4.04 eggs/0.05m^2 day^{-1}. Consequently, the larger spawning area surveyed in 2005 resulted in total egg production being greater in 2005 than in 2006 (1.26 compared with 1.05 eggs x 10^{12}). Consistency in daily egg production is indicative of overall similarity in spawning effort within the areas surveyed, a phenomenon that may have been influenced to some extent by the underlying similarity in environmental conditions for each of the two years. For example, average mid-water temperatures along the shelf break over the 2005 and 2006 spawning areas were 13.4 and 13.6^{o}C, respectively, while surface temperatures in both areas remained at around 13.7^{o}C.

Mean daily egg production levels for redbait lie within the range of those reported for other small pelagic species such as anchovy in Bay of Biscay, mackerel from Japan, and sardine from California and South Australia, i.e. 0.7-5.0 eggs/0.05m^2 day^{-1}. However, they fall below values typically recorded for anchovies in California, Chile, Peru and South Africa, i.e. 5.0-20.0 eggs/0.05m^2 day^{-1} (Fig. 3.19; refer to references under figure caption).

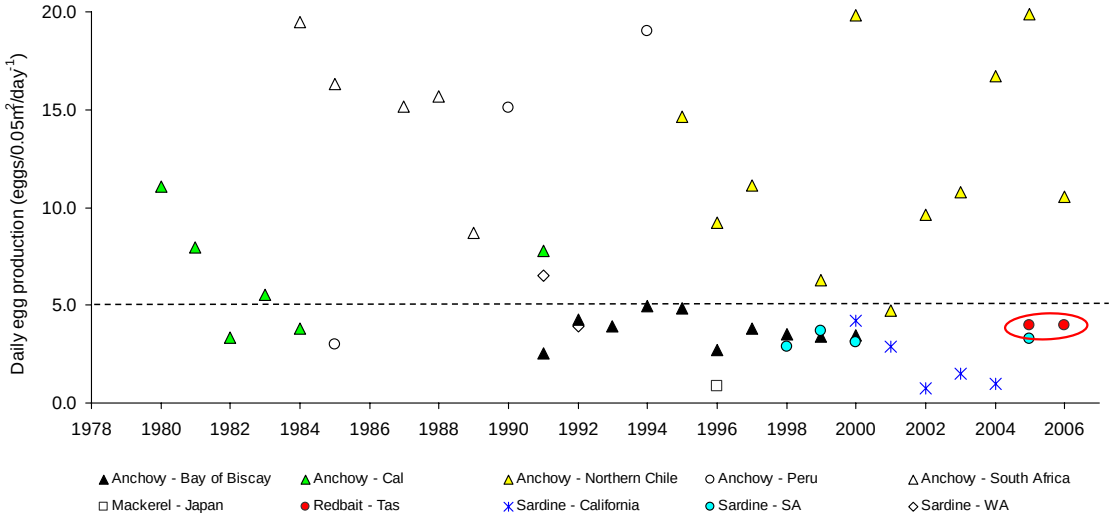

Fig. 3.19 Comparison of mean daily egg production (eggs/0.05m^2 day^{-1}) across selected pelagic fish taxa from different localities in different years. Dashed line draw at the 5 level is for reference; red oval points to redbait. References: Anchovy Bay of Biscay – ICES (2004); anchovy California, Peru and South Africa – Somarakis *et al*. (2004); 1991 anchovy California – Otero-Altamirano & Green-Ruiz (1997); anchovy Northern Chile – Claramunt *et al*. (2007); mackerel Japan – Watanabe *et al*. (1999); sardine California – Lo & Macewicz (2004); sardine South Australia – Ward & McLeay (1998, 1999), Ward *et al*. (2000), Ward & Rogers (2005); sardine Western Australia – Fletcher *et al*. (1996).

3.4.5 Implications for DEPM

Overall findings of this study indicate that redbait is a suitable species for the application of the DEPM (Stratoudakis *et al*., 2006). Besides fulfilling essential criteria in terms of adult reproductive biology (Chapter 1), redbait have a temporally-discrete spawning period, with spawning occurring along a well-defined area along the continental shelf break. In addition, the pelagic eggs (1.00-1.05 mm) are easily collected and identified, and can be staged and subsequently assigned ages using an existing temperature-dependent incubation model developed during this study (Chapter 2; Neira *et al*., 2008). Furthermore, mortality of egg cohorts follows the typical exponential decay curve described for eggs of other small pelagic fishes, implying that eggs of different ages are being sampled with similar probabilities of occurrence. At present, the application of GLM appears to be a suitable analytical approach to estimate daily egg production, however, techniques such as general additive models (GAMs) should also be tested to ascertain whether precision in P_0 estimates is improved (ICES, 2004; Castro *et al*., 2005; Stratoudakis *et al*., 2006). Additional ways to improve precision of P_0 estimates should also be investigated, including adaptive sampling, and use of yolk-sac larvae to anchor the upper end of the egg mortality curve and enable the slope to tilt (Hunter and Lo, 1997).

CHAPTER 4: SPAWNING BIOMASS ESTIMATES FOR REDBAIT OFF EASTERN TASMANIA

F.J. Neira and J.M. Lyle

Objective 4: To evaluate the use of the daily egg production method for estimating the spawning biomass of redbait on the east coast of Tasmania.

Objective 5: To produce a minimum biomass estimate of redbait in Zone A of the Small Pelagic Fishery.

Spawning biomass of redbait was estimated using the daily egg production method (DEPM) from egg and adult reproductive data collected off eastern Tasmania during October 2005 and 2006. Main spawning areas within the surveyed area off the east coast of Tasmania were identified between north-eastern Bass Strait (38.8°S) and south of the Tasman Peninsula (43.5°S) in 2005 (13,220 km^2), and between Cape Barren Is. (40.5°S) and the same southern boundary in 2006 (8,695 km^2). Mean daily egg production (P_0) was estimated using non-linear regression and a generalised linear model and was based on two data scenarios. Overall, the GLM that omitted eggs with assigned ages $\leq$4 hours and $\geq$98% of incubation time provided the best fit and was adopted as the preferred model. Mean P_0 (eggs/0.05m^2 day^{-1}) was estimated to be 4.04 in both 2005 (CV=0.14) and 2006 (CV=0.19). Estimates (CVs) of 2005 and 2006 adult parameters were, respectively: sex ratio, 0.30 (0.19) and 0.44 (0.05); average female weight (g), 71.7 (0.20) and 78.3 (0.06); average batch fecundity, 10,894 (0.24) and 11,441 (0.09); and average spawning fraction (day^{-1}) was 0.32 in both years (0.23, 0.14). Biomass estimates (CVs) within respective spawning areas were 86,990 t (0.37) in 2005 and 50,782 t (0.21) in 2006. These almost certainly represent minimum estimates of spawning biomass since redbait spawning is likely to have extended over much larger area than that surveyed. Such observation is strongly supported by the relatively high abundance of eggs at the northern survey boundaries in both years, coupled with the presence of redbait eggs as far north as Jervis Bay in southern NSW.

Our findings are discussed in relation to the harvest strategy developed for the Small Pelagic Fishery. With the move towards stock-based management and recognition that recommended biological catch levels for small pelagic species need to be conservative, our spawning biomass estimates provide a scientifically defensible basis for decision making in relation to sustainable catch levels for redbait off south-eastern Australia.

4.1 INTRODUCTION

The daily egg production method (DEPM) is becoming increasingly important worldwide as a fishery-independent technique used to estimate biomass of pelagic, multiple (batch) spawning fishes with indeterminate fecundity, such as clupeoids and scombrids. The method combines mean daily egg production at spawning time (P_0) over a spawning area (A) and four adult parameters, namely mean female weight (W), batch fecundity (F), catch sex ratio (R) and spawning fraction (S). Parameters P_0 and A derive from intensive egg surveys over the assumed spawning area, while the other parameters are estimated from adults sampled concurrently with plankton surveys in the same area (Lasker, 1985; Picquelle and Stauffer, 1985; Priede and Watson, 1993; Lo *et al*., 1996; Watanabe *et al*., 1999; Stratoudakis *et al*., 2006).

Besides clupieods, scombrids and their like, the DEPM has not been tested to estimate spawning biomass in a species of the family Emmelichthyidae (Stratoudakis *et al*., 2006). In this chapter we provide DEPM-based spawning biomass estimates of redbait in eastern Tasmania, based on egg and adult parameters derived from intensive egg and adult surveys conducted during peak spawning time of the species in October 2005 and 2006 (Chapters 1-3). Redbait eggs have been positively identified and staged, and a temperature-dependent incubation model developed to assign ages to field-collected eggs based on stages and water temperatures from positive stations (Chapter 3). These data were then employed to compute P_0 and daily instantaneous mortality (Z) using an exponential decay model based on daily egg abundance-at-age data (Chapter 3). The P_0 and Z parameters were estimated by fitting a least squares non-linear regression (NLS) model and a generalized linear model (GLM) assuming a negative binomial error distribution error to the egg abundance-at-age data (Chapter 3). Parameter A was calculated for each egg survey from positive stations (Chapter 3), while parameters W, F, R and S came from extensive adult redbait collections (Chapter 1). Comparisons are made between biomass outputs derived from the two models under two data scenarios. Overall model results are subsequently discussed in relation to the suitability of applying the DEPM to estimate spawning biomass of redbait and in terms of the application of the harvest strategy developed for the Small Pelagic Fishery (SPF).

4.2 MATERIALS AND METHODS

4.2.1 Adult parameters

Details on how redbait adult parameters were estimated are provided in Chapter 1 of this report. Estimates and respective CVs are provided in Table 4.1. Average weight of mature redbait females (*W*) from eastern Tasmania were 71.7 g in 2005 (CV=0.20) and 78.3 g in 2006 (CV=0.06). Average batch fecundity of spawning redbait (*F*) was 10,894 oocytes for 2005 (CV=0.24) and 11,441 oocytes for 2006 (CV=0.09). Mean fraction of mature female by weight (sex ratio, *R*) was estimated as 0.30 for 2005 (CV=0.19) and 0.44 for 2006 (CV=0.05).

Average spawning fraction (*S*) was 0.32 in both 2005 (CV=0.23) and 2006 (CV=0.14). This parameter was estimated based on the presence of hydrated oocytes and/or fresh POFs, criteria which were deemed to appropriately reflect the parameter given the available data.

4.2.2 Spawning area

Procedures to estimate redbait spawning areas are provided in Chapter 3. The 2005 survey covered a total area of ~ 15,650 km^2 between north-eastern Bass Strait and south of the Tasman Peninsula. Of this, 13,220 km^2 was regarded as spawning area (84.4%). The 2006 survey covered a total area of ~ 21,351 km^2. However, examination of redbait egg abundances prompted the survey area to be divided into eastern and southern Tasmania at 43.5^{o}S, with eggs within the former area contributing 96.2% of the total caught during that survey (Chapter 3). Consequently, the area off eastern Tasmania between Cape Barren Is. and Tasman Peninsula was considered as the 2006 DEPM survey area (12,924 km^2), and within which the main spawning area was estimated in 8,695 km^2 (67.3%).

4.2.3 Mean daily egg production

Estimates of mean daily egg production (P_0) of redbait at spawning time (eggs/0.05m^2 day^{-1}), as well as total egg production per spawning area off eastern Tasmania in October 2005 and 2006, are provided in Table 4.1 together with individual CVs (refer to Chapter 3 for details on estimate procedures). The *R* package *Ichthyoanalysis* (www.r-project.org; http://sourceforge.net/projects/ichthyoanalysis) was employed to fit a least squares non-linear regression (NLS) model (Lo, 1996) and a generalized linear model (GLM) assuming a negative binomial error distribution (ICES, 2004; Cubillos *et al*., 2007) to estimate P_0. Weighted mean P_0 and *Z* estimates for 2005 and 2006, as well as biomass estimates (see section 4.2.4), are provided for two data scenarios, namely (i) all daily egg abundance-at-age data, and (ii) data omitting extreme egg cohorts, i.e. eggs aged $\leq$4 hours and $\geq$98% of incubation time (Chapter 3).

4.2.4 Spawning biomass model

Spawning biomass (*B*, tonnes) was estimated by the following equation (Parker, 1985):

$$B = \frac{P_0 \; A \; W}{R \; F \; S}$$

where P_0 is egg production at time zero per unit of area per day (eggs/0.05m^2 day^{-1}); *A* spawning area (km^2); *W* mean weight of mature females in the population (g); *F* batch fecundity (number of oocytes released per mature female per batch; eggs/batch); *R* fraction of mature females by weight (sex ratio); and *S* spawning fraction (proportion of mature females spawning each day).

The approximate variance of each spawning biomass estimate was computed using the following equation modified from Parker (1985):

$$Var(B) = B^2 * (VarP_0/P_0^2 + VarW/W^2 + VarR/R^2 + VarF/F^2 + VarS/S^2 + 2\{COVS\})$$

Where *COVS* corresponds to the sum of terms involving the covariance (*Cov*) of each adult parameter, in this case:

$$COVS = \{-Cov(WR)/WR - Cov(FW)/FW - Cov(WS)/WS + Cov(RF)/RF + Cov(RS)/RS + Cov(FS)/FS\}$$

The estimated variance was then employed to calculate standard deviation (SDev) for each biomass estimate (Table 4.1).

4.3 RESULTS

Redbait spawning biomass estimates computed using daily egg production estimates derived from NLS and GLM model fits varied between 66,000 - 143,000 t in 2005 and 43,000 - 58,000 t in 2006 (Table 4.1). The 2005 NLS-based estimates were 25-65% higher than GLM-based estimates depending on data scenario. By contrast, 2006 estimates were more similar in magnitude between models and data scenarios, although the GLM-based estimates tended to be slightly higher. Regardless of year or data scenario, coefficients of variation (CVs) were substantially greater for estimates based on NLS-fitted data compared with GLM-fitted data, i.e. 0.45-0.51 cf. 0.20-0.37, indicating that the latter provided an overall better fit to the egg data.

Table 4.1. Input parameters and spawning biomass estimates for redbait in 2005 and 2006. Data provided for adult parameters *W*, *F*, *R* and *S* comprise means (CVs) and variances; criterion employed to estimate mean spawning fraction (*S*) was hydrated oocytes and/or fresh POFs (refer to Chapter 1). Mean daily egg production (P_0) and total egg production, as well as spawning biomass estimates (tonnes) and respective CVs (in brackets) for positive spawning areas are provided for two statistical models and two data scenarios; no extremes refers to the exclusion of extreme egg cohorts, i.e. eggs aged ≤4 hours and ≥98% of incubation time (refer to Chapter 3, and section 4.2 for details).

Input data	**October 2005**		**October 2006**	
DEPM survey area/spawning area (km^2)	15,650 / 13,220		12,924 / 8,695	
Mean weight female (*W*)	71.7 (0.20)		78.3 (0.06)	
Variance *W*	196.2		22.3	
Mean fecundity (*F*)	10,894 (0.24)		11,441 (0.09)	
Variance *F*	7,084,349		1,004,943	
Sex ratio (*R*)	0.30 (0.19)		0.44 (0.05)	
Variance *R*	0.0034		0.001	
Spawning fraction (*S*)	0.32 (0.23)		0.32 (0.14)	
Variance *S*	0.005		0.002	
Data scenario	All data	No extremes	All data	No extremes
Model 1: ***Non-linear least squares***				
Weighted P_0 (eggs/0.05 m^2 day^{-1})	3.83	6.66	3.41	3.81
Total egg production (eggs x 10^{12})	1.20	2.08	0.88	0.98
Spawning biomass (tonnes) (CV)	**82,648** (0.45)	**143,434** (0.47)	**42,802** (0.47)	**47,792** (0.51)
Standard deviation	37,624	67,971	19 810	24,505
Model 2: ***GLM - negative binomial error distribution***				
Weighted P_0 (eggs/0.05 m^2 day^{-1})	3.08	4.04	4.60	4.04
Total egg production (eggs x 10^{12})	0.96	1.26	1.19	1.05
Spawning biomass (tonnes) (CV)	**66,276** (0.37)	**86,990** (0.37)	**57,808** (0.20)	**50,782** (0.21)
Standard deviation	24,439	32,592	11,331	10,939

4.4 DISCUSSION

4.4.1 Spawning biomass

Preliminary spawning biomass estimates obtained for redbait off eastern Tasmania varied between years, models applied and data scenarios tested, ranging between about 43,000 and 143,000 t. In terms of model suitability, the GLM with negative binomial error distribution has been shown to provide a better fit to egg abundance-at-age data than the traditional NLS model for pelagic, batch spawning fishes such as anchovy (Hewitt, 1985; Claramunt *et al*., 2007). In addition, the GLM accounts for variability of egg production at early ages better than the NLS model, resulting in P_0 values with lower variances (Claramunt *et al*., 2007; Cubillos *et al*., 2007). This is particularly relevant since Montecarlo simulations have indicated that nearly 80% of the variance in estimated biomass is associated with the variance of P_0 (G. Claramunt, Arturo Prat University, Chile, *pers. comm*.). Our findings are generally consistent with this observation, with substantially improved CVs for GLM-based P_0 estimates and a consequent marked reduction in CVs associated with biomass estimates.

The 2006 GLM-based biomass estimates were generally insensitive to the inclusion or removal of extreme egg cohorts (51,000 – 58,000 t). By contrast, the 2005 estimate excluding extreme egg cohorts (87,000 t) was about 31% higher than that when all egg data were included (66,000 t). However, although there were large CVs associated with these estimates (0.37), this difference was not statistically significant. These results indicate that, in certain situations, daily egg production and subsequent biomass estimates may be very sensitive to abundances of very young and old age classes of eggs. In the context of identifying the preferred data scenario, it has been recommended that eggs aged $\leq$4 hours be omitted given potential biases arising from encountering highly concentrated patches of newly-spawned eggs compared to older eggs (Hunter and Lo, 1997; ICES, 2004; Claramunt *et al*., 2007; Cubillos *et al*., 2007). Likewise, recent application of DEPM to anchovy excludes eggs with ages $\geq$95-98% of hatching age for each station to reduce biases (Claramunt *et al*., 2007). Thus, based on our findings and recommendations of other studies, we conclude that biomass estimates computed from GLM-derived P_0 runs that exclude extreme egg cohorts are appropriate for redbait.

Spawning biomass estimates derived from the preferred model differed by 71% between years, i.e. ~ 87,000 t in 2005 and ~ 51,000 t in 2006. The higher 2005 estimate was expected given the influence of the increased spawning area surveyed in that year (52% larger than in 2006), coupled with consistency in the level of daily egg production between years. Besides differences in spawning area, the lower female sex ratio in 2005 compared with 2006 (0.30 cf 0.44) was also a contributing factor to the higher 2005 estimate. Notwithstanding this, it is noteworthy that the CV associated with the 2005 estimate (0.37) was substantially greater than that for 2006 (0.21) and as a consequence estimates were not statistically different.

DEPM-based spawning biomass estimates generally have CVs >30% and, as such, are considered rather imprecise though potentially unbiased (Stratoudakis *et al*., 2006). Since the CVs associated with daily egg production in 2005 (0.14) and 2006 (0.19) were relatively low and not substantially different, the higher CV associated with the 2005 biomass was clearly influenced by the comparatively higher variances associated with each of the adult parameters in 2005 (Chapter 1). This highlights the need to ensure that adult sampling is robust and comprehensive and presents a challenge for further assessments involving redbait, noting that commercial-scale mid-water trawls appear to represent the only feasible capture method.

4.4.2 Spawning stocks

Overall, the biomass estimates are considered conservative given that they were derived from areas likely to cover less than half of the actual redbait spawning area in south-eastern Australia. Supporting evidence for a significantly larger spawning area, and hence a larger spawning stock, comes from the finding of redbait eggs and larvae along shelf waters off southern NSW (Jervis Bay to Cape Howe) in October 2002 and 2003, and the fact that spawning boundaries could not be identified either to the south of this NSW area (refer to Appendix 7), nor to the north of the areas surveyed off eastern Tasmania in 2005 and 2006 (Chapter 3).

The above findings, combined with adult reproductive data (Chapter 1) and distribution of redbait eggs and larvae (Chapter 3), point to two scenarios which call for further investigation to support current stock management strategies in place for redbait in south-eastern Australia. These are (a) discrete eastern and western stocks that split to the south of Tasmania; or (b) one continuous stock distributed along the outer continental shelf from at least north-western Tasmania around southern Tasmania and up to mid southern NSW. At present, our egg and larval data support the two-stock scenario, based mainly on the abrupt decline in egg and larval counts south and westwards from the Tasman Peninsula in 2006 (Chapter 3). This drop in spawning activity was likely to be associated to the lower average water temperatures around southern Tasmania (~11.7^{o}C) compared to those where the bulk of spawning activity occurred off mid-eastern Tasmania (~13.6^{o}C). Additional supporting evidence for a discrete eastern redbait stock is the fact that reproductively active females trawled from the east coast differed substantially from those from the south-west coast of Tasmania in terms of size, average GSI, and size at maturity (Chapter 1). The possibility of a separate eastern redbait stock was raised by Bulman *et al*. (2008) in a review of management zones from stock structure of small pelagic species in southern Australia.

Although very few eggs and larvae of redbait originated from the lower south-west coast in 2006 (Chapter 3), their occurrence neither support nor refute the existence of a separate western spawning stock. In the case of a two-stock scenario, both stocks may continue to display some low-intensity shelf spawning activity off southern Tasmania during October through to November, as our 2006 data seem to suggest. In the case of a single-stock scenario, on the other hand, spawning activity off southern Tasmania could be delayed until the onset of suitable habitat requirements, e.g. higher temperatures resulting from the southwards EAC inflow into the region during summer (Ridgway,

2007a). However, given the abrupt decline in eggs and larvae south of the Tasman Peninsula (Chapter 3), coupled with the discrete spring spawning season derived from the adult reproductive data (Chapter 1), this scenario seems unlikely.

4.4.3 Implications for management

The Commonwealth's Fisheries Harvest Strategy Policy and Guidelines were released in late 2007 as a response to a Ministerial Direction[1] calling for the Australian Fisheries Management Authority (AFMA) to take a more science based approach to setting total allowable catches. In accordance with this policy the Small Pelagic Fishery Resource Assessment Group (SPFRAG) developed a harvest strategy for the fishery (AFMA, 2008), with a guiding principle being that exploited stocks are managed for long-term biological sustainability and economic profitability.

The SPF harvest strategy is based on a tiered approach that has been implemented to accommodate fishery expansion, recognising that the fishery is still in an early developmental phase. Underpinning this approach is the need to balance risk with knowledge by establishing exploitation rates that are initially conservative but which can be increased as information becomes available. The strategy explicitly allows the level of investment in research and types of assessment to be varied to match commercial interest in exploiting the resource.

The harvest strategy has been developed around three tiers; namely Tier 3, applying to stocks for which little is known (low information requirement and low harvest levels applied); Tier 2, where fishery and biological information are available (moderate information requirements and conservative harvest rates); and Tier 1, where quantitative biomass assessments are available (relatively high information requirements, less conservative harvest rates). The DEPM provides fishery-independent estimates of spawning biomass and, as such, stocks assessed by this method meet Tier 1 criteria. In the context of the harvest strategy, the maximum harvest rate to be applied to Tier 1 stocks is 20% of spawning biomass. It was recognised, however, that the relevancy of biomass estimates will degrade over time and, in the absence of up-to-date assessments, an increasingly precautionary approach should be adopted with harvest rates being reduced progressively over time until new information becomes available.

A management strategy evaluation (MSE) of the harvest strategy for the SPF concluded that the 20% maximum harvest rate was well below the level required to meet maximum sustainable yield (MSY) and maximum economic yield (MEY) benchmarks for most SPF species (Knuckey *et al.*, 2008) and thus was overtly conservative. For redbait, however, the comparatively high longevity (refer Appendix 3) and resultant low rate of natural mortality, meant that the 20% harvest rate was closer to that required to achieve MEY targets and less precautionary than for other SPF species. While further refinement of the MSE is required it has raised the issue of whether a more conservative harvest rate approach is necessary for redbait because of the species broad ecological significance.

[1] A Direction issued to AFMA under section 91 of the *Fisheries Administration Act 1991* on 14 December 2005 by the Minister for Fisheries, Forestry and Conservation.

4.4.4 Recommended biological catch levels

The harvest strategy provides the basis for determining Recommended Biological Catch (RBC) and Total Allowable Catch (TAC) levels, where RBCs apply to fish stocks throughout their range and to mortality resulting from all types of fishing and TACs are fishery specific. The AFMA Board recently agreed to manage SPF species as having separate stocks east and west of 146^{o} 30'E, based on a review of likely stock structuring for the key SPF species (Bulman *et al.* 2008).

Within the eastern management zone redbait meets Tier 1 criteria, with two spawning biomass estimates available, and this information has been used by SPFRAG (May 2008) to recommend the 2008/09 RBC for 'eastern zone' redbait. By applying the maximum harvest rate[2] to the spawning biomass averaged over the two years (~ 69,000 t), the RBC was calculated as 13,800 t. However, since our surveys covered only part of the spawning area off south-eastern Australia, it was recognised that the actual eastern zone spawning biomass would be higher than estimated. In order to partially account for this, the provisional spawning biomass estimate for NSW of about 20,500 t (Appendix 7) was taken into consideration. This involved discounting the estimate by 50% in view of the high uncertainties surrounding it, and then applying a harvest rate of 10%[3]. This resulted in an additional 1,000 t which was combined to produce the 2008/09 eastern zone redbait RBC of 14,800 t.

As the primary focus of the present study was eastern Tasmania, little is known about the size of the redbait population in the western management zone, the presumed western zone 'stock'. Significant commercial quantities (up to 4,500 t) have been taken from south-western Tasmania in recent years, along with occasional catches from waters adjacent to South Australia. Under the harvest strategy framework, and in the absence of biomass indicators, the western zone stock was assessed as a Tier 2 species with SPFRAG recommending that the maximum Tier 2 level RBC of 5,000 t be applied for the 2008/09 fishing year. Both eastern and western redbait RBCs have been approved by the AFMA Board and implemented for the 2008/09 fishing year.

In order to further develop and expand the fishery for redbait under the present harvest strategy, it will be necessary to develop a program of fishing and research that will provide biomass estimates/indices for eastern and western zone stocks. Of critical importance to industry will be the balance between costs and benefits, particularly given the trade-offs between operational costs and low product value and the ability to support the costs of research.

[2] According to the harvest strategy the maximum harvest rate of 20% is applied in situations where there have been two robust DEPM assessments within the past three years or three assessments over the past five years.

[3] The harvest strategy specifies that if the DEPM assessment is five years old then the maximum applicable harvest rate is 10%.

BENEFITS

This study has established the efficacy of the DEPM to assess spawning biomass of redbait and, significantly, has produced robust biomass estimates for the stock off eastern Tasmania. These achievements have particular benefit to the future management and development of the SPF, especially at this early stage in the development of the fishery. This study has provided a scientific basis on which to determine recommended biological catch levels for eastern zone redbait. The outputs of this study also have relevance to the development of the SPF harvest strategy.

It is noteworthy that the success of this study was underpinned by the high level of industry support and engagement throughout all aspects of the work, and as such provides a model for this and other developing or established fisheries. The full engagement of industry has enhanced acceptance of findings, including their implications for future catch levels and fishery opportunities. As industry will be expected to assume more of the financial burden of future assessment research, the need to develop partnerships to reduce costs will be especially critical, this study has confirms the credibility and benefits of such partnerships.

FURTHER DEVELOPMENT

Various aspects of this project have identified areas that would benefit from further development. These have been classified under (a) reproductive biology; (b) incubation model; (c) egg surveys; (d) egg production model; and (e) stock delineation.

Reproductive biology

Additional histological analyses need to be undertaken to refine the protocol to age postovulatory follicles (POFs) for redbait. This is required to increase precision of spawning fraction estimates. Regular sampling of spawning females over a 24-hour period from the same area would also enable peak spawning time to be refined.

Ideally, spawning females from different locations across the entire spawning area are needed to augment precision of DEPM-based biomass estimates. Future surveys should plan for a greater number of samples to be collected over the whole survey area.

Incubation model

On board egg rearing trials during this study resulted in a development data set based on a narrow temperature range. Thus, egg rearing experiments conducted in facilities equipped with suitable temperature-controlled incubating rooms and water baths is likely to yield a more extensive egg incubation dataset over a wider range of temperatures. In particular, better controlled rearing would result in additional egg stage data that could be employed to increase the aging accuracy of early-stage eggs, and help

lower uncertainties associated with the current temperature-dependent development model. This would also permit the application of an alternative ageing option that fits a continuation-ratio logit model which considers the data as a multinomial distribution, i.e. models the natural probability of an egg being at a specific stage given certain age and temperature.

Egg surveys

Using information regarding spatial patchiness in egg abundances, it would be worth modelling the impacts of different sampling intensities (i.e. stations per unit area) on data precision. This has significance both for data utility and survey costs, especially if biomass estimates are to be obtained for the entire suspected spawning area of redbait in south-eastern Australia. When possible, future redbait egg surveys could also include adaptive sampling in areas of high egg concentrations to improve precision.

In the event of locating a large patch of spawning females, it is recommended that egg samples by taken every hour from the same site to provide a precise estimate of the spawning time. This information is required by the DEPM to accurately age eggs. Such data would also provide important insights on vertical/horizontal dispersal of eggs if vertically-stratified samples are taken.

Egg production model

General additive models (GAMs) should be tested to ascertain whether precision in daily egg production (P_0) estimates is improved. Furthermore, adaptive egg sampling, and use of yolk-sac larvae to anchor the upper end of the egg mortality curve, should be investigated as ways to improve precision of P_0 estimates (e.g. Lo *et al*., 1996, 2001; Hunter and Lo, 1997; Lo, 2004; Somarakis *et al*., 2004; Claramunt *et al*., 2007).

Stock delineation

Although fish off eastern and south-western Tasmania exhibit some biological differences, redbait stock structure remains a major uncertainty for the assessment and management of the species. Stock delineation is therefore an issue that warrants further research. There is also a need to assess the size and extent of redbait populations to the west of Tasmania. With the exception of the relatively small area off south-western Tasmania, there has been limited fishing activity in that region to date.

PLANNED OUTCOMES

The primary outcome of this project has been the foundation for the sustainable management of redbait early in the development of the fishery. Specifically, this study has demonstrated the suitability of the DEPM to determine spawning biomass of redbait, thereby providing a sound basis for the implementation of a harvest strategy developed around a precautionary approach to exploitation. Underpinning this approach is recognition of the ecological significance of the target species, the state of

knowledge about the stocks and the need for industry to be profitable. Ultimately, whether on-going fishery-independent assessment occurs will be determined by the trade-off between the costs of data collection (industry investment into research) set against the potential risks to the stocks and ecosystem of not having reliable biomass estimates and hence lower catch allocations. This project has provided a firm basis for industry and management to make decisions about the future development of the fishery for redbait.

CONCLUSION

This study represents the most comprehensive biological study ever conducted on redbait, and possibly the only one of such wide scope for a member of the family Emmelichthyidae. Redbait in Tasmania reach sexual maturity between 2 and 4 years of age depending on region, and spawn mostly during the spring months in deep waters (>100 m) along the continental shelf break. Females spawn once every three nights, producing batches of pelagic eggs (1.0-1.1 mm diameter) which hatch into pelagic larvae after a period of 96-84 hours at 13.1-14.4^{o}C, i.e. the temperature range in the spawning area (Chapter 2). Reproductively active females trawled from eastern Tasmania differed substantially from those caught off south-western Tasmania in terms of size, mean GSI, and size and age at sexual maturity, with south-west coast redbait maturing at 4 years of age and at lengths some 100 mm greater than fish from eastern Tasmania (Chapter 1).

Adult redbait reproductive data (Chapter 1), coupled with information on egg and larval distribution off southern NSW and Tasmania (Chapter 3), support the hypothesis that redbait off south-eastern Australia belong to a single spawning stock. Evidence in support of such a discrete eastern redbait stock is based three key aspects: (i) marked differences in size and age at maturity of redbait between eastern and south-western Tasmania; (ii) sharp decline in the abundance of redbait eggs and larvae off southern Tasmania; and (iii) presence of redbait eggs and larvae off southern NSW during the peak spawning period off Tasmania. The current stock management strategy for the SPF assumes separate stocks east and west of Tasmania and is thus broadly consistent with this hypothesis.

Until new evidence becomes available, the occurrence of redbait eggs and larvae off south-western Tasmania cannot unequivocally support or refute the existence of a separate western spawning stock. On the balance of probabilities, however, both these and the adult data from this study (Chapter 1), together with past larval records from western Victoria/eastern South Australia (Chapter 3), point to the presence of a western redbait stock. If this were the case, then a key factor driving a split between eastern and western stocks is probably temperature requirements for spawning. Evidence of such thermal spawning boundary appears to be demonstrated by the sharp drop in redbait spawning activity off southern Tasmania in October, which coincided with the lower water temperatures in that region compared to those where the bulk of spawning occurred along the north-east coast. In the case of a two-stock scenario, both stocks may continue to display some low-intensity spawning activity along the shelf break off

southern Tasmania during and possibly after October. Alternatively, spawning activity of a common redbait stock around southern Tasmania could be delayed until the onset of suitable habitat requirements, such as higher temperatures resulting from the encroachment of EAC water into the region. The likelihood of this occurring would need to be assessed during surveys undertaken after the main spring spawning season.

Overall, the combined findings of this study indicate that redbait is a suitable mid-water species for the application of the DEPM to estimate spawning biomass. Besides fulfilling essential DEPM criteria in terms of adult reproductive attributes (Chapter 1), redbait have a temporally-discrete spawning period along a known shelf area, and the pelagic eggs are easily collected and identified. In addition, eggs can be staged, and subsequently assigned ages using a temperature-dependent incubation model developed during this study (Chapter 2). Furthermore, mortality of egg cohorts follows the typical exponential decay curve described for eggs of small pelagic fishes, implying that eggs of different ages were sampled with similar probabilities of occurrence (Chapter 3). The application of generalised linear modelling appears to be a suitable approach to estimate daily egg production.

Consistency in egg production over the two surveyed years provides confidence in their accuracy and reliability, with resultant biomass estimates (50,000 - 90,000 t) varying mainly on the basis of the actual size of the spawning area surveyed (Chapter 4). However, an important implication of a widely distributed eastern stock is that spawning biomass estimates provided in this study only apply to that proportion of the spawning stock that was present within the surveyed areas off eastern Tasmania and, as such, are conservative. In turn this implies that future ichthyoplankton surveys for DEPM-based biomass estimates would need to be extended northward to include as much of the redbait distribution as possible.

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APPENDIX 1: INTELLECTUAL PROPERTY

This is not applicable to this project.

APPENDIX 2: STAFF

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APPENDIX 3: ESTIMATION OF AGE AND GROWTH OF REDBAIT IN TASMANIAN WATERS

G.P. Ewing and J.M. Lyle

Introduction

Ageing methods have not been validated for any Emmelichthyid species. However, unvalidated ageing of rubyfish (*Plagiogeneion macrolepis*), using thin otolith sections, has produced age estimates in excess of 80 years for fish over 400mm (Paul *et al.*, 2000). Estimates of growth for redbait, derived from scales (Roschin, 1985) or whole otoliths (Williams *et al.*, 1987), suggest that it is rapid in the first years of life. Williams *et al.* (1987) reported that redbait from Tasmanian waters reached a mean FL in excess of 200 mm in the first three years, with growth slowing thereafter. These authors estimated a maximum age of at least 7 years. A maximum age of 10 years old was estimated from otolith analyses of redbait captured in eastern Victorian waters (Furlani *et al.*, 2000) which reached a maximum size of 335mm FL. Redbait have also been reported to grow to 344mm SL of the coast of Chile (Melendez and Cespedes, 1986) and 493mm TL in South African waters (Heemstra and Randall, 1977; Meyer and Smale, 1991). Redbait are observed to school by size class, and also stratify by depth, with larger (>200mm) redbait often found deeper and closer to the seafloor than schools of smaller fish (Markina and Boldyrev, 1980).

The aims of this study were to generate an ageing protocol to derive age estimates for redbait collected off eastern and south-western Tasmania. Reader precision was estimated and validation of the periodicity of increment structure was attempted through size cohorts and marginal increment analysis. Age at the first enumerated zone was estimated through comparison of the size and structure of the otoliths from juveniles with the inner zones of otoliths from adults. Growth was modelled by sex and by region using the von Bertalanffy growth function.

Methods

Sampling and laboratory procedures

Redbait were collected off the east and south-west coasts of Tasmania between January 2003 and November 2006. Fish were sub-sampled from commercial mid-water trawl catches taken by the FV *Ellidi* on the coastal shelf break, generally in depths from 100 to 180m. Random samples of at least 100 redbait per shot were collected and frozen fresh. Fish were later thawed and measured for fork length (FL, ±1mm), total weight and sex. Sagittal otoliths were removed, cleaned and stored dry from a subsample of these fish chosen to ensure that sexes, size classes and regions were well represented in the ageing dataset (Table A1). One otolith from each fish was mounted in a block of polyester casting resin and sectioned with a diamond lapidary saw transversely through the primordium to a thickness of 250 to 300 μm. Sections were mounted on a glass slide with polyester resin.

Table A1. Summary of otolith samples processed by region and year sampled.

Region	Year	N aged	N shots
Eastern Tasmania	2003	165	13
	2004	349	18
	2005	217	30
	2006	70	7
	Total	**801**	**68**
South-western Tasmania	2003	4	1
	2004	178	4
	2005	170	17
	2006	112	5
	Total	**460**	**27**
Grand total		**1,261**	**95**

Reading criteria and precision

A reference library of 100 otolith sections was randomly sampled from the complete collection of otoliths. Initially, two experienced otolith readers, working together, examined each section in the reference library to develop a standardised approach to generating an increment count. Otoliths were viewed with transmitted light under a stereo-microscope at 25X magnification. From this process criteria were derived to identify which increment structure to count, the selection of the first enumerated structure, and interpretation of increment structure in the vicinity of the margin. Subsequent counts of the reference library, using these criteria, were assessed for precision and bias using age bias plots and the index of average percentage error (IAPE) (Beamish and Fournier 1981; Campana, 2001). The criteria were refined and recounts were conducted until an IAPE of less than 10% (and with no obvious bias) was achieved.

Otolith sections were then read using these criteria and the zone count, the width of the margin relative to the width of the last complete increment (ie. wide, medium or narrow), and the diameter from the primordium to the first enumerated zone were recorded for every otolith. A random subsample of 50 otolith sections from the reference library was read after every 200 reads of otoliths from the full otolith collection to monitor drifts in interpretation. Otoliths were rejected if increment structure was optically unclear or confusing, or if the primordium could not be identified. Otoliths were examined with no reference to fish length or date sampled.

Periodicity and timing of increment structure formation
The periodicity and timing of formation of increment structure was validated by analysing temporal trends in material on the otolith growing edge.

Interpretation of inner structure

Fish from the juvenile dataset were "aged" on the basis of size cohort structure and then the visual characteristics and dimensions of their otoliths were used to assign an age to components of the inner structure of otoliths from the sample of larger fish.

Growth modelling

Growth was described by sex and by region using the von Bertalanffy (VB) growth function. Model parameters were estimated by minimizing squared residuals and a likelihood ratio test (LRT) (Kimura 1980) was used to test for differences in growth rates between sexes and regions.

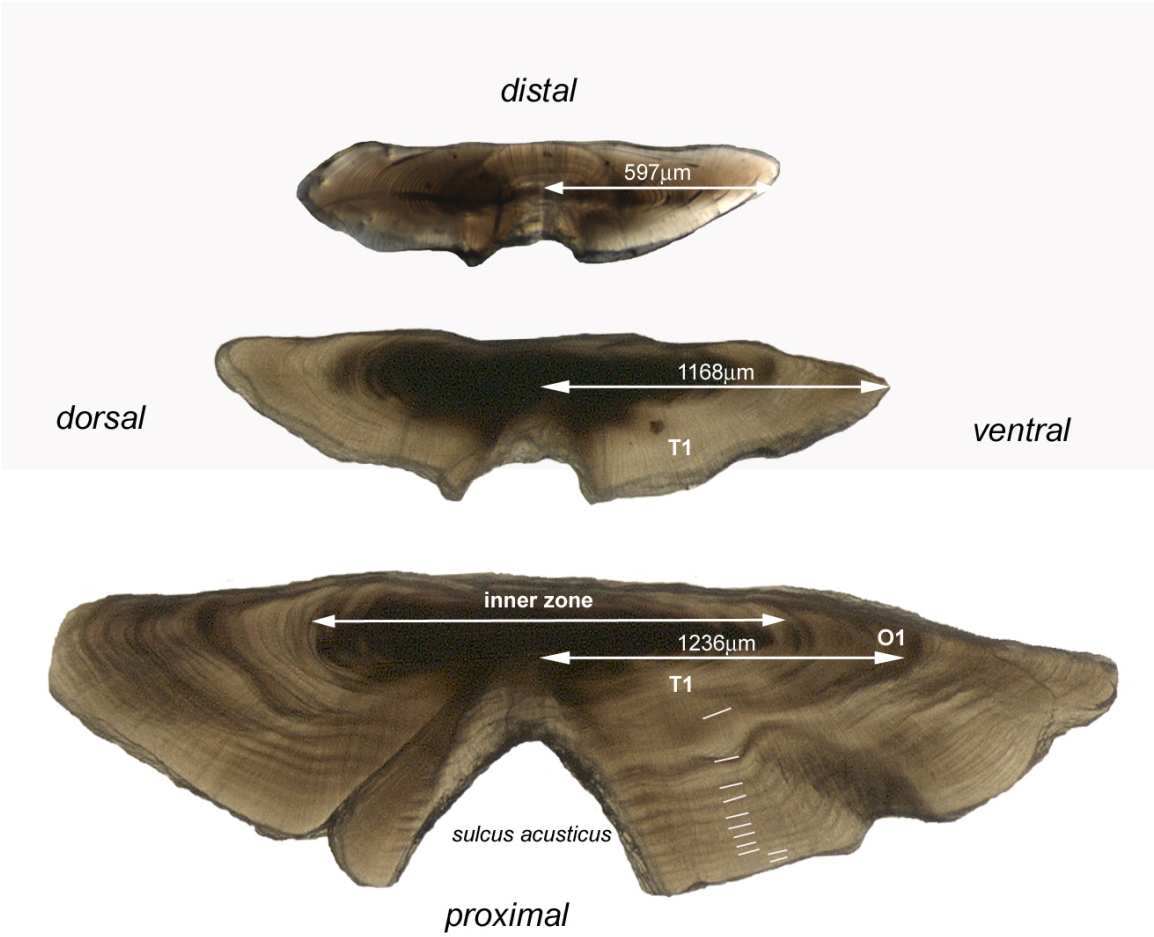

Fig. A1. Photomicrographs, taken using transmitted light, of transverse otolith sections. The top image is a juvenile sampled in January (55mm FL), the middle is a representative of the smallest clear size cohort (110mm FL sampled in June) and at the bottom is an adult redbait (282mm FL sampled in October). T1 refers to the distinctive translucent zone used to assist in the detection of O1 which is the first enumerated zone. The scale on the middle otolith section is the mean radius of otoliths from that size cohort. The scale on the adult otolith section is the mean distance from the primordium to O1. Counted opaque zones are marked and the counts were 0, 0 and 11.

Results and Discussion

Sampling and laboratory procedures

When viewed with transmitted light the transverse, sagittal otolith sections showed alternating bands of translucent and opaque material radiating from an inner zone that was generally uniformly opaque (Fig. A1). Opaque and translucent zones adjacent to the inner zone were broad and decreased in width rapidly out to around the third or fourth increment, after which consecutive increment cycles gradually narrowed in width out to the margin. Every otolith margin displayed a band of optically confusing material of around five microns wide, presumably from diffraction of light through structure damaged by the sectioning process.

Reading criteria and precision

The criterion for identifying zones to count was defined as opaque zones (when viewed under transmitted light), that were continuous through the region ventral and adjacent to the sulcus acusticus (Fig. A1). As opaque material on the margin could not always be discriminated from the optically confusing material on the growing edge, opaque zones on the margin were not counted. Consequently, the last enumerated opaque zone was always followed by a band of translucent material. The criterion for selecting the first enumerated zone (O1) was defined as the first opaque zone immediately exterior to a distinctive wide translucent zone (T1) that was immediately exterior to the inner zone (Fig. A1).

In some otolith sections the inner zone was not uniformly opaque and displayed more complex zonation. This made it more difficult to locate the first enumerated zone despite the consistency of the appearance of T1 across most sections. Fortunately, the radius from the primordium to O1 in the ventral plane was quite stable in otolith sections in which T1 (and hence O1) was easy to identify (mean = 1236μm, N = 1083, SD = 105) (Fig. A1) and could be used as an indicator of the position of these structures in less clear sections. Consequently, O1 was identified by a combination of the visual characteristics of the inner zone, T1 and O1, and the radius out to O1. Twenty seven otoliths displayed structures that were not sufficiently clear to identify O1, and were excluded from the dataset. The excluded sections generally reflected the temporal, spatial and age distribution of the full dataset.

A different randomly selected sample of 50 sections from the reference library was read on each of five occasions during the reading of the complete otolith collection and yielded IAPE values of less that 7.5% on each occasion. The maximum difference between re-reads of the reference library was 3 rings (Fig. A2), with a slight bias towards a lower count on re-reads. However bias plots (Fig. A3) suggest that this was mainly in fish older than 10 years of age where increment structure is compressed and the margin is more difficult to interpret.

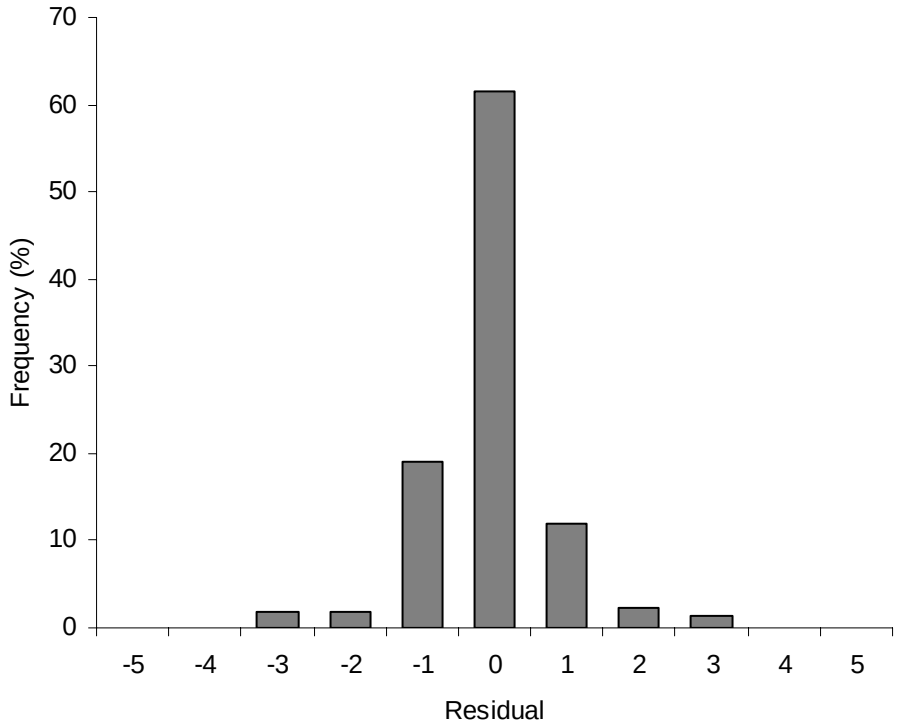

Fig. A2. Plot of the residuals of rereads of the otolith reference collection from the reference read.

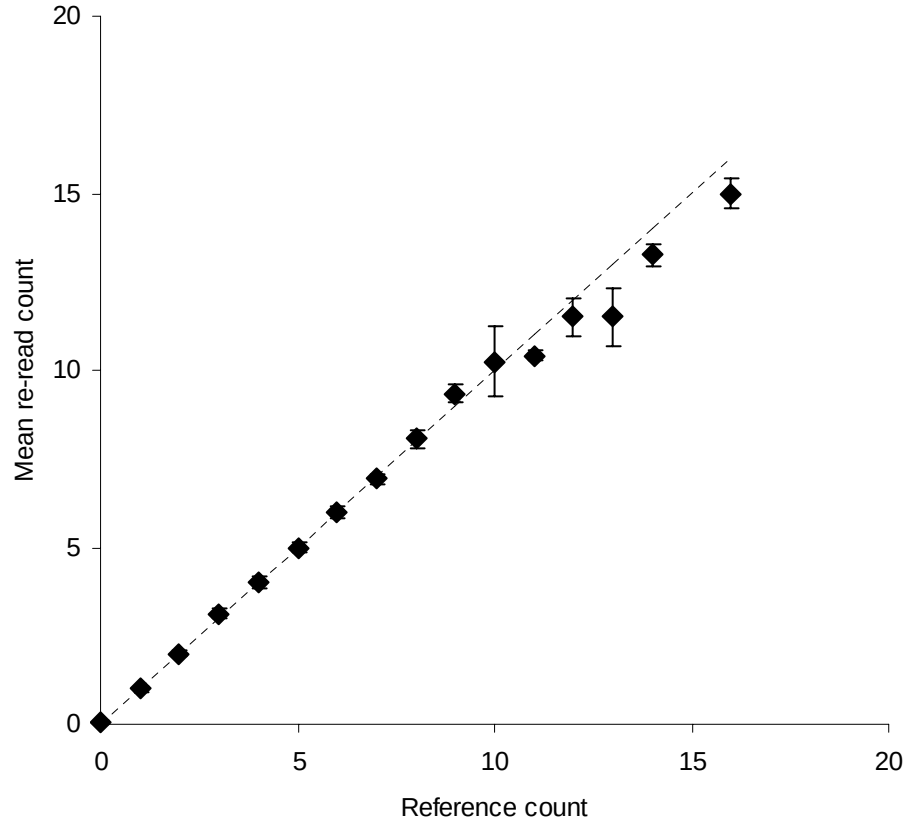

Fig. A3. Age bias plot of mean counts of re-reads of the otolith reference library by the reference count. Error bars are standard error. Dotted line indicates equal ages.

Periodicity and timing of increment structure formation

Validation of the periodicity of increment structure should, where possible, include reference to known-age fish (eg. reared in captivity or bomb radiocarbon analysis) (Campana, 2001). Unfortunately, it was not within the scope of this study to rear captive redbait and the short lifespan and absence of otolith samples from the 1970's precluded bomb radio-carbon analyses. Robust mark recapture techniques were precluded by the low likelihood of survival and recapture (detection) of marked pelagic fish. Due to the prevalence of samples from around the spawning season (September to November), attempts to plot marginal increments by month and by age class were not successful. Consequently, the frequency of margin categories pooled across age and sample year was used to assess trends in margin width.

Narrow translucent margins were present in otoliths sampled throughout the year with an annual minimum of 7% in November and December and an annual peak of 46% in January and February (Fig. A4). Medium width translucent margins were present in more than 50% of otoliths throughout the year, with the exception of January and February where they plunged to less than 13%. Wide margins were present in less than 20% of otoliths from March to August then climbed steadily to a maximum in January and February (Fig. A4).

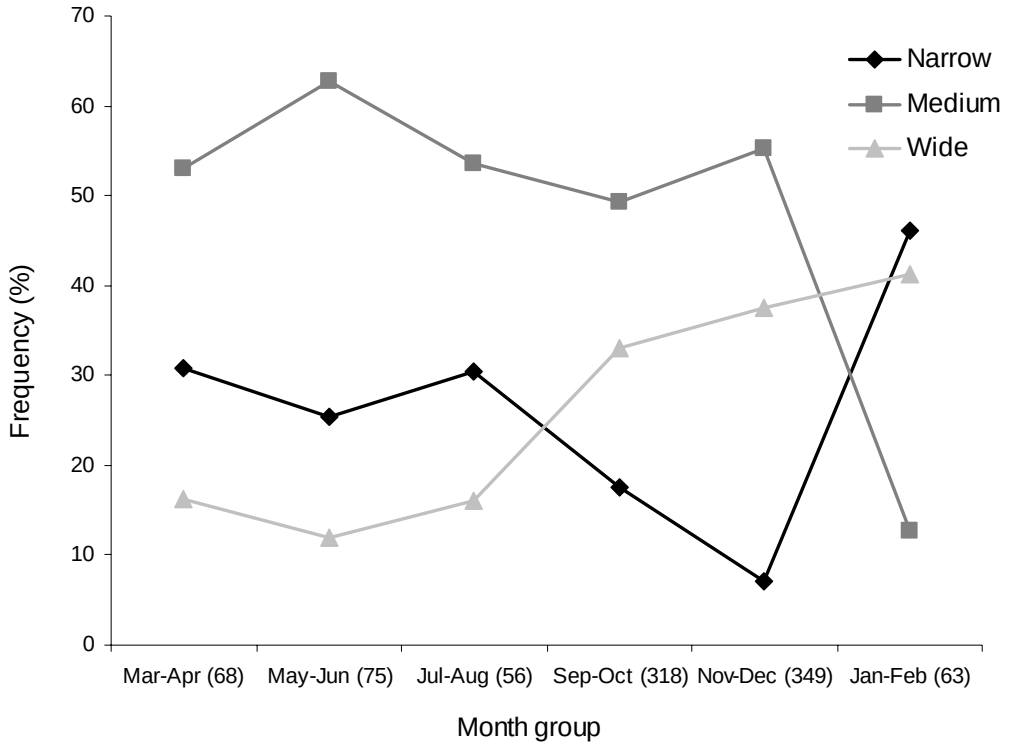

Fig. A4. Frequency of occurrence of translucent zone width categories by month groups. The number of otoliths read in month groups are in brackets.

Given that increment widths decreased in width for the first 3 or 4 cycles, that over 77% of the otoliths counted have fewer than 5 opaque zones, and that margin widths are categorized relative to the width of the previous complete increment; most of the *medium* width margins are likely to be margins that have reached their maximum width. Further, *wide* margins are only likely to have been recorded for otoliths of older fish or for otoliths that have a completed opaque zone on their margin and for which newly deposited translucent material is not yet visible due to the optically unclear area at the growing edge. For these reasons, temporal patterns in the relative proportions of otoliths with *narrow* and *medium* width margins were more useful for determining the periodicity and timing of increment formation than comparisons between *narrow* and *wide* margins. Consequently, the presence of single and distinct annual minima in both *narrow* and *medium* width translucent margins implies that opaque zone formation is annual (Fig. A4). Further, the dominance of *medium* width margins over *narrow* margins in November and December indicates that the formation of translucent material on the growing edge has ceased and conversely, the dominance of *narrow* margins over *medium* margins in January and February indicates that the formation of opaque zones has ceased.

Thus, we conclude that formation of opaque material on the margin is likely to be annual, extending into November and December and ceasing before January and February. Annual periodicity and timing of opaque zone completion around spring/summer is a common finding in Tasmanian and temperate waters in general (Choat and Axe, 1996; Fowler and Short, 1998; Cappo *et al.*, 2000; Ewing *et al.*, 2003; Smith and Deguara, 2003; Ewing *et al.*, 2007). Consequently, despite the validation of the periodicity and timing of opaque zone formation being based on categorical data which was pooled across years and age classes, the agreement with other local species for which robust validations have been performed has improved confidence in this conclusion.

Interpretation of inner structure

Age at the first enumerated zone can be inferred from comparisons of the radius of otoliths in known age 0+ and 1+ cohorts with the radius of the inner structures of otoliths from older fish (Ewing *et al.*, 2003). Ages can be assigned to juveniles through validated daily aged otoliths, length cohorts or captive rearing of juveniles (Campana, 2001). Unfortunately, attempts to count daily rings were unsuccessful and it was impractical to rear captive juvenile redbait. Size cohorts had potential for assigning ages to juvenile redbait, however catches of redbait were observed to be structured by size, and the fishing operation did not target aggregations of smaller fish. In addition, the mesh size of the trawl net was unlikely to target very small fish, further reducing the occurrence of small juveniles in catches. However, fishing was conducted in shallower water (average depth 92 m) during May, June, August and September of 2003 compared with an average depth of 130 m for other shots in 2003. Samples from these shallower shots yielded an identifiable size cohort centered at 110mm FL (Fig. A5). When aged, over 98% of the otoliths from fish in this cohort were yet to deposit the first enumerated zone (O1 in Fig. A1) and consequently were attributed a count of zero. The mean radius in the distal plane of the otoliths from fish in this cohort was 1168 μm (N=70, SD=77) (Fig. A1). The close similarity of this radius to the mean radius to O1 in older

fish of 1236 μm indicates that O1 is deposited during, or soon after the May to September period. This timing of completion of opaque zone formation is consistent with estimates from margin categories in adult fish.

Small redbait were also been retrieved from gut contents of adults caught in trawl samples in early 2002. These fish were 55 mm FL with a distal otolith radius of 597 μm (caught in January) (Fig. A1), 65 mm FL (caught in February, no otoliths available) and 70 mm FL with an otolith radius of 748 μm (caught in March). Larval redbait have been collected in Tasmanian waters at the postflexion stage in October at 6.5mm (Chapter 2). Given the continuity of the size progression from 6.5 mm larvae in October, through 55 mm in January, 65 mm in February, 70 mm in March and around 110 mm from May to September, it is likely that these redbait represent the first year of growth. Thus, redbait reach a size of around 110 mm FL and a distal otolith radius of around 120 0μm in their first year of growth. Consequently, the inner zone represents the first year of otolith growth and the deposition of O1 is concluded at around one year of age (Fig. A1).

Whilst this interpretation is plausible, it is based on a very small sample of early 0+ fish, which reflects the difficulty and opportunism encountered when sampling juvenile pelagic fish. It is conceivable that this conclusion is biased through factors such as schooling behaviour or gear selectivity. For example, the small individuals found in the stomachs of larger redbait may have been vulnerable to predation because they were very small for their age and hence may mask a full annual cohort between them and the larval samples. The attainment of approximately 30% of the maximum recorded size in the first year is rapid growth; however, it is not unusual in pelagic and semi-pelagic perciformes (Collins *et al.*, 1989; Naish *et al.*, 1991; Hernandez and Ortega, 2000; Lyle *et al.*, 2000). In summary, it is considered likely that this interpretation of the first year of somatic and otolith growth is correct, however, further research targeting young of the year redbait is required to provide a more robust estimate of early growth.

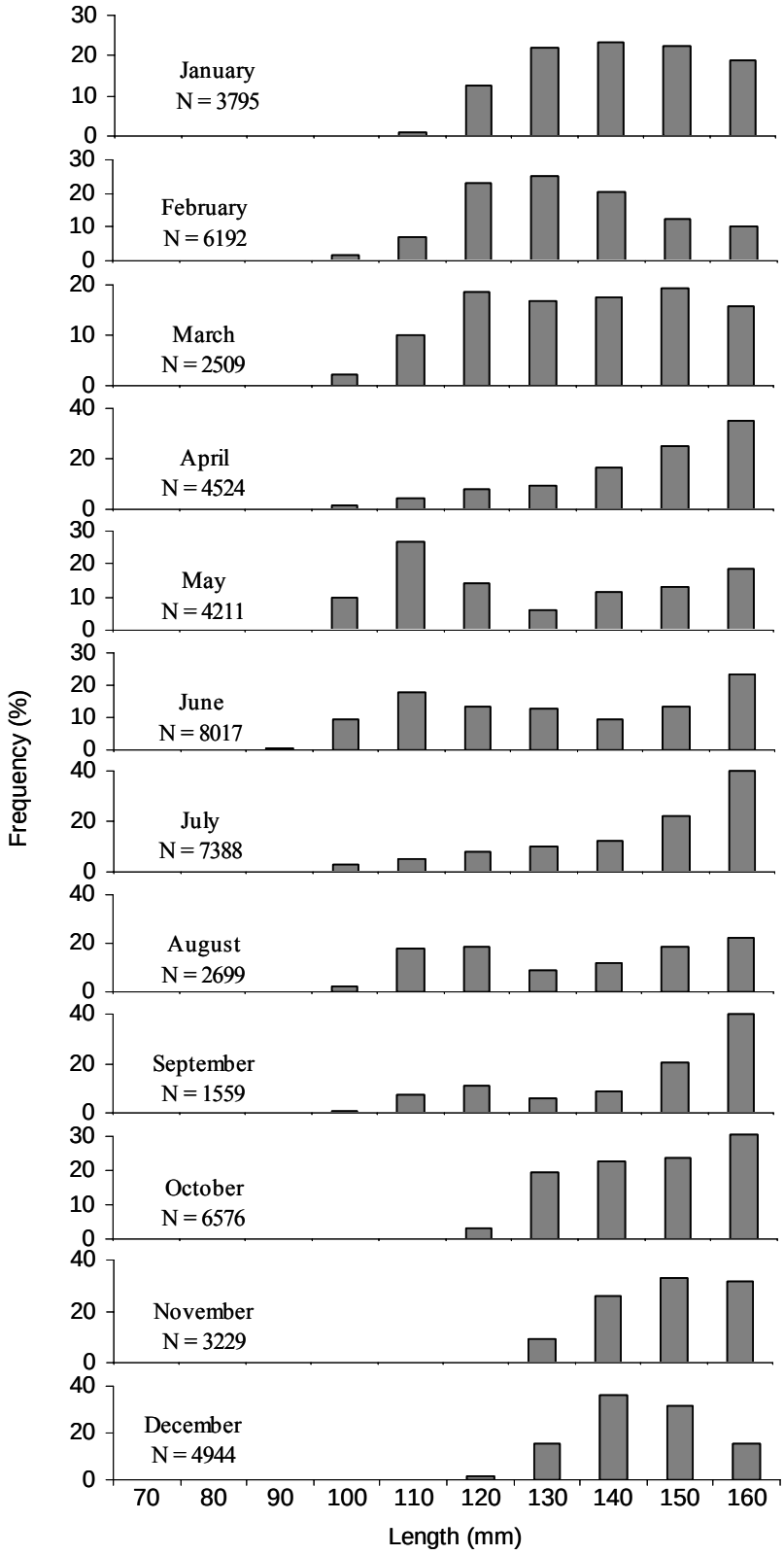

Fig. A5. Length frequency histograms of redbait less than 170 mm FL by month sampled (data pooled across years).

Assignment of age estimates

Conversion of an opaque zone count to an age involved consideration of opaque material in the vicinity of the margin, with reference to the timing of opaque zone formation, the month sampled and the arbitrary birthdate. An arbitrary birthdate of 1st October was chosen as it corresponded with the annual fall in GSI which indicated that spawning had commenced (Chapter 1). The margin width and timing of zone formation must be considered because fish collected at around the time when opaque zones are completed with a *narrow* otolith margin will have a count one greater than fish born in the same year with a *wide* margin despite being the same age. This lack of detection of the latest opaque zone in the fish with a *wide* margin (or the early detection in those with a *narrow* margin) is due to variation in the onset and rate of translucent otolith growth or differences in the width of the optically confusing layer on the otolith margin. Consequently, otoliths with a *wide* or *medium* margin and sampled between the arbitrary birth date (1st October) and the end of February were assumed to have an undetected opaque zone in the vicinity of their margin and had their count increased by one. The first of October was chosen as the cut-off time for opaque-zone formation in this correction because its approximate conformance with the arbitrary birthdate simplifies the equation for generating an age estimate from an otolith count. The end of February was chosen as the other limit for applying this correction because it is plausible that by this time *medium* margins could occur after the deposition of the previous years opaque zone due fast translucent growth over the summer period and because *medium* margins have passed their annual minima. Otoliths with a *narrow* margin sampled between the 1st of September and the arbitrary birthdate had their count decreased by one to account for early detected opaque zones. September was chosen for the commencement of this correction as it is unlikely that *narrow* margins would persist until that time.

Counts were converted to age estimates using the following formula:

$$t = count + (t_{cap} - t_{arb}) + C_{Mar}$$

Where t is the age estimate, *count* is the number of opaque zones commencing from O1 out to the margin but not including opaque material on the margin itself. The subtraction of t_{arb} (the arbitrary birthdate immediately prior to the capture date) from t_{cap} (capture date) is expressed in decimal years and accounts for the time elapsed between the deposition of the last counted opaque zone and the growing edge. C_{mar} is the correction for variation in detection of opaque zones formed around the time of completion of opaque zone formation and is only applied to fish sampled from September to February. It has a value of zero for *wide* and *medium* margins sampled in September and for *narrow* margins sampled from October to February. It has a value of one for *wide* margins sampled from October to February and a value of minus one for *narrow* margins sampled in September.

Maximum assigned ages for females and males for the entire dataset were 21 and 18 years, respectively, the largest female encountered being 317 mm and the largest male being 304 mm. These sizes are somewhat smaller than recorded elsewhere throughout

the distributional range. Redbait have been reported to grow to 335 mm FL off eastern Victoria (Furlani *et al.*, 2000), 344 mm SL off the coast of Chile (Melendez and Cespedes, 1986) and 493 mm TL and possibly larger in South African waters (Meyer and Smale, 1991). The capture of much larger redbait in African waters (Meyer and Smale, 1991) indicate that maximum age in this species may be higher than suggested from Tasmanian or Victorian samples, or that growth is highly variable by region.

Growth modelling

Model parameters for the VB growth function and likelihood ratio test (LRT) results by sex and by region are presented in Table A2. Growth modelling which included the juvenile samples collected on the East Coast yielded a good fit (Fig. A6) with biologically interpretable parameters (Table A2 and Fig. A7). The negative t_0 (-1.5) reflected fast initial growth. The juvenile dataset was distributed randomly between the male and female datasets for growth comparisons by sex for eastern Tasmania but was excluded from comparisons with growth between eastern and south-western Tasmania (no small juveniles were encountered off the south-west coast).

Table A2 Summary of VB parameters and LRT results by sex and by region.
EC and SW refer to eastern and south-western Tasmania and * refers to eastern Tasmanian samples with unsexed juveniles excluded.

		Pooled	EC ♂	EC ♀	EC ♀*	SW ♀	SW ♂	EC ♂*
N		1,260	312	489	386	290	169	209
VB parameters	L_∞	284	281	301	346	307	308	282
	K	0.27	0.26	0.21	0.11	0.17	0.12	0.23
	t_0	-1.54	-1.4	-1.9	-4.6	-4.7	-5.7	-2.3
Coincident curves	χ_2		13.1		71.3	40.3	17	
	df		3		3	3	3	
	P		<0.01		<0.01	<0.01	<0.01	
L_∞ constraint	χ_2		5.6		3.1	0.03	2.4	
	df		1		1	1	1	
	P		0.02		0.08	0.8	0.13	
K constraint	χ_2		3.5		1.6	0.59	6.4	
	df		1		1	1	1	
	P		0.06		0.2	0.44	0.01	
t_0 constraint	χ_2		3.6		0.03	0.36	11.6	
	df		1		1	1	1	
	P		0.06		0.9	0.55	<0.01	

Whilst all comparisons yielded significant differences on the coincident curves comparison, sex comparisons within region yielded no significant differences between any parameters with the exception of males from eastern Tasmania which had a lower asymptotic length than east coast females. Comparisons by sex between regions yielded significant differences, however, the absence of small young fish in the samples from the southwest and their subsequent exclusion from samples from the east coast may have biased curve fit.

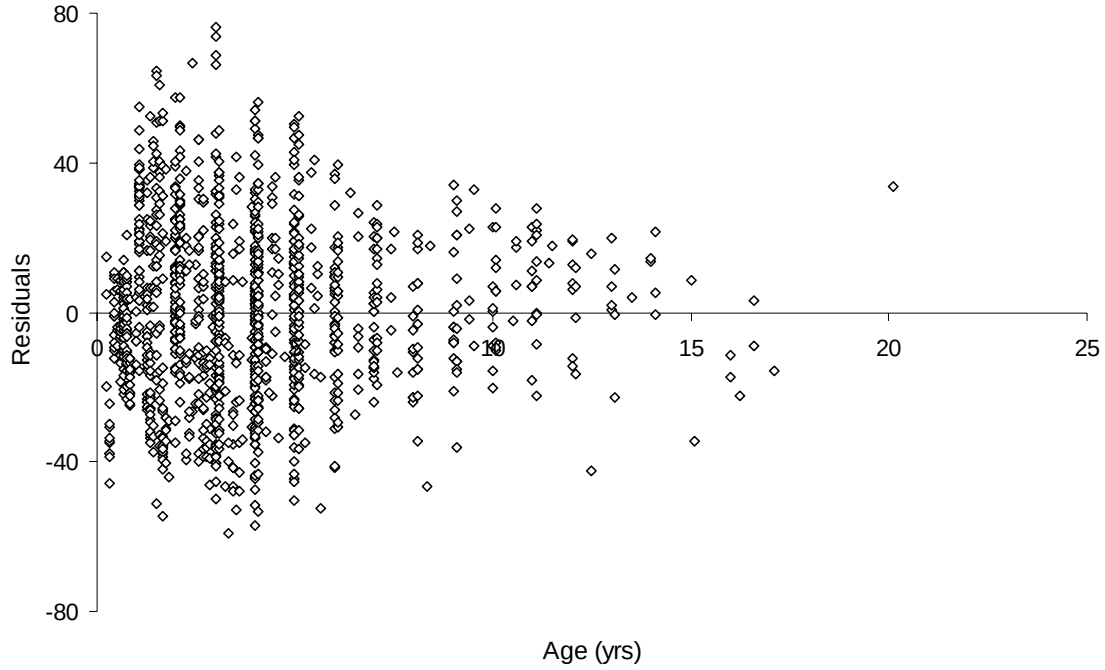

Fig. A6. Plots of the residuals of the length data by age data for redbait against VB growth function.

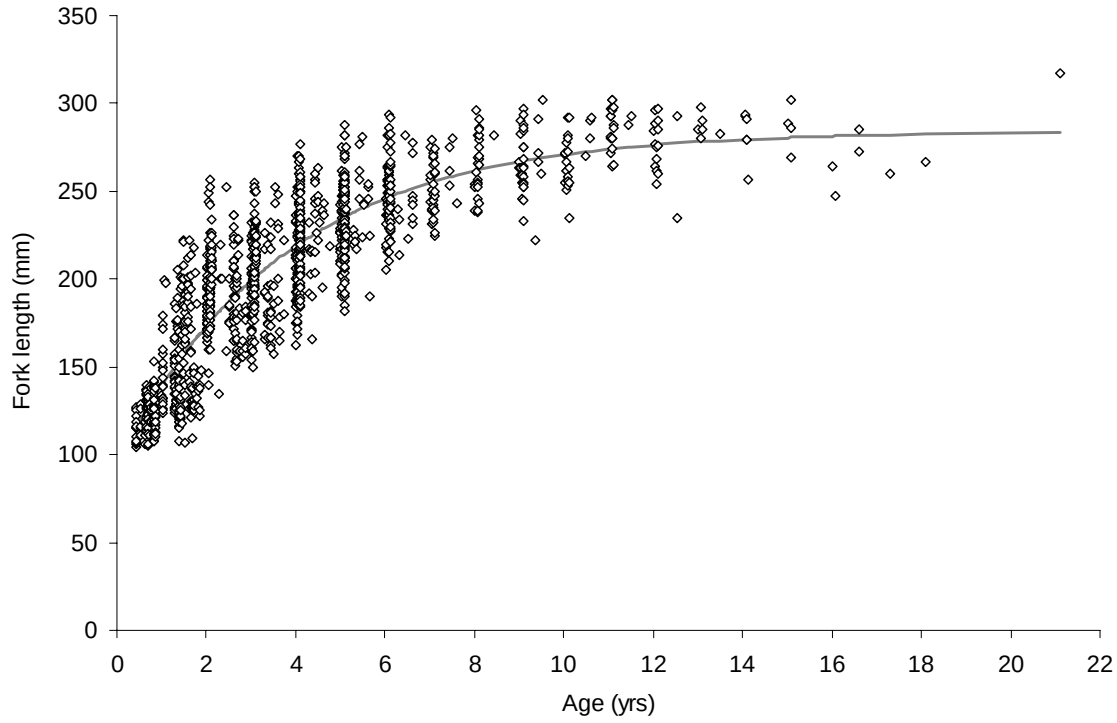

Fig. A7. Length at age data for redbait with sexes and regions pooled. The grey line represents the VB growth function.

Conclusions

The ageing protocol developed for redbait in this study has provided plausible age estimates. The use of the reference library to standardise otolith interpretation and then to track precision during the course of routine reads ensured that age estimates were generated with a high level of repeatability. However, the use of opportunistic sampling from a commercial fishing operation and the difficulty of collecting samples of juvenile pelagic fish have necessitated compromises in the validation of the periodicity of increment structure and the interpretation of the inner structures. Consequently, age estimates for redbait under this protocol have a high likelihood of being reliable estimates of relative age between fish, but should be used as an estimate of absolute age with caution.

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APPENDIX 4: 2005 PLANKTON SURVEY STATIONS

Transect	Station	Latitude S	Longitude E	Transect	Station	Latitude S	Longitude E
1	1	-38.83	148.08	13	1	-40.83	148.52
1	2	-38.83	148.20	13	2	-40.83	148.63
1	3	-38.83	148.32	13	3	-40.83	148.75
1	4	-38.83	148.43	14	1	-41.00	148.47
2	1	-39.00	148.23	14	2	-41.00	148.63
2	2	-39.00	148.35	14	3	-41.00	148.70
2	3	-39.00	148.47	15	1	-41.17	148.41
2	4	-39.00	148.58	15	2	-41.17	148.53
3	1	-39.17	148.40	15	3	-41.17	148.64
3	2	-39.17	148.52	16	1	-41.33	148.51
3	3	-39.17	148.63	16	2	-41.33	148.63
3	4	-39.17	148.75	17	1	-41.50	148.37
3	5	-39.27	148.74	17	2	-41.50	148.48
4	1	-39.33	148.38	17	3	-41.50	148.60
4	2	-39.33	148.50	18	1	-41.67	148.46
4	3	-39.33	148.62	18	2	-41.67	148.58
4	4	-39.33	148.73	18	2	-41.67	148.58
5	1	-39.50	148.51	18	2	-41.67	148.58
5	2	-39.50	148.63	E1	Extra	-41.57	148.60
5	3	-39.50	148.75	E2	Extra	-41.57	148.60
5	4	-39.50	148.86	19	1	-41.83	148.36
6	1	-39.67	148.35	19	2	-41.83	148.48
6	2	-39.67	148.47	19	3	-41.83	148.59
6	3	-39.67	148.58	20	1	-42.00	148.51
6	4	-39.67	148.70	20	2	-42.00	148.62
7	1	-39.83	148.59	21	0	-42.17	148.39
7	2	-39.83	148.71	21	1	-42.17	148.45
7	3	-39.83	148.83	21	2	-42.17	148.57
7	4	-39.83	148.94	22	1	-42.33	148.43
8	1	-40.00	148.54	22	2	-42.33	148.55
8	2	-40.00	148.66	23	1	-42.50	148.27
8	3	-40.00	148.78	23	2	-42.50	148.38
8	4	-40.00	148.89	23	3	-42.50	148.50
9	1	-40.17	148.67	24	1	-42.67	148.30
9	2	-40.17	148.78	24	2	-42.67	148.42
9	3	-40.17	148.90	25	1	-42.83	148.11
9	4	-40.17	149.02	25	2	-42.83	148.23
10	1	-40.33	148.65	25	3	-42.83	148.35
10	2	-40.33	148.77	26	1	-43.00	148.12
10	3	-40.33	148.88	26	2	-43.00	148.24
11	1	-40.50	148.62	27	1	-43.17	148.08
11	2	-40.50	148.73	27	2	-43.17	148.20
11	3	-40.50	148.82	28	1	-43.33	148.04
11	4	-40.50	148.85	28	2	-43.33	148.16
12	1	-40.67	148.55	29	1	-43.50	147.74
12	2	-40.67	148.67	29	2	-43.50	147.86
12	3	-40.67	148.78	29	3	-43.50	147.97

APPENDIX 5: 2006 PLANKTON SURVEY STATIONS

Transect	Station	Latitude S	Longitude E	Transect	Station	Latitude S	Longitude E
1	1	-40.50	148.66	10	2	-42.75	148.30
1	2	-40.50	148.78	10	3	-42.75	148.36
1	3	-40.50	148.83	10	4	-42.75	148.42
1	4	-40.50	148.89	10	5	-42.75	148.53
1	5	-40.50	149.01	11	1	-43.00	148.08
2	1	-40.75	148.59	11	2	-43.00	148.19
2	2	-40.75	148.71	11	3	-43.00	148.25
2	3	-40.75	148.77	11	4	-43.00	148.31
2	4	-40.75	148.82	11	5	-43.00	148.42
2	5	-40.75	148.94	12	2	-43.25	148.11
3	1	-41.00	148.52	12	3	-43.25	148.17
3	2	-41.00	148.63	12	4	-43.25	148.23
3	3	-41.00	148.69	12	5	-43.25	148.34
3	4	-41.00	148.75	13	1	-43.50	147.83
3	5	-41.00	148.87	13	2	-43.50	147.94
4	1	-41.25	148.48	13	3	-43.50	148.00
4	2	-41.25	148.60	13	4	-43.50	148.06
4	3	-41.25	148.66	13	5	-43.50	148.17
4	4	-41.25	148.71	14	1	-43.68	147.67
4	5	-41.25	148.83	14	2	-43.71	147.78
5	1	-41.50	148.43	14	3	-43.72	147.83
5	2	-41.50	148.55	14	4	-43.73	147.89
5	3	-41.50	148.61	14	5	-43.75	148.00
5	4	-41.50	148.67	15	1	-43.82	147.52
5	5	-41.50	148.78	15	2	-43.88	147.58
6	1	-41.75	148.40	15	3	-43.92	147.62
6	2	-41.75	148.52	15	4	-43.95	147.65
6	3	-41.75	148.58	15	5	-44.03	147.73
6	4	-41.75	148.63	16	1	-43.87	147.27
6	5	-41.75	148.75	16	2	-43.95	147.28
7	1	-42.00	148.47	16	3	-44.00	147.30
7	2	-42.00	148.58	16	4	-44.04	147.31
7	3	-42.00	148.64	16	5	-44.12	147.33
7	4	-42.00	148.70	17	1	-44.00	147.02
7	5	-42.00	148.82	17	2	-44.08	147.02
8	1	-42.25	148.43	17	3	-44.13	147.02
8	2	-42.25	148.55	17	4	-44.17	147.02
8	3	-42.25	148.61	17	5	-44.25	147.02
8	4	-42.25	148.67	18	1	-43.88	146.72
8	5	-42.25	148.78	18	2	-43.96	146.72
9	1	-42.50	148.33	18	3	-44.00	146.72
9	2	-42.50	148.44	18	4	-44.04	146.72
9	3	-42.50	148.50	18	5	-44.13	146.72
9	4	-42.50	148.56	19	1	-43.85	146.42
9	5	-42.50	148.67	19	2	-43.94	146.39
10	1	-42.75	148.18	19	3	-43.98	146.38

APPENDIX 5: cont.

Transect	Station	Latitude S	Longitude E
19	4	-44.02	146.36
19	5	-44.11	146.33
20	1	-43.73	146.19
20	2	-43.81	146.12
20	3	-43.84	146.08
20	4	-43.88	146.06
20	5	-43.95	146.00
21	1	-43.63	145.92
21	2	-43.65	145.88
21	3	-43.68	145.83
21	4	-43.73	145.74
22	1	-43.44	145.72
22	2	-43.47	145.68
22	3	-43.49	145.63
22	4	-43.54	145.53

APPENDIX 6: DAILY EGG PRODUCTION MODEL RUNS

Summary of *R* (*Ichthyoanalysis* package) model runs employed to compute mean daily egg production (P_0) of redbait off eastern Tasmania in 2005 and 2006. Model outputs are provided for runs undertaken using two data scenarios, and applying a least squares non-linear regression (NLS) model and GLM with a negative binomial error distribution (glm.nb). Model diagnostic plots are shown only for GLM runs. Ages of each egg cohort were estimated using the temperature-dependent incubation model developed in Chapter 2, based on a staging protocol described in same chapter. Ages were assigned using a purposely-coded Excel Visual Basic macro, with mid-water temperatures of each station and time of spawning considered being early evening (21:00 hrs). Extreme egg cohorts correspond to eggs aged ≤4 hours and ≥98% incubation time. The NLS model (Lo, 1985) assumes that all eggs are spawned and fertilized at a fixed hour, and that all are affected by a constant mortality rate (Z).

2005 model run scenarios:

- Entire sampled region off eastern Tasmania considered
- 84 stations
- **13 stations omitted** due to being outside spawning area (0 catch)
- Egg abundance data unweighted for station area (i.e. assumed all same area)
- All zeros included – as per current work in Mediterranean/Chilean waters
- Total redbait eggs used for runs = 12 899 (standardized)

Scenario 1 – all data (n = 332)

Scenario 2 – Excludes extreme egg cohorts (n = 280)

2006 model run scenarios:

- Only data from eastern Tasmania region (transects 1-13; 64 stations) included
- 47 stations
- **17 stations omitted** from runs due to being outside spawning area
- Egg abundance data unweighted for station area (i.e. assumed all same area)
- All zeros included – as per current work in Mediterranean/Chilean waters
- Total eggs used for runs = 10 622 (standardized)

Scenario 1 – all data (n = 209)

Scenario 2 – Excludes extreme egg cohorts (n = 175)

RESULTS 2005 – All transects; 13 stations omitted

Scenario 1 - model runs with all data (n = 332)

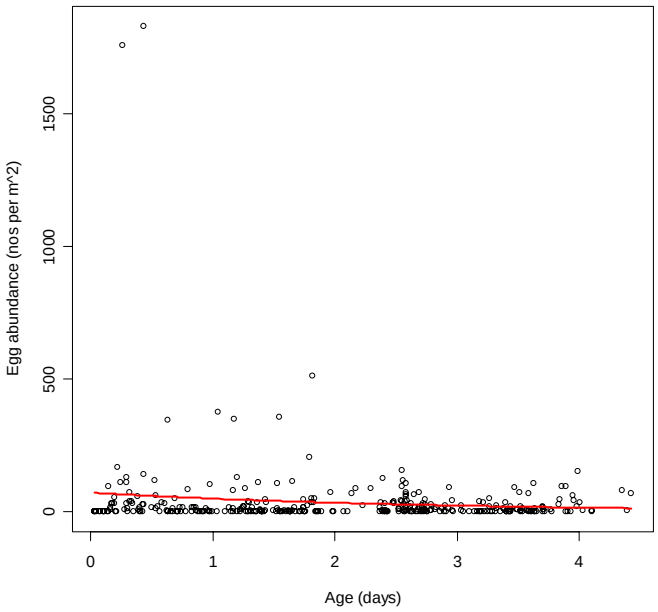
1.1. NLS

```
   Estimate Std. Error t value Pr(>|t|)
p0  90.8948    24.2896   3.742 0.000215 ***
z   -0.5196     0.2216  -2.344 0.019647 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 147 on 330 degrees of freedom
Number of iterations to convergence: 6
Achieved convergence tolerance: 9.705e-06
```

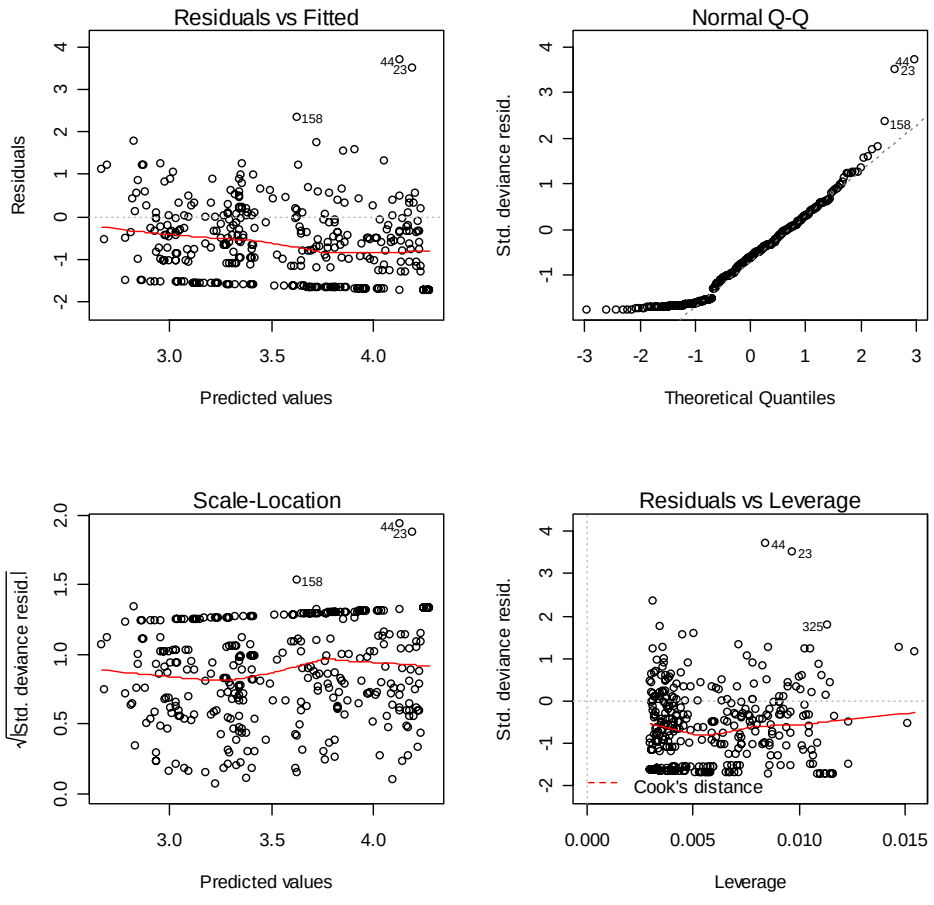
1.2. GLM

```
glm.nb(formula = (Eggs) ~ Age, data = survey.data, init.theta =
0.27527,link = log)
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-1.75152  -1.35980  -0.62057  -0.02185   3.71298
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  4.28894    0.10924  39.262  < 2e-16 ***
Age         -0.36622    0.04674  -7.836 4.66e-15 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for Negative Binomial(0.2753) family taken to be
0.2753)
    Null deviance: 403.76  on 331  degrees of freedom
Residual deviance: 383.72  on 330  degrees of freedom
AIC: 2640.5
Number of Fisher Scoring iterations: 1
              Theta:  0.2753
          Std. Err.:  0.0207
2 x log-likelihood:  -2634.5490
```

Plot of raw data with superimposed fitted GLM (red line)



GLM diagnostic plots



Scenario 2 - model runs excluding extreme egg cohorts (n = 280)

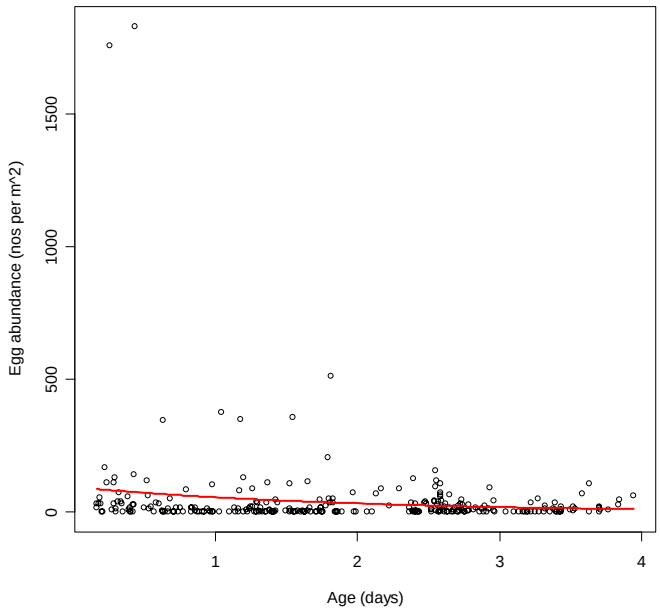
2.1 NLS

```
   Estimate Std. Error t value Pr(>|t|)
p0 157.7463    46.2269   3.412  0.00074 ***
z   -1.0138     0.3769  -2.690  0.00758 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 157.8 on 278 degrees of freedom

Number of iterations to convergence: 12
Achieved convergence tolerance: 9.964e-06
```

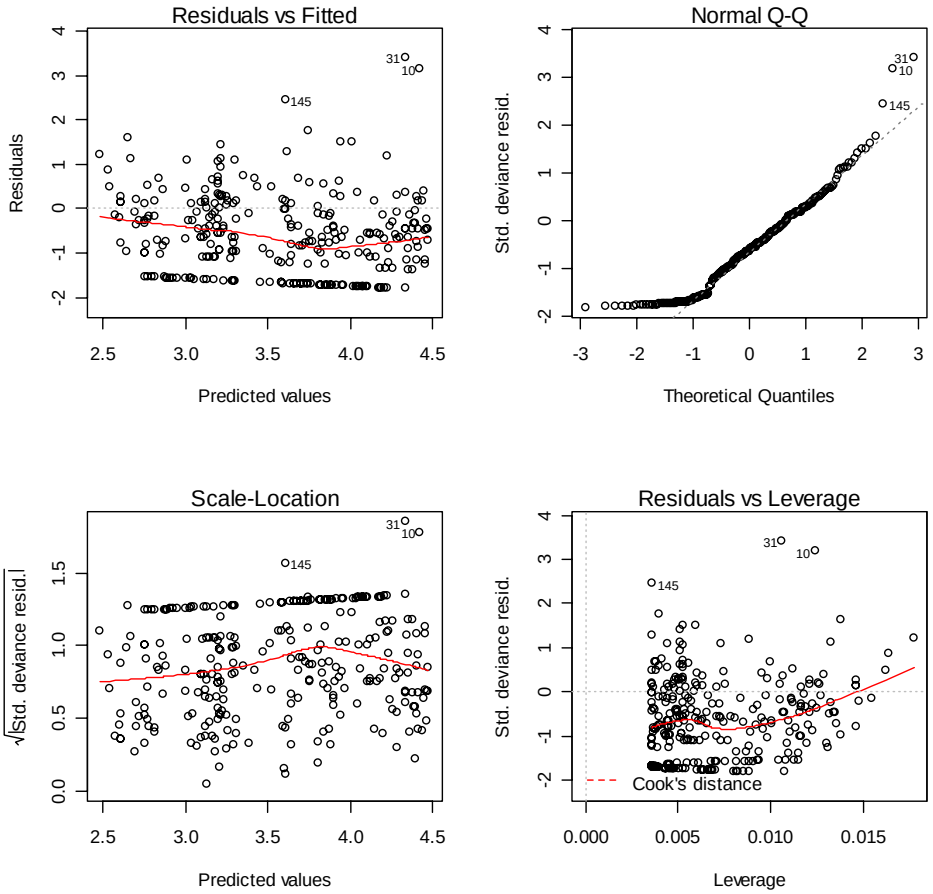
2.2 GLM

```
glm.nb(formula = (Eggs) ~ Age, data = survey.data, init.theta =
0.2925, link = log)
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-1.80536  -1.34197  -0.63797   0.02792   3.40984

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  4.56095    0.12491  36.515   <2e-16 ***
Age         -0.52671    0.05831  -9.032   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Dispersion parameter for Negative Binomial(0.2925) family taken to be
0.2925)
    Null deviance: 354.31  on 279  degrees of freedom
Residual deviance: 326.45  on 278  degrees of freedom
  (52 observations deleted due to missingness)
AIC: 2272.2

Number of Fisher Scoring iterations: 1
              Theta:  0.2925
          Std. Err.:  0.0239
2 x log-likelihood:  -2266.2420
```

Plot of raw data with superimposed fitted GLM (red line)



GLM diagnostic plots



RESULTS 2006 – Eastern Tasmania (transects 1-13; 17 stations omitted)

Scenario 1 - 2006 model runs with all data (n = 209)

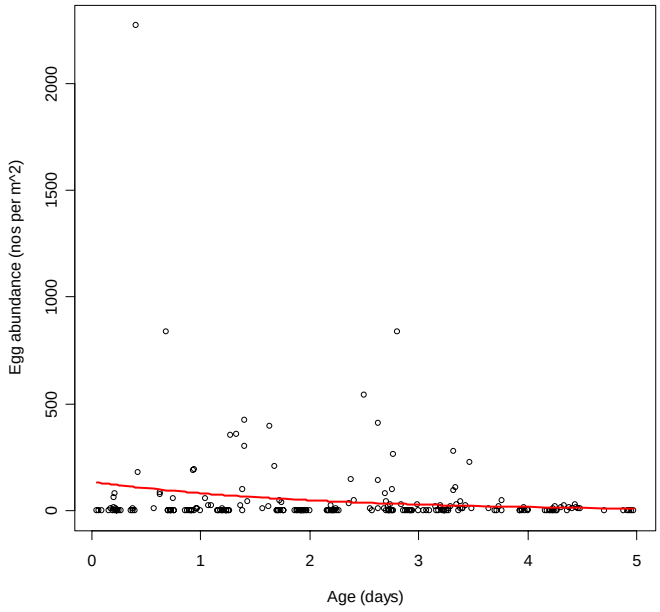
1.1 NLS

```
   Estimate Std. Error t value Pr(>|t|)
p0 101.3995    37.2349   2.723  0.00702 **
z   -0.3381     0.2210  -1.530  0.12762
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 191 on 207 degrees of freedom
Number of iterations to convergence: 7
Achieved convergence tolerance: 3.393e-06
```

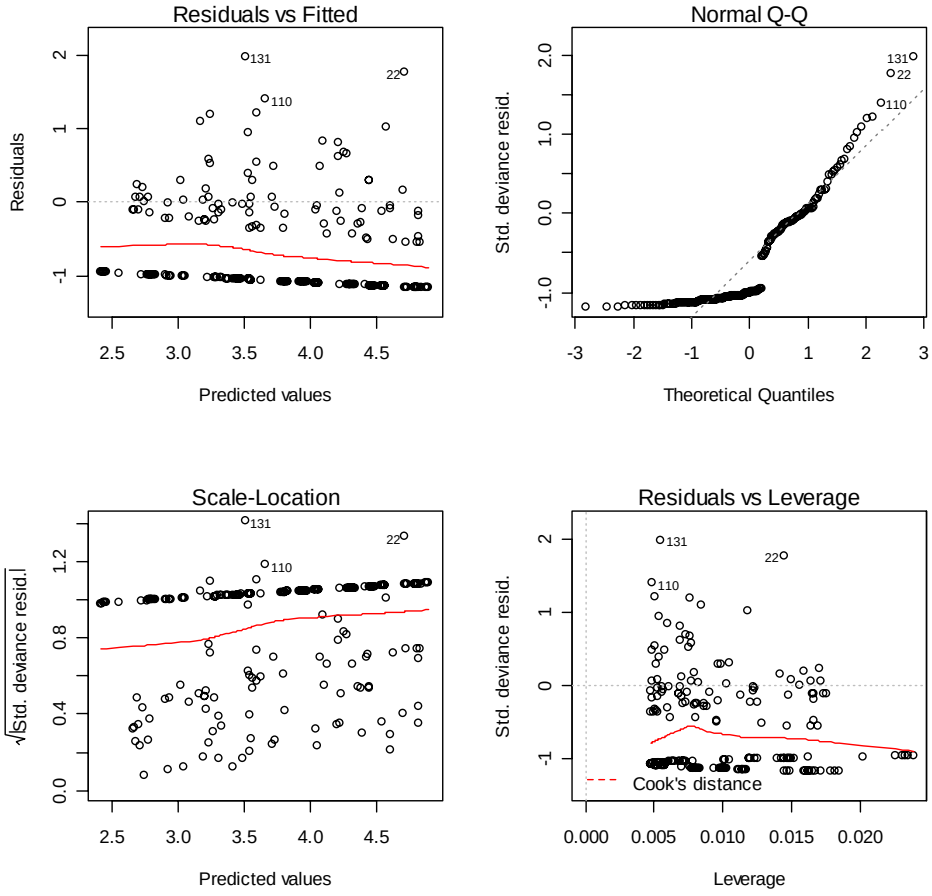
1.2 GLM

```
glm.nb(formula = (Eggs) ~ Age, data = survey.data, init.theta = 0.094,
link = log)
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.1687  -1.0905  -0.9977  -0.1154   1.9798
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  4.91962    0.13797   35.66   <2e-16 ***
Age         -0.50244    0.05191   -9.68   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for Negative Binomial(0.0941) family taken to be
0.094)
    Null deviance: 171.38  on 208  degrees of freedom
Residual deviance: 164.93  on 207  degrees of freedom
AIC: 1269.9
Number of Fisher Scoring iterations: 1
              Theta:  0.0941
          Std. Err.:  0.0114
2 x log-likelihood:  -1263.9250
```

Plot of raw data with superimposed fitted GLM (red line)



GLM diagnostic plots



Scenario 2 - 2006 model runs excluding extreme egg cohorts (n = 175)

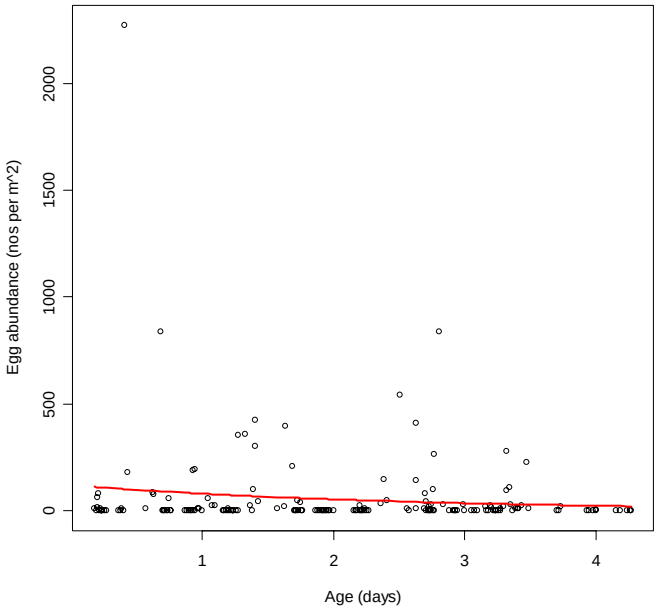
1.1 NLS

```
   Estimate Std. Error t value Pr(>|t|)
p0 113.2216    46.2588   2.448   0.0154 *
z   -0.3622     0.2659  -1.362   0.1749
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 208.2 on 173 degrees of freedom

Number of iterations to convergence: 6
Achieved convergence tolerance: 8.477e-07
```

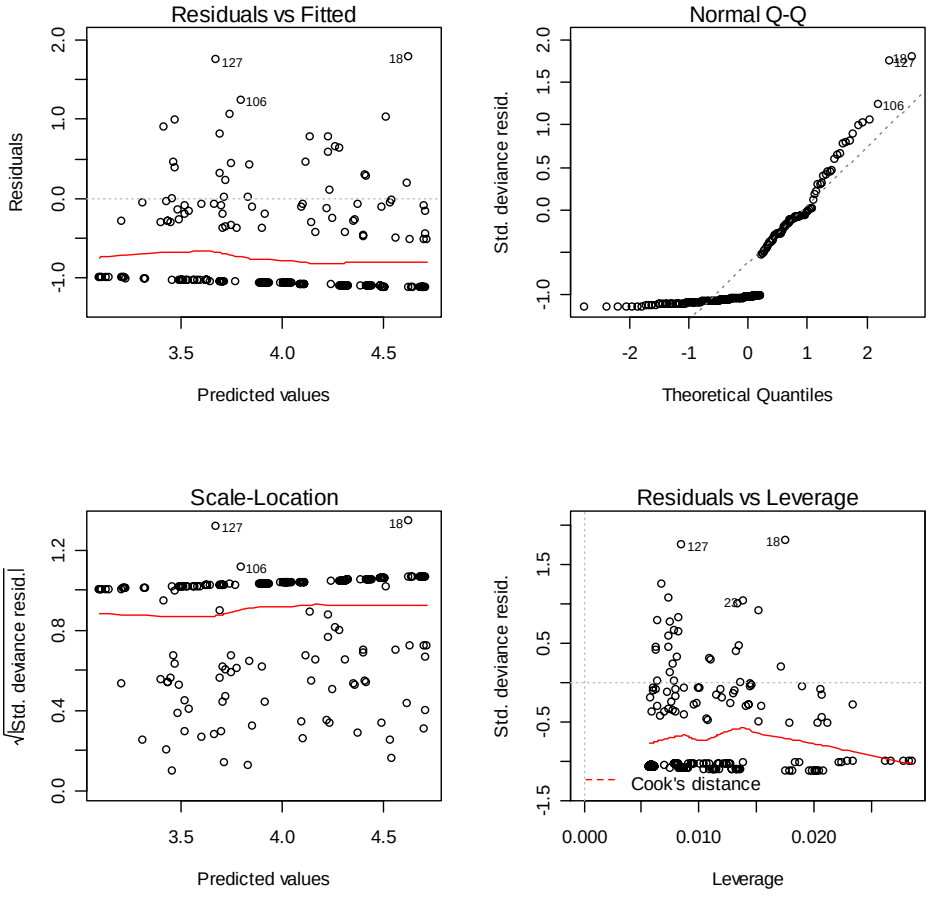
1.2 GLM

```
glm.nb(formula = (Eggs) ~ Age, data = survey.data, init.theta =
0.08837,link = log)
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.1233  -1.0718  -1.0245  -0.1594   1.7871
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  4.79003    0.15587  30.730  < 2e-16 ***
Age         -0.39554    0.06738  -5.871 4.34e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for Negative Binomial(0.0884) family taken to be
0.0884)
    Null deviance: 139.36  on 174  degrees of freedom
Residual deviance: 136.66  on 173  degrees of freedom
AIC: 1085.3
Number of Fisher Scoring iterations: 1
              Theta:  0.0884
          Std. Err.:  0.0117
2 x log-likelihood:  -1079.2700
```

`Plot of raw data with superimposed fitted GLM (red line)`



`GLM diagnostic plots`



APPENDIX 7: PROVISIONAL SPAWNING BIOMASS ESTIMATES FOR REDBAIT OFF SOUTHERN NSW

F.J. Neira and J.M. Lyle

Adapted from a report prepared for the Australian Fisheries Management Authority (May 2008)

Summary

Eggs of redbait (*Emmelichthys nitidus*) were caught during plankton surveys carried out in October 2002 and 2003 between Jervis Bay and Cape Howe in southern New South Wales (NSW). The surveys formed part of a FRDC-funded project to evaluate the daily egg production method (DEPM) to estimate spawning biomass of blue mackerel (*Scomber australasicus*) in south-eastern Australia. Data on egg abundance-at-age from October 2003, combined with adult reproductive data of redbait from eastern Tasmania pooled across 2005 and 2006, were employed to provide an estimate of the spawning biomass of redbait off southern NSW. Based on egg distribution of alone, the spawning area of redbait off southern NSW in 2003 was estimated to be 4,062 km^2 (1,271 nm^2), which comprised 92.8% of the area surveyed. The weighted mean daily egg production (P_0) computed using a GLM with a negative binomial distribution error was estimated to be 3.13 eggs/0.05m^2 day^{-1} (CV=0. 54). Estimated redbait spawning biomass for the above area and P_0 was 20,563 t (CV=0.86). Key factors underpinning the large variances of both estimates comprise the limited redbait egg data (positive stations) and the fact that the surveys were not designed to provide DEPM-based spawning biomass estimates. Overall results indicate that the biomass estimate is largely imprecise, though it may be overly conservative given the constraints in the approach employed in this evaluation to estimate spawning area. Additional uncertainty in this estimate arises from the use of adult reproductive parameters based on redbait from eastern Tasmania.

The redbait eggs collected during the 2002 and 2003 surveys off southern NSW, combined with egg and larval abundances obtained from eastern Tasmania during 2005 and 2006, provide strong evidence of a single, continuous spawning redbait stock distributed between Jervis Bay (NSW) and Tasman Peninsula (Tas). Confirmation of the existence of this eastern redbait spawning stock is urgently required to strengthen the management of this developing fishery in south-eastern Australia.

Background

The daily egg production method (DEPM) constitutes, at present, the preferred technique to estimate spawning biomass of small pelagic fishes worldwide, especially clupeoid species (Stratoudakis *et al*., 2006). The method was demonstrated to be appropriate to estimate spawning biomass of redbait (*Emmelichthys nitidus*) off eastern Tasmania, in what constituted the first attempt to successfully apply DEPM to a mid-water species of the family Emmelichthyidae (Neira *et al*., 2008a). Reproductive attributes which make redbait a suitable species for such approach include the release of batches of pelagic, buoyant eggs once about every three nights during its relatively short spawning spring period. Based on DEPM parameters obtained during egg and adult surveys, redbait spawning biomass within the surveyed areas off eastern Tasmania in 2005 and 2006 was estimated in 86,990 and 50,782 t, respectively (Neira *et al*., 2008a).

The DEPM is a fishery-independent method applied to provide biomass estimates of pelagic fishes that are batch spawners, i.e. release batches of eggs into the water column throughout the spawning season. The method assumes that the biomass of a fish population can be estimated from the daily production of eggs (P_0) over the entire spawning area (A), the average female weight (W), the proportion of females (R), the fraction of females (S) that spawned the previous night, and the average fecundity (F) of reproductively active females. Parameters P_0 and A derive from intensive egg surveys over the assumed spawning area, while the other four parameters are estimated from adults sampled concurrently with plankton surveys in the same area (Lasker, 1985; Picquelle and Stauffer, 1985; Lo *et al*., 1996; Stratoudakis *et al*., 2006). As such, one of the primary objectives of the completed redbait study was to collect the required information on reproductive biology and spawning dynamics of this species, including eggs development, before applying the DEPM (Neira *et al*., 2008a).

Amongst the key findings reported in the above study was that the spawning area of redbait in southeastern Australia extended from the lower south-east coast of Tasmania (Tasman Peninsula) as far north as Jervis Bay in southern New South Wales (NSW). This finding was based on a relatively large number of redbait eggs and larvae caught in plankton samples taken over shelf waters off southern NSW during October 2002 and 2003 as part of a FRDC-funded project (2002/61) to evaluate the DEPM in blue mackerel, *Scomber australasicus* (F.J. Neira, *unpublished data*). The availability of these egg samples prompted managers of the Commonwealth-managed Small Pelagic Fishery (AFMA) to commission a provisional redbait spawning biomass estimate for this NSW area, utilizing egg development and adult reproductive information available for this species from the original study off Tasmania.

The main objective of this study is to provide an estimate of the spawning biomass of redbait for southern NSW, including associated coefficient of variation (CV), based on samples collected during the blue mackerel egg survey conducted over that area in 2002 and 2003. This report complements the final FRDC report to evaluate the DEPM to estimate spawning biomass of redbait off eastern Tasmania (Neira *et al*., 2008a).

Methodology

Material available and sampling procedures

A total of 962 redbait eggs were available for this evaluation. The eggs were collected in vertical plankton samples taken along shelf waters between Jervis Bay and Cape Howe in southern NSW in October 2002 and 2003 (Fig. 1), and identified as redbait using the description of Neira *et al*. (2008b). It is worth noting that while sampling in both surveys extended north to Fraser Island in southern Queensland, no redbait eggs or larvae were found in shelf waters north off Jervis Bay.

Samples were obtained with a bongo sampler equipped with 3-m long, 0.6-m diameter 300 and 500 μm mesh plankton nets, encased within a custom-built, weighted stainless steel frame to facilitate vertical drops. The survey area comprised 25 stations, four across four transects perpendicular to the coastline and 50 nm apart, and 9 in sets of three along-shelf stations between transects. Along-transect stations were positioned 10 and 5 nm inshore from the shelf break, at the break and 5 nm offshore from the break. Tows were conducted from within 5 m of the seabed, or from a maximum depth of 200 m, with sampling depth regulated using a Scanmar depth sensor fitted to the sampler frame. Volume of water filtered (m^3) was estimated from a mechanical General Oceanic flowmeter fitted to the mouth of each net. All samples were fixed in 98% ethanol, and all redbait eggs removed for analyses. Number of redbait eggs were converted into abundance (numbers per m^2) and plotted by station for the 2002 and 2003 survey areas off southern NSW using SURFER.

Vertical data on conductivity, temperature and depth were obtained simultaneously with each plankton sample using a CTD (Conductivity-Temperature-Depth) profiler fitted to the sampler's frame. The mid-water temperature of each station employed to assign an age to each staged egg (see below) corresponded to the median value to 100 m or to the maximum depth when site depth was <100 m.

Aging of eggs

Of all available redbait eggs, each of the 902 eggs collected during October 2003 was staged and subsequently assigned an age (hours) using an existing temperature-dependent egg incubation model developed for redbait (Neira *et al*., 2008b). Based on reproductive information of redbait from eastern Tasmania, the eggs were assigned ages using a peak spawning time of 21:00 hrs. The 60 eggs collected in October 2002 were omitted from the analysis as they were too few to justify the application of DEPM.

Adult parameters

Data of all four adult reproductive parameters used in this report, namely sex ratio, female weight, spawning fraction and fecundity, correspond to averages estimated for redbait in eastern Tasmania pooled across October 2005 and 2006 (Neira *et al*., 2008a). Estimates and respective variances are provided in Table 1.

Spawning area estimation

The procedure to estimate the sizes of the survey and redbait spawning areas off southern NSW followed that employed to evaluate the 2005 and 2006 spawning biomass of redbait off eastern Tasmania (Neira *et. al.*, 2008a). However, given the layout of the three along-shelf stations sampled between transects, both estimates included these stations within a narrow corridor (10 and 5 nm respectively) rather than assuming the entire shelf area comprised between the across-shelf transects as spawning area. Consequently, survey and spawning area estimates are likely to be underestimated. Employing this approach, the survey along southern NSW covered a total area of ca 6,498 km^2 between Jervis Bay and Cape Howe (Figs 1, 2). Of this area, 4,602 km^2 was regarded as spawning area in 2003 (92.8%).

Mean daily egg production and mortality

Redbait egg abundance-at-age-data were employed to estimate mean daily egg production (P_0; eggs/0.05m^2 day^{-1}) and mortality (Z) by fitting a generalised linear model (GLM) assuming a negative binomial distribution error (ICES, 2004). This model was applied to estimate mean P_0 of redbait from eastern Tasmania and found to fit the data better than Lo's (1986) least squares non-linear regression model (Neira *et al*., 2008a). However, unlike runs performed for redbait off eastern Tasmania, estimates provided in this report employed all egg abundance-at-age data as there were no extreme data (e.g. eggs <4 hours old) that could be eliminated. Weighted mean P_0 and Z estimates, as well as total egg production per spawning area and spawning biomass off southern NSW for October 2003, are provided in Table 1 together with individual CVs. Model fitting was carried out using the *R* library *Ichthyoanalysis* (www.r-project.org; *http://sourceforge.net/projects/ichthyoanalysis*). The egg mortality curve (Fig. 4), model output and diagnostic plots for the model run (Appendix I), constitute *R* outputs.

Spawning biomass model

Spawning biomass (**B**, tonnes) was estimated by the following equation (Parker, 1985):

$$\boldsymbol{B} = \frac{P_0\ A\ W}{R\ \ F\ \ S}$$

where P_0 is egg production at time zero estimated per unit of area per day (eggs 0.05m^2 day^{-1}); A total spawning area (km^2); W mean weight of mature females in the population (g); F batch fecundity (number of oocytes released per mature female per batch; eggs/batch); R fraction of mature females by weight (sex ratio); and S spawning fraction (proportion of mature females spawning each night; night^{-1}).

The variance of the spawning biomass estimate was computed using the following equation modified from Parker (1985), and subsequently employed to calculate standard deviation (SDev) for the biomass estimate (Table 1):

$$Var(B) = B^2 * (VarP_0/P_0^2 + VarW/W^2 + VarR/R^2 + VarF/F^2 + VarS/S^2 + 2\{COVS\})$$

where *COV* corresponds to the covariance of each adult parameter, in this case:

$$COVS = \{-Cov(WR)/WR - Cov(FW)/FW - Cov(WS)/WS + Cov(RF)/RF + Cov(RS)/RS + Cov(FS)/FS\}$$

Results

Distribution of eggs

Redbait eggs occurred during the 2002 and 2003 surveys along the region of southern NSW between Jervis Bay Cape Howe (Fig. 3). However, eggs were significantly more abundant during 2003, with the highest abundance (651 eggs/m^2) obtained at a station 5 nm inshore from the shelf break in water depth of 125 m (36.7^{o}S; 150.2^{o}E; Fig. 3).

Egg production and spawning biomass estimates

Daily egg abundances plotted against their respective ages (days) followed the typical exponential decay model described for redbait eggs from eastern Tasmania, with the fitted mortality curve sloping down from young to old cohorts (Fig. 4). Weighted mean daily egg production (P_0) in shelf waters off southern NSW in 2003 was estimated in 3.13 (CV=54%). Total egg production over the spawning area was 4.07 eggs x 10^{11}. Estimated redbait spawning biomass within the positive area (4,360 km^2) was 20,563 t (CV=86%; Table 1).

Table 1. **Input parameters employed to compute spawning biomass of redbait off southern NSW in October 2003.**

Means (CVs) and variances of adult parameters *W*, *F*, *R* and *S* correspond to estimates derived from redbait off eastern Tasmania pooled across October 2005 and 2006 (see Neira *et al*., 2008a for details). Criterion employed to estimate mean spawning fraction (*S*) was hydrated oocytes and/or fresh POFs. Intercept and corresponding standard error, as well as mortality (*Z*), derive from a GLM assuming a negative binomial error distribution.

Adult input data - Tasmania	**October 2005/2006**
Mean weight female (*W*)	76.6 (0.06)
Variance *W*	23.6
Mean fecundity (*F*)	11,807 (0.09)
Variance *F*	1,106,335
Sex ratio (*R*)	0.40 (0.07)
Variance *R*	0.0007
Spawning fraction (*S*)	0.32 (0.12)
Variance *S*	0.0014
Egg input data – New South Wales	
DEPM survey area/spawning area (km^2)	6,498 / 4,360
Data scenario	No extremes
Number of stations included in run	12
Data points	30
Intercept	4.5364
Std error of intercept	0.4235
Age (*Z*)	-0.98
CV (*Z* – R output matrix)	0.61
AIC	199
Weighted P_0 (eggs/0.05 m^2 day^{-1})	**3.13**
CV – weighted P_0	0.54
Total egg production (eggs x 10^{11})	4.07
Spawning biomass (tonnes) (CV)	**20,536** (0.86)
SDev (t)	17,694

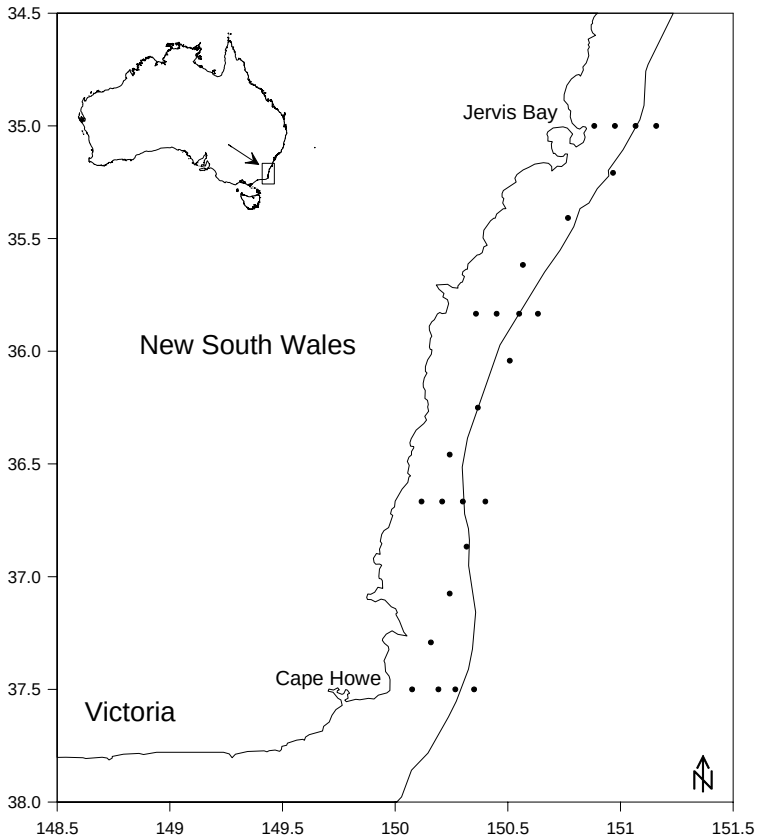


Fig. 1. Map of southern New South Wales showing position of stations sampled during the blue mackerel surveys in October 2002 and 2003. Line bordering coastline corresponds to the approximate position of the 200 m depth contour.

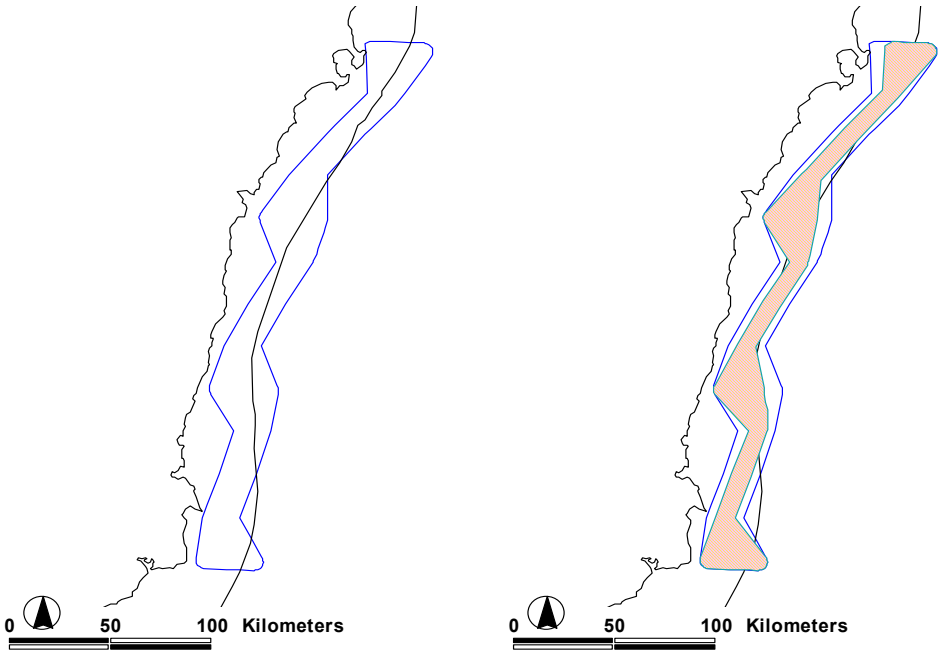


Fig. 2. Geographical limits of the survey area (A) and redbait spawning area (B) off southern NSW in October 2002/2003.

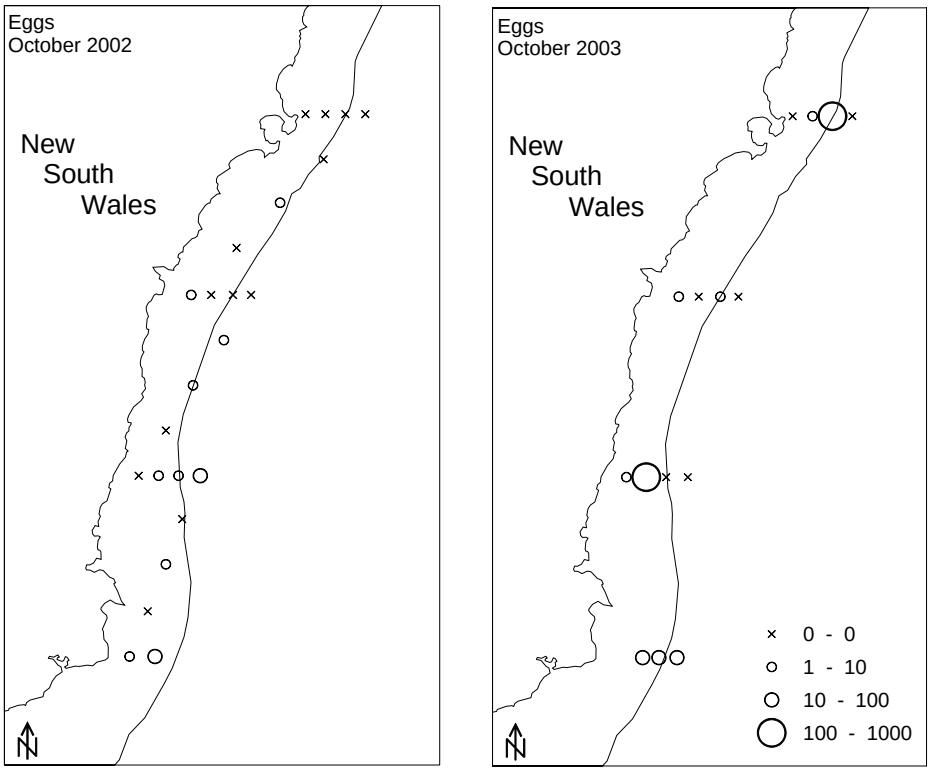


Fig. 3. Distribution of redbait eggs (numbers per m^2) along shelf waters off southern NSW during October 2002 and 2003.

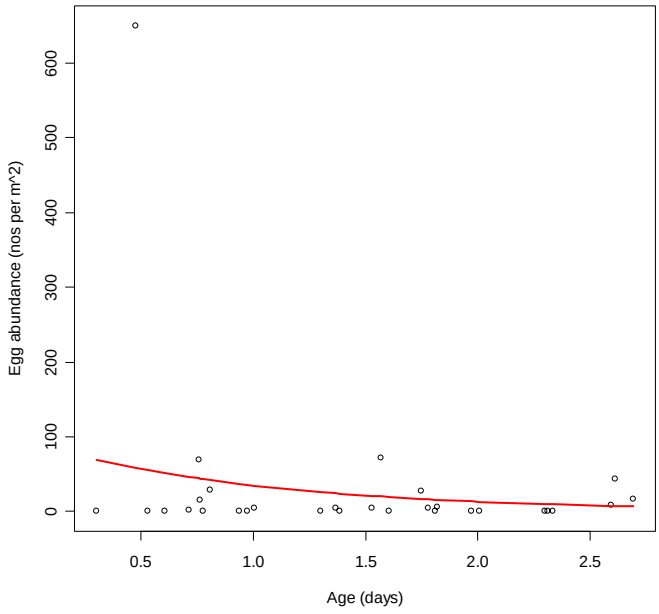


Fig. 4. Egg mortality model for redbait off southern NSW based on October 2003 data. Superimposed fitted mortality curve was derived from a GLM with negative binomial error distribution.

Discussion

The spawning biomass estimate provided for redbait off southern NSW in this report is considered as highly imprecise and, as such, it needs to be treated with caution. This imprecision is clearly evident in the large CVs associated both with the mean daily egg production (P_0) (54%) and the biomass (B) estimate (86%). Furthermore, such values are significantly greater than the CVs associated to P_0 and B estimates obtained for redbait off eastern Tasmania for the spawning period in October 2005 (14% and 37%) and October 2006 (19% and 21%) based on the same GLM technique (Neira *et al*., 2008a). Despite the large CVs, however, it is noteworthy that the mean P_0 estimated for redbait off southern NSW is only marginally lower than that obtained for redbait off eastern Tasmania, i.e. 3.13 vs. 4.04 eggs/0.05m^2 day^{-1} (Neira *et al*., 2008a).

There are two main sources of error identified as contributing to the large variances in the P_0 and B estimates, namely the limited number of stations in which redbait eggs were present and the underlying survey design. The egg data available for this evaluation originated from surveys designed to delineate the spawning area of blue mackerel and hence sub-optimal for the purpose of providing DEPM-based spawning biomass estimates of redbait. Thus, the survey area comprised a few stations along several offshore transects each placed at considerable distance apart, with three well-spaced stations between transects. Furthermore, there is uncertainty due to the fact that no adult redbait data were available from NSW and consequently all four adult reproductive parameters employed in the final biomass estimate, i.e. average female weight (W), sex ratio (R), batch fecundity (F) and spawning fraction (S), had to be based on combined 2005-2006 estimates for redbait from eastern Tasmania.

The redbait eggs collected during the 2002 and 2003 surveys off southern NSW, combined with the redbait egg and larval abundances obtained along eastern Tasmania in 2005 and 2006, provide compelling evidence of a single, continuous spawning redbait stock distributed between Jervis Bay (NSW) and Tasman Peninsula (Tas). This view is supported by the fact that no spawning boundaries could be defined based on egg distributions either south of the Cape Howe (NSW) during the 2002 and 2003 surveys, or north of the areas off eastern Tasmania surveyed during 2005 and 2006 (Neira *et al*., 2008a).

Confirmation of the existence of a single eastern redbait spawning stock is urgently required to strengthen the management of this developing fishery in south-eastern Australia. In the meantime, the biomass estimate provided in this evaluation (20,563 t) should be viewed as provisional, though it may be overly conservative given the constraints in the approach employed in this evaluation to estimate spawning area. In this context, a purposely-designed DEPM survey covering the shelf area from southern NSW to south-eastern Tasmania should provide a more comprehensive evaluation of redbait spawning biomass that is required for appropriate management of this currently growing mid-water fishery. Such survey would in turn provide evidence of the extent of interannual variability both in spawning distribution and intensity off southern NSW and elsewhere, as suggested by the significantly lower abundances of redbait eggs obtained in 2002 compared to 2003.

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Appendix I

R output of GLM with negative binomial error distribution:

```
glm.nb(formula = (Eggs)~Age,data=survey.data, init.theta = 0.19392,link = log)
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.5107  -1.2829  -0.7653  -0.2058   1.7287

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   4.5364     0.4235  10.712  < 2e-16 ***
Age          -0.9832     0.2648  -3.713 0.000204 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.1939) family taken to be 0.1939)
    Null deviance: 35.434  on 29  degrees of freedom
Residual deviance: 31.396  on 28  degrees of freedom
AIC: 199.53
Number of Fisher Scoring iterations: 1
              Theta:  0.1939
          Std. Err.:  0.0521
2 x log-likelihood:  -193.5300
```

Diagnostic plots: