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Estimating the spawning fraction of Blue Mackerel off eastern Australia: Stage 2: Spatiotemporal variability in spawning patterns and implications for future DEPM surveys.

Report to the Australian Fisheries Management Authority

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Contents

About this document..... 2

Contents..... 3

Figures..... 4

Tables 5

Appendices..... 5

Acknowledgements..... 7

Executive Summary..... 8

1 Introduction..... 11

 1.1 Background..... 11

 1.2 Need..... 15

2. Methods..... 17

 2.1 Adult sampling and initial processing 17

 2.2 Gonosomatic Index 17

 2.3 Histological processing 17

 2.4 Staging and ageing postovulatory follicles (POFs) 20

 2.5 Estimating spawning fraction 27

3 Results..... 28

 3.1 Gonosomatic Indices..... 28

 3.2 Duration of POF cohorts 29

 3.3 Estimates of the spawning fraction 30

4. Discussion 35

5. References 37

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Figures

Figure 1.1 Sub-areas of the Commonwealth Small Pelagic Fishery. Source: SPF-Harvest-Strategy_April-2017_FINAL.pdf (afma.gov.au))	11
Figure 1.2. Areas open and closed to fishing in the mid-water trawl sector of the Small Pelagic Fishery. The catch grids (G101, G103 and G105) where most catches have been taken are marked in black. (Source: Small Pelagic Fishery Australian Fisheries Management Authority (afma.gov.au))	12
Figure 2.1 Locations where adult Blue Mackerel were collected in 2022 and 2023 (pink stars) are shown in relation to distributions of eggs (circles), trawl locations (red dots) and Sea Surface Temperatures recorded in DEPM surveys conducted in 2014 and 2019. (Source: Ward et al. 2015, 2021b).	18
Figure 2.2. Macroscopic stages I–V (1-5) and their microscopic characteristics. A: Immature Stage I (1) ovary with developing unyolked (UY) oocytes. B: Maturing Stage II (2) ovary with UY and partially yolked (PY) oocytes. C: Mature Stage III (3) ovary with UY, PY and fully yolked oocytes (Y). D: Hydrated Stage IV (4) ovary with UY, PY and partially hydrated oocytes (H). E: Spent Stage V (5) ovary with post-ovulatory follicles (POFs). Scale bar = 150µm. Source: Rogers et al. (2009), <i>Marine and Freshwater Research</i>	19
Figure 2.3. Histological sections of <i>S. australasicus</i> ovaries showing coexistence of recent postovulatory follicles (POFs) and imminent spawning markers (e.g., germinal vesicle break-down oocyte stage, GVBD; late terminal vesicle migratory oocyte stage, GVM or MN2). A) GVBD oocytes together with POFs. B) GVM oocytes with POFs. C) Coexistence of newer and older POFs in a late vitellogenic ovary	21
Figure 2.4. Application of Weibel stereological method on the ovaries of <i>S. australasicus</i> using Imagej.	23
Figure 2.5 Histological sections of <i>S. australasicus</i> ovaries including different daily POF cohorts. A) Day0 POFs in late migratory nucleus stage (MN2) ovary. B) Day1 POFs in early migratory nucleus stage (MN1) ovary. C) Day2 POFs in early migratory nucleus stage (MN1) ovary. D) Day2* POFs in Early Migratory nucleus stage (MN1) ovary.	24
Figure 2.6. Mean measured area of the recent spawning marker, postovulatory follicle (POF _{xsa} , mm ²) assigned to each daily POF cohort.	26
Figure 3.1 Gonosomatic index (GSI) by month in both years (A), and in the different locations (colour), and years (shape) (B).	28
Figure 3.2. The mean relative number of postovulatory follicles (RF _{POF}) (A), and the mean measured POF area (POF _{xsa}) (B) in each ovarian developmental stage. MN0= very early stage of migratory nucleus, MN1= early migratory nucleus stage, MN2= late migratory nucleus stage, GVBD= germinal vesicle break-down stage, HYD= hydrated stage.	31

Figure 3.3. Spawning fraction estimated by two methods, POFdur33h* (red), POFdur48h (green) by year, by year and month, and by year and both years combined (both) in Coffs Harbour and Port Stephens..... 34

Tables

Table 3.1 Mean monthly gonosomatic index (GSI) by year and Location. The total number of fish, the mean GSI, the standard error (SE), and the lower and upper bounds (95% CI) are also presented. 29

Table 3.2. The number of ovaries with POFs assigned in each daily POF cohort (Day0, Day1, Day2 and Day2*), the number of migratory nucleus (MN) oocytes, and the total number of fish analysed are shown for each location. 32

Appendices

Appendix 1. Estimates of spawning fraction obtained in other studies 41

Appendix 2. Summary of samples collected to estimate the spawning fraction of Blue Mackerel off eastern Australia in 2024. 43

Appendix 3. Conceptual model of developed of female *Scomber japonicus* developed by Dickerson et al. (1992)..... 44

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

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Executive Summary

Background and Need

Blue Mackerel (*Scomber australasicus*) is a target species of the Commonwealth Small Pelagic Fishery (SPF). Catches from the Eastern sub-area reached 11,079 tonnes in 2023/24, which was 95.5% of the Total Allowable Catch (TAC) of 11,600 tonnes.

Estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM) are used to set TACs. Limited understanding of spawning fraction (i.e., the proportion of mature females that spawn each day (24 hours) during the spawning season) off eastern Australia is a major contributor to uncertainty in estimates of the spawning biomass of Blue Mackerel in this region.

In December 2020, the SPF Resource Assessment Group (RAG) identified that robust estimates of the spawning fraction of Blue Mackerel off eastern Australia were needed to provide more robust and accurate estimates of spawning biomass and ensure confidence in the setting of TACs.

Stage 1 of the current project (Ward et al. 2023) had three important outcomes:

1. Established methods for sampling large adult Blue Mackerel in offshore waters
2. Identified several locations (at different latitudes) where sample could be collected
3. Developed preliminary estimates of spawning fraction off eastern Australia

The study also confirmed that the main spawning season of Blue Mackerel off eastern Australia extends from August to October and that the main spawning area extends from Sandy Cape to about Wollongong. Ward et al. (2023) emphasized the strong effect that ongoing uncertainties about how long post-ovulatory follicles (POFs) persist in the ovaries of Blue Mackerel had on estimates of spawning fraction and spawning biomass.

Objectives

This objectives of Stage 2 of this project are to:

1. Apply the method(s) for sampling large adult Blue Mackerel developed in Stage 1 at four locations along the east coast.

2. Determine how long POFs remain present in the ovaries of Blue Mackerel off eastern Australia (additional objective)
3. Investigate the hypothesis that the spawning season of Blue Mackerel varies with latitude off eastern Australia and identify implications for the design of future DEPM surveys.
4. Estimate the spawning fraction of Blue Mackerel off eastern Australia and assess the need for additional adult sampling in future DEPM surveys.

Methods

This study is based on samples collected from Tweeds Heads, Coffs Harbour, Port Stephens and Wollongong from August to October 2022 and 2023. Samples collected in 2024 will be included in the 2024 DEPM survey report (AFMA 2024/0806). Ovaries from mature females were preserved in 10% buffered formaldehyde solution and analysed using standard histological procedures.

We investigated the duration of POFs by determining the number of POF cohorts in each ovary. The rationale was that: if POFs persist for 24 hours, only one daily POF cohort would be present; if POFs persist for 24-48 hours two daily cohorts would be present; and if POFs last for more than 48 hours, more than two cohorts would be present. We demonstrated that POFs remain visible in the ovaries for approximately two days, which suggests that the spawning dynamics of *S. australasicus* conforms to the conceptual model that Dickerson et al. (1992) developed for *Scomber japonicus*.

Following Dickerson et al. (1992), Day0 POFs were assumed to be up to 10 hours old, Day1 POFs 10-24 hours old, Day2 POFs 24-33 hours old and Day2*POFs more than 33 hours old. Spawning fraction was calculated from both POFs that last for 48 hours (i.e. Day0, Day1, Day2 and Day2*POFs) and POFs that last for 33 hours (i.e. Day0, Day1, and Day2 POFs).

Results and Discussion

Estimates of spawning fraction calculated using the two methods provided similar results. The overall estimates of spawning fraction based on POFs that last for 48 hours of 0.121 ± 0.043 95%CI was slightly lower than the estimate from POFs that last 33 hours of 0.151 ± 0.057 95%CI. Estimates of spawning fraction differed among months and locations. However, due to the opportunistic nature of the sampling and limited sample sizes the significance of these differences could not be tested statistically. The only other estimates of spawning fraction available for *S. australasicus* are

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

from north-eastern Taiwan by Sinaga et al. (2021) who followed the methods described by Rogers et al. (2009) and Ward et al. (2009). Estimates of spawning fraction provided for individual months during the peak spawning season ranged from 0.03 in March 2018 to 0.16 in April 2017 (Sinaga et al. (2021). Estimates of spawning fraction for other species of *Scomber* are based on a large variety of different methods and are highly variable (0.081 to 0.50 see Appendix 1).

The results of this study show that the assumption of Rogers et al. (2009) and Ward et al. (2009) that POFs persist for two days in the ovaries of Blue Mackerel in Australian waters was appropriate. The estimates of spawning fraction obtained in the present study are similar to the estimate of 0.135 (0.102–0.167 95% CI) obtained from southern Australia by Ward et al. (2009) that has been used in previous applications of the DEPM to Blue Mackerel off eastern Australia. Applying the method of Ward et al. (2009) to samples collected in the present study provides the same value that we obtained using POFs that last for 48 hours. The two methods are effectively identical. The main difference is that in the present study we compiled evidence that demonstrated (rather than assumed) that POFs persist for two days.

The similarity of the estimates of spawning fraction obtained in 2022 of 0.114 (± 0.075 95% CI) and 2023 of 0.128 (± 0.063 95% CI) suggest that this parameter may be relatively stable among years. As a result, the costs and benefits of conducting large-scale adult sampling programs in each future application of the DEPM to Blue Mackerel should be evaluated (as Ward et al. 2021b did for Sardine off southern Australia). This issue will be assessed in the report on the DEPM survey conducted off eastern Australia in September 2024 (AFMA 2024/0806) that will use methods developed in this study and present results of adult sampling undertaken in 2022, 2023 and 2024.

The present study resolved several important technical limitations and key knowledge gaps related to the application of the DEPM to Blue Mackerel off eastern Australia. Stage 1 of this study developed effective methods for sampling large spawning Blue Mackerel off eastern Australia and identified several locations where samples could be collected reliably. Stage 2 resolved uncertainties related to the time that POFs persist in the ovaries and as a result provided the most well justified estimates of spawning fraction currently available for Blue Mackerel in Australian waters.

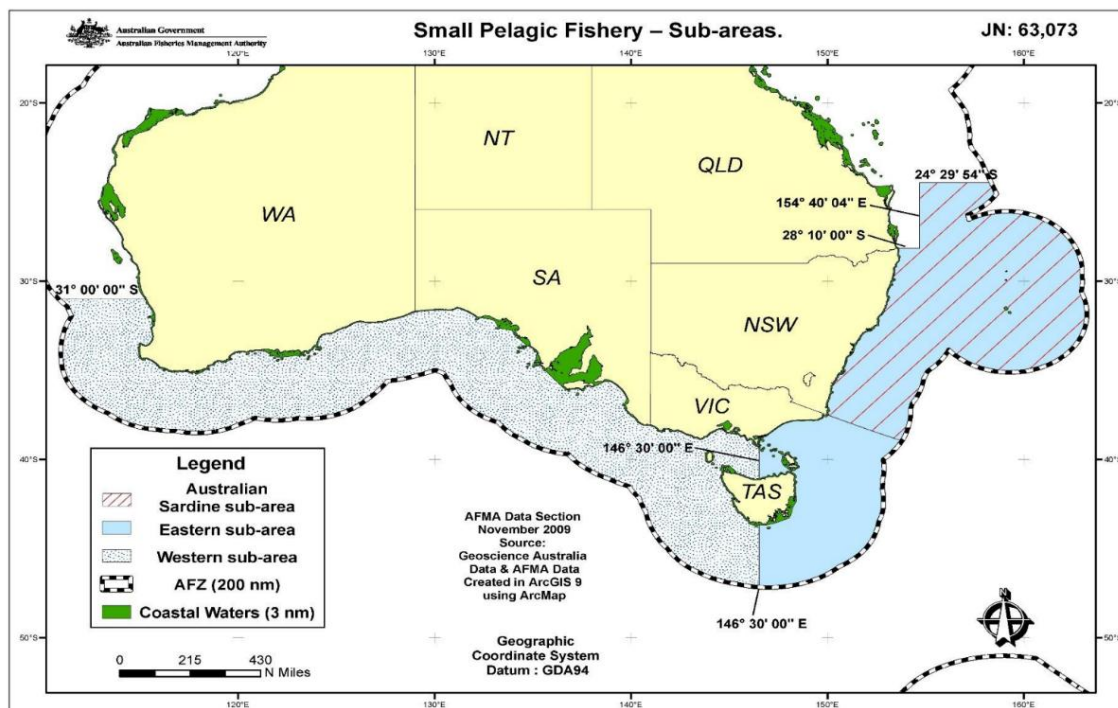
Keywords: Daily Egg Production Method, pelagic fishes, spawning fraction, *Scomber australasicus*

1 Introduction

1.1 Background

Blue Mackerel (*Scomber australasicus*) is a key target species of the mid-water trawl sector of the Commonwealth Small Pelagic Fishery (SPF). Catches of Blue Mackerel from the Eastern sub-area of the SPF (Figure 1.1) have grown rapidly since 2016 and reached 11,079 tonnes in 2023/24, which was 95.5% of the Total Allowable Catch (TAC) of 11,600 tonnes (e.g. Butler et al. 2024). Most of the recent catch has been taken from three catch grids off southern New South Wales, near Ulladulla and Eden (Figure 1.2). Blue Mackerel is also a target species within the NSW Ocean Hauling Purse-Seine Fishery, currently managed under an annual TAC of 1,010 t.

Figure 1.1 Sub-areas of the Commonwealth Small Pelagic Fishery. Source: [SPF-Harvest-Strategy April-2017 FINAL.pdf \(afma.gov.au\)](#)

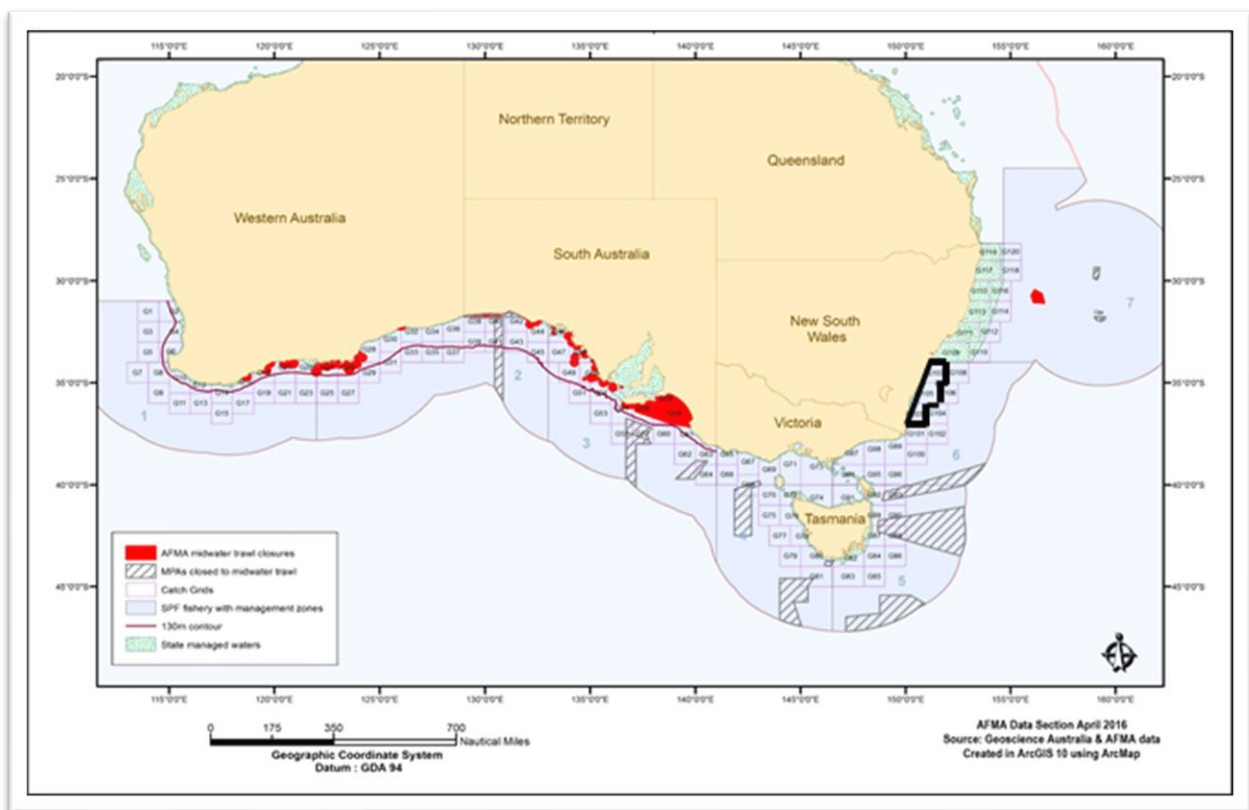


Blue Mackerel, also known as Slimy Mackerel, is a key target species for recreational fishers off eastern Australia (e.g., Murphy et al. 2020), especially game fishers, who consider “slimies” to be the premier bait for a range of large pelagic fishes, including tunas and marlin. Recreational fishers have expressed concerns about the ecological sustainability of commercial fishing for Blue Mackerel

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

and potential impacts of disturbance of bait schools on the availability of game fish in key recreational fishing hotspot ([spf panel meeting minutes 17 january 2019 final.pdf\(afma.gov.au\)](#)).

Figure 1.2. Areas open and closed to fishing in the mid-water trawl sector of the Small Pelagic Fishery. The catch grids (G101, G103 and G105) where most catches have been taken are marked in black. (Source: [Small Pelagic Fishery | Australian Fisheries Management Authority \(afma.gov.au\)](#))



Like other species in the SPF (AFMA 2008, 2009), the primary source of information used to determine Recommended Biological Catches (RBCs) and set TACs for Blue Mackerel are estimates of spawning biomass obtained using the Daily Egg Production Method (DEPM, Parker 1980, Lasker 1985, AFMA 2008). The premise of the DEPM is that spawning biomass can be estimated by dividing the mean number of pelagic eggs produced per day throughout the spawning area (i.e., total daily egg production) by the mean number of eggs produced per unit mass of adult fish (i.e., mean daily fecundity) (Parker 1980, Lasker, 1985). Total daily egg production is calculated from estimates of mean daily egg production and spawning area obtained from broad-scale plankton surveys. Mean daily fecundity is calculated from samples of adult fish collected during the spawning season.

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

Ward (2021 a, b) showed that mean daily fecundity can be calculated most precisely from three adult parameters (Parker 1980), i.e. sex ratio, relative fecundity and spawning fraction, rather than the four parameters identified by Lasker (1985). Under this formulation, variation in mean daily fecundity is driven almost entirely by variation in spawning fraction because sex ratio (by weight) is usually close to 0.5 (1:1 males versus females by number) and relative fecundity is virtually constant across a wide range of female weights because the relationship between female weight and batch fecundity is linear. Ward (2021 a, b) also showed that for Australian Sardine (and perhaps Blue Mackerel) spawning fraction can be relatively stable among years in comparison to sampling error and may not need to be estimated annually. This finding has the potential to substantially reduce survey costs by potentially eliminating and at least reducing the need for ongoing adult sampling once reliable estimates of spawning fraction are established.

The DEPM has been applied to Blue Mackerel in the East sub-area three times (Ward et al. 2009, 2015, 2021b). The initial application of the method in 2003 and 2004 suggested that the spawning biomass was at least 30,000 t (Ward et al. 2009). The estimates of spawning biomass obtained in 2014 and 2019 were ~83,300 (95% CI = 35,100–165,000 t) and 88,265 t (95% CI = 33,320–143,209), respectively (Ward et al. 2015, 2021b). The authors of all these studies suggested that the results should be interpreted with caution because of the lack of data available to estimate key adult reproductive parameters. The similarity of the estimates of spawning biomass obtained in 2014 and 2019 reflects, at least in part, the fact that both estimates were based on adult reproductive data (especially spawning fraction) obtained from samples collected off South Australia. The use of the estimate of spawning fraction obtained from these samples (i.e., 0.135 (0.102–0.167 95% CI)) was justified by its relative similarity to the mean spawning fractions of 0.081 and 0.169 reported for a closely related species, i.e., Chub Mackerel (*Scomber japonicus*) off and California (Dickerson et al. 1992) and Japan (Shiraishi et al. 2009), respectively (also see Appendix 1 for a list of estimates of spawning fraction obtained in other studies of *Scomber* spp.). Because no other estimates of spawning fraction are available of Blue Mackerel in Australian waters there was also no alternative but to use the South Australian data.

The lack of information available on the spawning fraction of Blue Mackerel off eastern Australia is important because uncertainty in this parameter has a strong effect on the reliability of estimates

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

of spawning biomass obtained using the DEPM, especially for species with low spawning fractions (e.g., Stratoudakis et al. 2006). Sensitivity analyses presented in Ward et al. (2021a) showed that varying spawning fraction across the range of values that have been reported for Chub Mackerel (*Scomber japonicus*) off Japan (i.e., 0.087 to 0.386) resulted in a more than threefold variation in estimates of spawning biomass, i.e., from approximately 140,000 t to 40,000 t. If the actual spawning fraction of Blue Mackerel off eastern Australia during the previous surveys was higher than the assumed value of 0.135, then the spawning biomass would have been lower than the estimates provided by Ward et al. (2015, 2021b), and vice versa.

The lack of information available on the reproductive biology of Blue Mackerel off eastern Australia reflects the limitations of previous sampling programs. Blue Mackerel in Australian waters can attain lengths up to 650 mm (Hutchins and Swainston 1986) and ages of 8+ years (Stevens et al. 1984;). Blue Mackerel in New Zealand have been reported to attain more than 20 years of age (Marriott and Manning 2011). Samples collected from the east coast of Australia have been obtained mainly from vessels operating in inshore waters and dominated by small (<300 mm FL, Ward et al. 2015, 2021, 2023), young (≤ 2 years old, Stewart and Ferrell 2001; Ward and Rogers 2007) fish. Only a few larger, older fish from offshore waters have been collected through opportunistic sampling by commercial fishers (John Stewart, NSW DPI, unpublished data). The lack of data available from larger, older fish constrains our knowledge of the growth rates, longevity, reproductive biology and spawning biomass of Blue Mackerel off eastern Australia.

Samples collected from inshore waters off NSW by Ward et al. (2021) included female fish that were around the mean size at maturity off South Australia (i.e., ~287 mm, Rogers et al. 2009) and macroscopically mature (i.e., ovaries with yolked oocytes), but their ovaries displayed no histological signs of recent spawning (i.e., post-ovulatory follicles, POFs), so the spawning fraction was zero. Low spawning rates inshore and high spawning rates offshore have been observed in other species in the genus *Scomber*. For example, the spawning fraction of the western stock of Atlantic Mackerel (*Scomber scombrus*) to vary from 0.1 in inshore waters to around 1.0 offshore (Priede et al. 1995). The spawning rates of *Scomber* species also vary with latitude. For example, the spawning fraction of Atlantic Mackerel varies from 0.18 in the (cool) north, through 0.34 in the central region

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

to 0.62 in the (warmer) south (Priede and Watson 1993). This suggests that the spawning fraction of Blue Mackerel off eastern Australia (25-34°S) could be higher than off South Australia (32-36°S).

Estimates of spawning fraction are strongly influenced by assumptions about how long POFs persist in ovaries (e.g., Ganiyas 2012). The estimate of spawning fraction provided by Ward et al. (2009) was based on the assumption that POFs persist in ovaries of Blue Mackerel for two days. However, in some scombrids, such as Skipjack Tuna, *Katsuwonus pelamis*, (Hunter et al. 1986) and Yellowfin Tuna, *Thunnus albacares*, (Schaefer, 1996), POFs can degenerate in one day or less.

1.2 Need

At its meeting in December 2020, the SPF RAG highlighted the need “to collect adult Blue Mackerel in order to obtain parameters used to calculate stock biomass” and listed this a high research priority ([July 2021 SPF Resource Assessment Group Meeting 03](#)). The RAG considered that this project was needed to provide a more robust and accurate stock assessment and ensure confidence in setting of TACs for Blue Mackerel East.

The project was assessed as relatively high-risk, with the main risk being that large spawning fish would not be caught in sufficient numbers to provide robust estimates of spawning fraction. For this reason, the project was to be conducted in two stages. The first stage (AFMA Project RR2021/0810, Ward et al. 2023) focused on establishing an effective sampling method and identifying sampling locations. Stage 1 was successful and had three important outcomes:

1. Established methods for sampling large adult Blue Mackerel in offshore waters
2. Identified several locations at different latitudes where samples could be collected
3. Developed preliminary estimates of spawning fraction off eastern Australia

The study also confirmed that the main spawning season of Blue Mackerel off eastern Australia extends from August to October and that the spawning area extends from Sandy Cape to about Wollongong. Ward et al. (2023) also emphasized the strong effect that ongoing uncertainties about how long POFs persist in ovaries of Blue Mackerel had on estimates of spawning fraction.

Objectives

This objectives of Stage 2 of this project were to:

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

1. Apply the methods for sampling large adult Blue Mackerel developed in Stage 1 at four locations along the east coast.
2. Determine the period of time that POFs remain present in the ovaries of Blue Mackerel off eastern Australia (additional objective)
3. Investigate the hypothesis that spawning season of Blue Mackerel varies with latitude off eastern Australia and identify implications for the design of future DEPM surveys.
4. Estimate the spawning fraction of Blue Mackerel off eastern Australia and assess the need for additional adult sampling in future DEPM surveys

Samples collected in 2024 (Appendix 2) are not analysed in the current report. Those results will be presented in the conjunction with the results of the ichthyoplankton survey for Blue Mackerel that was conducted off eastern Australia in September 2024 (AFMA 2024/0806).

2. Methods

2.1 Adult sampling and initial processing

In 2022 and 2023, Blue Mackerel were taken by line-fishing at Tweed Heads, Coffs Harbour and Port Stephens (Figure 2.1) using the methods described by Ward et al. (2023). Fish were dissected at the end of each sampling trip to determine their sex and reproductive status. Ovaries were removed from mature females (Stage III-V) and preserved in a 10% buffered formaldehyde solution (Figure 2.2). Males, immature females and carcasses of dissected females (individually labelled) were frozen and transported to the Institute of Marine and Antarctic Studies (IMAS, Hobart, Tasmania) for further processing.

Fish taken by the purse-seine vessels operating from Wollongong (Figure 2.1) were placed on ice and transported to Sydney Fish Markets. Samples taken from the markets were processed at the Sydney Institute of Marine Science. Ovaries were removed from mature females (Stage III-V) and preserved in 5% buffered formaldehyde solution (Figure 2.2). Males and immature females were processed as described above. Preserved ovaries of all fish were transported to IMAS for processing.

2.2 Gonosomatic Index

All fish were measured (fork length, nearest mm) and weighed (nearest g) and ovaries were staged (Figure 2.1) and weighed (± 0.01 g). The gonosomatic index (*GSI*) was estimated using the equation:

$$GSI = \left(\frac{GW}{BW_{GF}} \right) * 100$$

where *GW* is gonad weight (g) and *BW_{GF}* is gonad-free female weight.

2.3 Histological processing

Preserved ovaries were processed using standard histologically procedures, embedded in paraffin, cut into 4 μ m sections, and stained using haematoxylin and eosin. Histological sections were examined using an Imaging Compound Fluorescence Leica DMLB2 Microscope connected to a camera (Leica Microsystems Ltd, CH-9435 Heerbrugg, DFC450 C). Images were analysed using Leica Application Suite Version 4.

Figure 2.1 Locations where adult Blue Mackerel were collected in 2022 and 2023 (pink stars) are shown in relation to distributions of eggs (circles), trawl locations (red dots) and Sea Surface Temperatures recorded in DEPM surveys conducted in 2014 and 2019. (Source: Ward et al. 2015, 2021b).

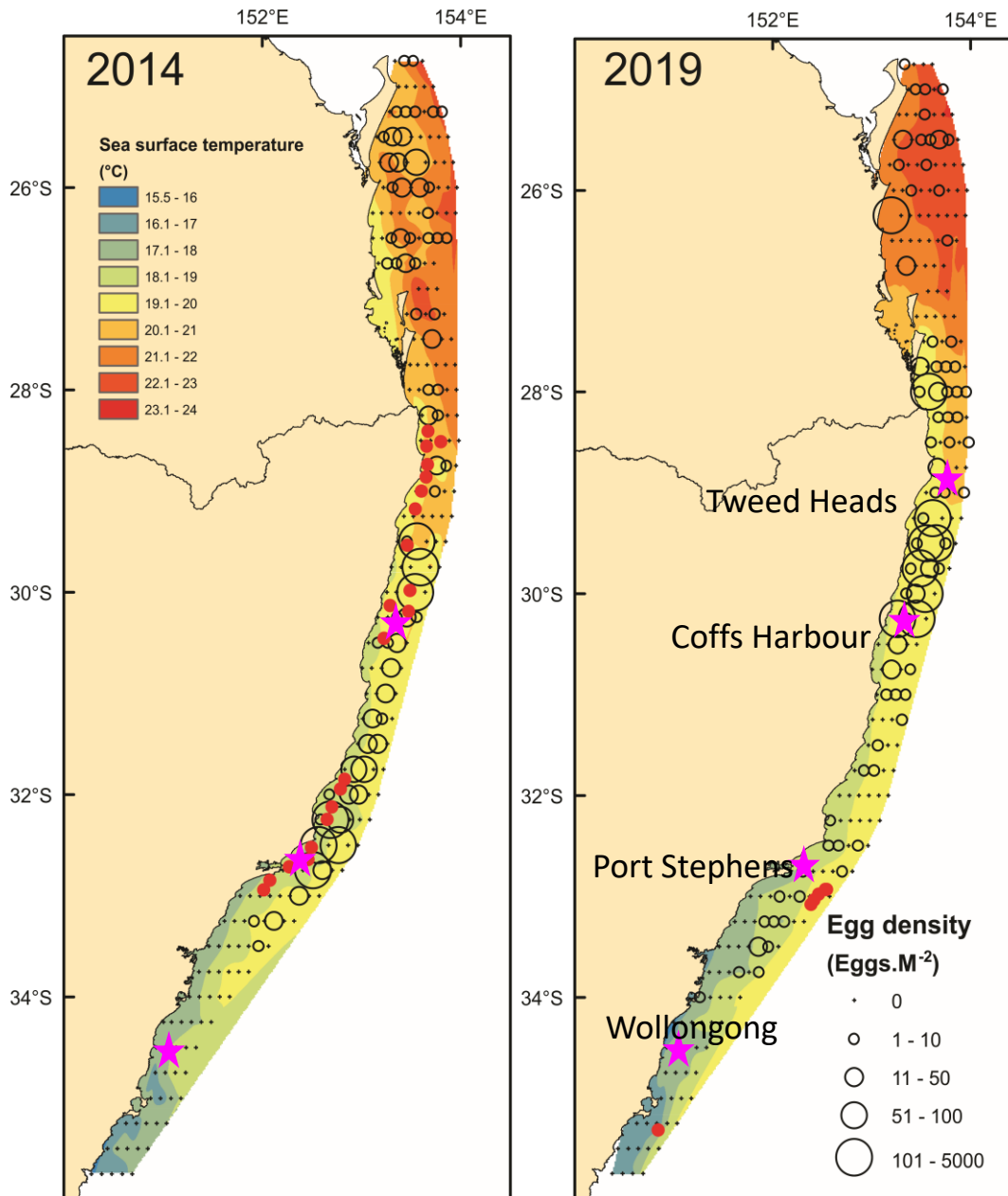
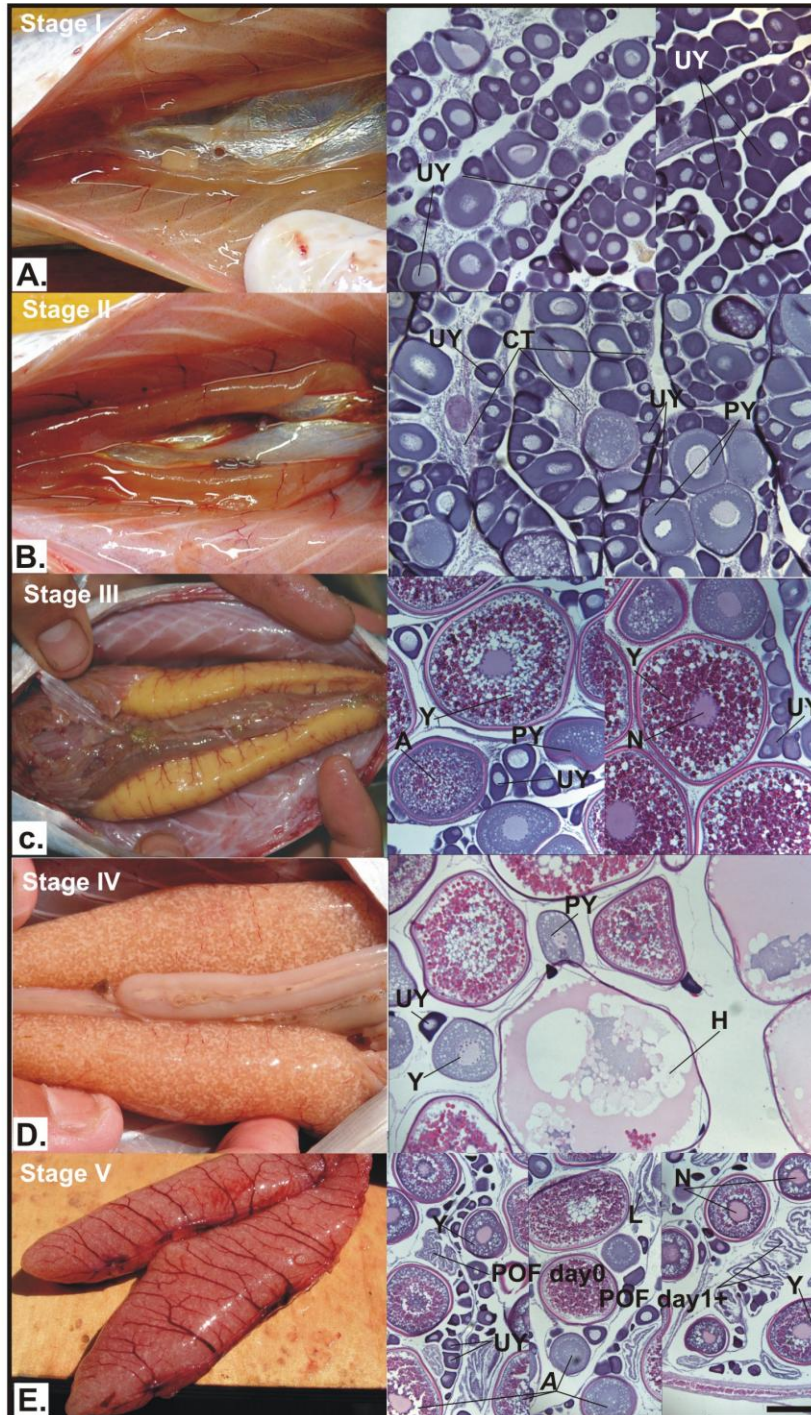


Figure 2.2. Macroscopic stages I–V (1-5) and their microscopic characteristics. **A:** Immature Stage I (1) ovary with developing unyolked (UY) oocytes. **B:** Maturing Stage II (2) ovary with UY and partially yolked (PY) oocytes. **C:** Mature Stage III (3) ovary with UY, PY and fully yolked oocytes (Y). **D:** Hydrated Stage IV (4) ovary with UY, PY and partially hydrated oocytes (H). **E:** Spent Stage V (5) ovary with post-ovulatory follicles (POFs). Scale bar = 150µm. Source: Rogers et al. (2009), *Marine and Freshwater Research*.



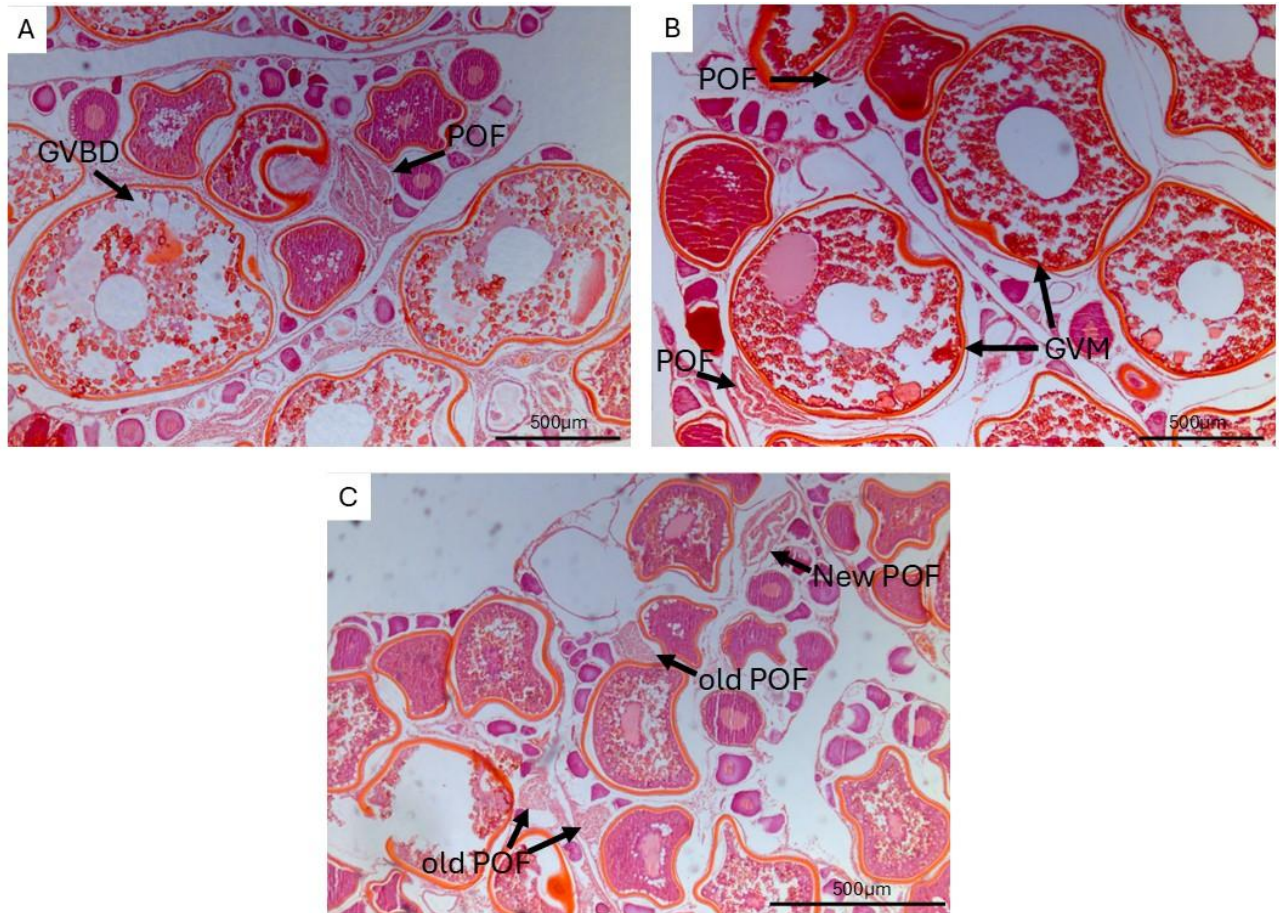
2.4 Staging and ageing postovulatory follicles (POFs)

The developmental stage of each ovary was determined from the most advanced oocyte mode present including cortical alveoli (CA), different stages of vitellogenesis (VTG1, VTG2, VTG3), germinal vesicle migration (MN1, MN2), germinal vesicle break down (GVBD) and hydration (HYD) (see Brown-Peterson et al. 2011 for details). For examining the spawning cycle, the earliest stage of migratory nucleus oocytes was characterised as MN0. Histological sections of each ovary were examined to identify presence/absence of POFs. POFs were initially classified into daily cohorts based on studies by Dickerson et al. (1992) and Shiraishi et al. (2009) for *S. japonicus*; Rogers et al. (2009), and Ward et al. (2021, 2023) for *S. australasicus*; and Hunter and Macewicz (1985) for northern anchovy *Engraulis mordax*.

No experimental studies have been done to investigate the duration of POFs in *S. australasicus*. However, Dickerson et al. (1992) created a conceptual model (Appendix 3) of the degeneration and resorption process of POFs of *Scomber japonicus* based on laboratory studies conducted at 20°C, which is similar to the SSTs recorded between Tweed Heads and Port Stephens during the plankton surveys conducted in 2014 and 2019, and where most samples were collected during the present study (Figure 2.1). Dickerson et al. (1992) observed the coexistence of up to two daily POF cohorts in *S. japonicus*. More than one daily POF cohort was observed in ovaries of *S. australasicus* collected in this study (Figure 2.3).

To assess the suitability of the conceptual model proposed by Dickerson et al. (1992) for *S. australasicus*, we investigated the duration of POFs by determining the number of POF cohorts present in ovaries. The rationale underpinning this approach is that: if POFs persist for 24 hours, only one daily POF cohort would be present; if POFs persist for 24-48 hours two daily cohorts would be present; and if POFs last for more than 48 hours, more than two cohorts would be present.

Figure 2.3. Histological sections of *S. australasicus* ovaries showing coexistence of recent postovulatory follicles (POFs) and imminent spawning markers (e.g., germinal vesicle break-down oocyte stage, GVBD; late terminal vesicle migratory oocyte stage, GVM or MN2). A) GVBD oocytes together with POFs. B) GVM oocytes with POFs. C) Coexistence of newer and older POFs in a late vitellogenic ovary.



BLUE MACKEREL SPAWNING FRACTION: STAGE 2

The Weibel stereological method (Weibel et al. 1966) was used to estimate the relative number of POFs in each ovary, and the number of daily POF cohorts during the spawning cycle (i.e., between different spawning events) (Figure 2.4). The rationale was that because POFs are remnants of oocytes after ovulation, the relative number of POFs present should be equal to the relative number of oocytes that were released (i.e. relative batch fecundity). This approach has been used in Atlantic sardine, *Sardina pilchardus* and Atlantic mackerel, *Scomber scombrus* (Charitonidou et al. 2020), and albacore (*Thunnus alalunga*) (Saber et al. 2015).

The Weibel method is based on the Delesse Principle that the fractional area of a particle (or group of particles) measured in a random section of a structure (e.g. tissue) equals the fractional volume (V_i) of that particle in the same structure. The V_i of POFs was estimated from histological micrographs obtained using the image analysis software imageJ (Schneider et al. 2012) applying a standard grid of 256 points within 4 or 5 counting fields of 5mm^2 each (see Charitonidou et al. 2020) (Figure 2.4). The number of POFs in V_i (N_{vi}) was estimated using in the following equation:

$$N_{vi} = \frac{K}{\beta} * \frac{Na_i^{\frac{3}{2}}}{V_i^{\frac{1}{2}}}$$

where Na_i is the number of POFs sectioned per unit of area, and β and K are coefficients reflecting POF shape and size distribution, respectively (Emerson et al. 1990; Charitonidou et al. 2020). The number of POFs was estimated as N_{vi} times ovary volume. Ovary volume was estimated by dividing ovarian weight by the assumed ovarian specific gravity of 1.026 (Ganias et al. 2015). The relative number of POFs was calculated by divided the number of POFs by the total body weight.

Figure 2.4. Application of Weibel stereological method on the ovaries of *S. australasicus* using Imagej.

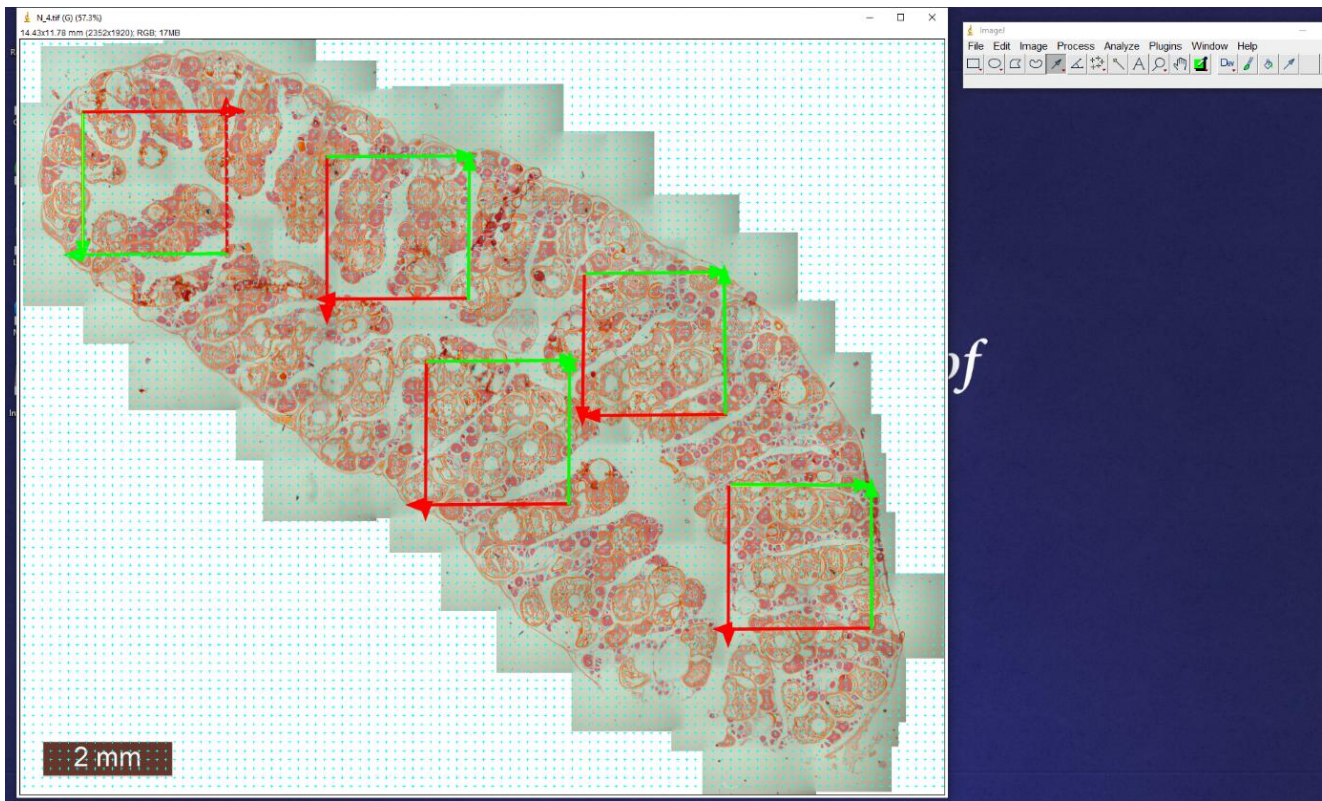
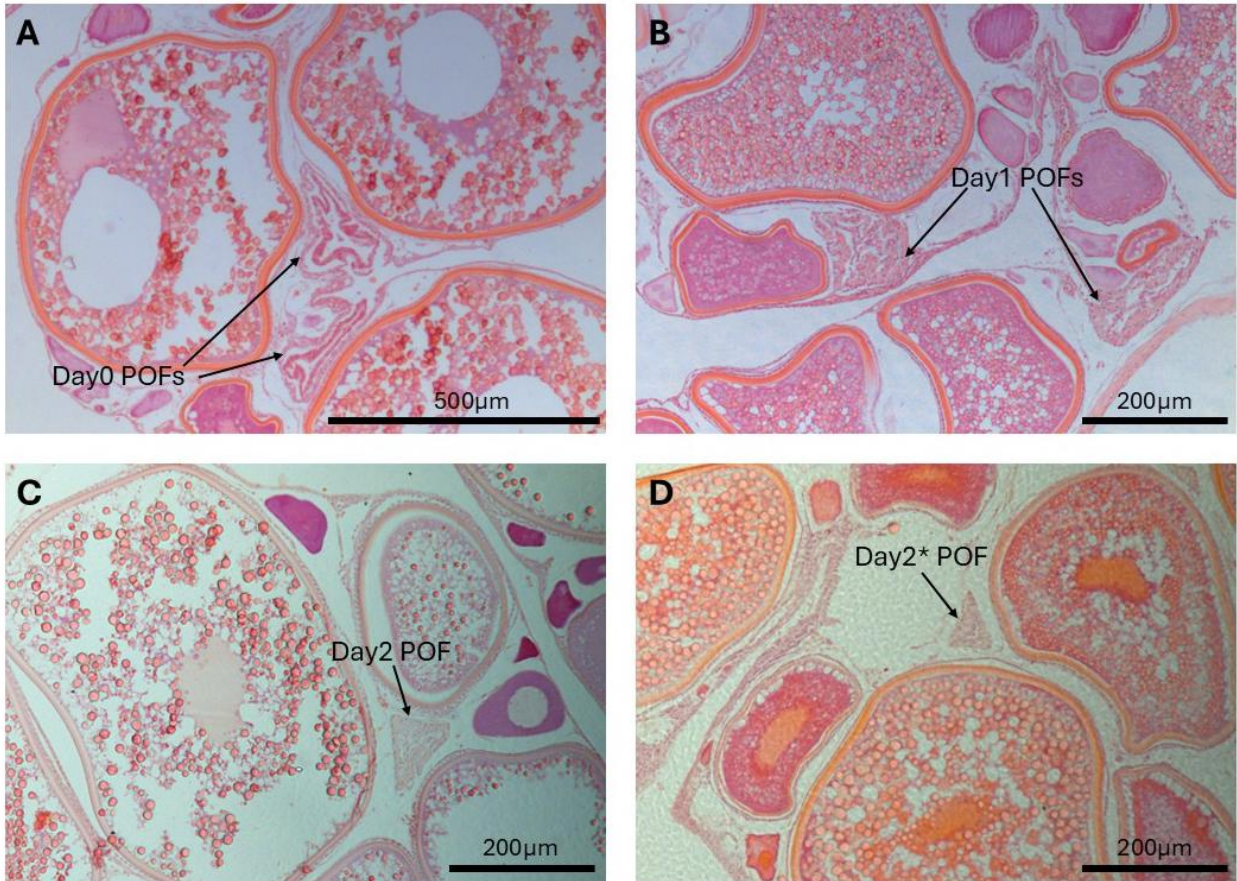


Figure 2.5 Histological sections of *S. australasicus* ovaries including different daily POF cohorts. A) Day0 POFs in late migratory nucleus stage (MN2) ovary. B) Day1 POFs in early migratory nucleus stage (MN1) ovary. C) Day2 POFs in early migratory nucleus stage (MN1) ovary. D) Day2* POFs in Early Migratory nucleus stage (MN1) ovary.

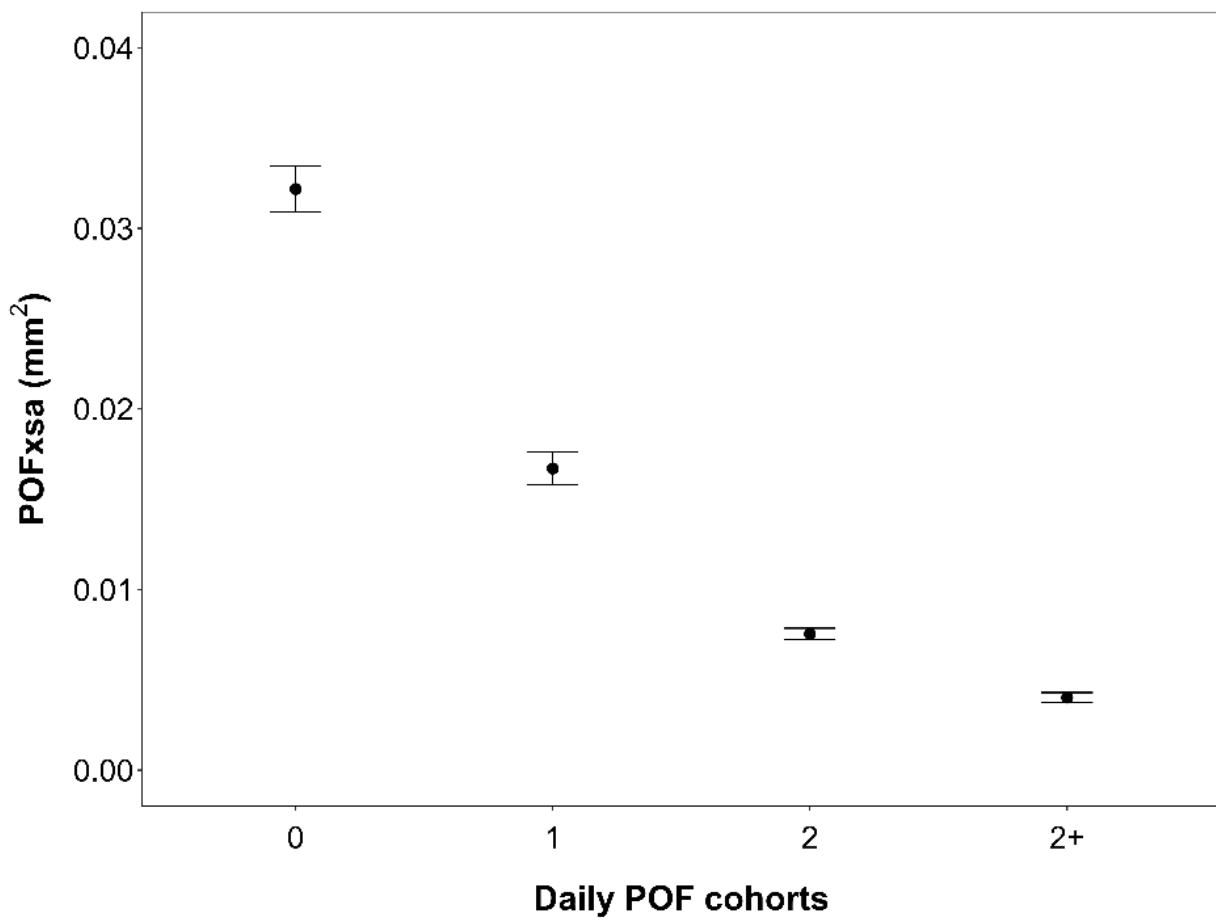


BLUE MACKEREL SPAWNING FRACTION: STAGE 2

A total of 21 ovarian histological sections from Port Stephens 2022 were analysed using the stereological method and covering ovarian dynamics throughout the spawning cycle (see Dickerson et al. 1992). The ovaries analysed were in different developmental stages including the very early migratory nucleus stage (MN0) (n=9) with oocytes with fused oil droplets still located around the nucleus, the early migratory nucleus stage (MN1) (n=6) with more advanced fusion of oil droplets, fewer in number than before, larger in size and unevenly located on one side of the nucleus, and late stage migratory nucleus stage (MN2) (n=6), with nucleus at the animal pole and a large oil droplet at the vegetal pole.

Ovaries were assigned to daily cohorts based on the most recent and largest POFs (Charitonidou et al. 2020). The newest (up to approximately 10 hours old) and largest POFs were assigned as Day0 (as per Dickerson et al. 1992, Appendix 3). The main characteristics of Day0 POFs were granulosa cells arranged in a single narrow line, large lumen size and theca stretched and separated from the granulosa cells (Figure 2.4A). POFs up to 24 hours old were assigned as Day1 (Dickerson et al. 1992). Day1 POFs were not as large as Day0 POFs and more compact, with the lumen small or absent, granulosa cells more disordered than before, and the theca closer to the granulosa (Figure 2.5B). POFs assigned as Day2, with duration between 24-33 hours (Dickerson et al. 1992), were half size than Day0 POFs, more compact, with a smaller lumen with some blood cells present, the theca layer remaining as a distinct thick band of cells, granulosa cells having degenerated more and vacuoles present (Figure 2.5C). POFs assigned as Day2*, with a duration 33-48 hours (Dickerson et al. 1992), were smaller than Day2 with thicker theca and few granulosa cells and were sometimes difficult to distinguished from atresia β (Figure 2.5D). The area of the largest POF in each section was measured from photomicrographs. The mean size of each daily POF cohort (i.e., Day0, Day1, Day2, and Day2* POFs) is shown in Figure 2.6.

Figure 2.6. Mean measured area of the recent spawning marker, postovulatory follicle (POF_{xsa}, mm²) assigned to each daily POF cohort.



2.5 Estimating spawning fraction

Spawning fraction was estimated using two approaches.

Firstly, spawning fraction in each sample (S_i) was estimated from the total number of all daily POF cohorts present in the sample, based on the assumption that POFs remain visible in the ovaries up to 48 hours, and following the equation:

$$S_i = (Day0 + Day1 + Day2 + Day2^*) / (Time_D * ni)$$

where, $Day0$, $Day1$, $Day2$, and $Day2^*$ are the numbers of fish assigned to each specific daily POF cohort, $Time_D$ is the assumed duration of POFs in the ovaries, ni is the number of fish in each sample.

Secondly, the previous equation was applied to the sum of all daily POF cohorts except for the small POFs assigned as $Day2^*$. The rationale for this approach is that if small POFs $Day2^*$ are excluded then the duration of POFs in the ovaries should be set at approximately 33h following Dickerson et al. (1992). This approach removes the potential for confusion of $Day2^*$ POFs with atresia β .

The mean spawning fraction of the population was calculated from the average of sample means weighted by the proportional sample size, using the following equation:

$$S = \frac{\sum[S_i * ni]}{N}$$

Where, S_i is the spawning fraction of each sample, ni is the number of fish in each sample, N is the total number of fish collected in all samples and S is the estimate mean spawning fraction for the population.

The coefficients of variation (CV) for the spawning fraction parameter were calculated from the following equation:

$$CV = \frac{\sqrt{var(S)}}{S}$$

3 Results

3.1 Gonosomatic Indices

The monthly gonosomatic indices (GSI) estimated from samples collected during this study are shown in Figure 3.1. Mean monthly GSIs in 2022 and 2023 combined were above 3.5 in August, September and October which supports previous findings that suggest the peak spawning season occurs during these months (Figure 3.1). There was a high level of variation of mean GSI within and among sampling locations and months (Table 3.1).

Figure 3.1 Gonosomatic index (GSI) by month in both years (A), and in the different locations (colour), and years (shape) (B).

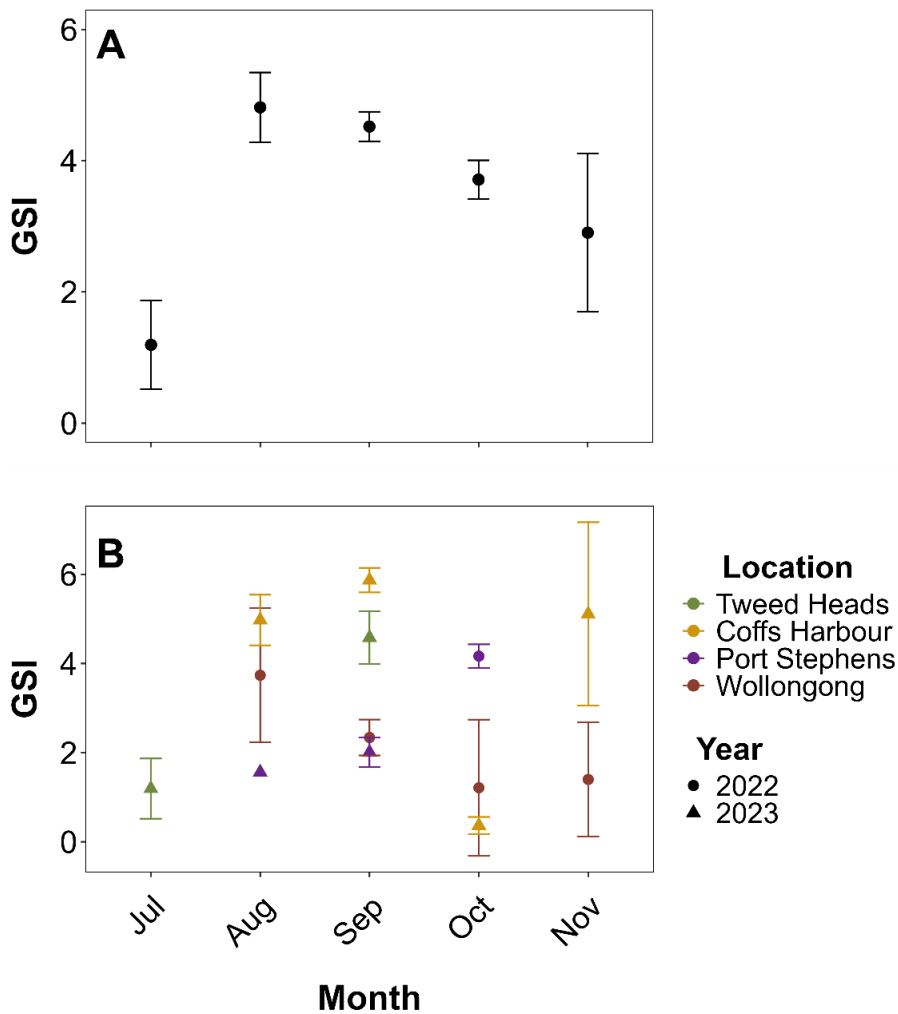


Table 3.1 Mean monthly gonosomatic index (GSI) by year and Location. The total number of fish, the mean GSI, the standard error (SE), and the lower and upper bounds (95% CI) are also presented.

Location	Year	Month	No. fish	Mean GSI	SE	Lower bound	Upper bound
Tweed Heads	2023	Jul	14	1.2	0.312	0.5	1.9
Tweed Heads	2023	Sep	103	4.6	0.298	4.0	5.2
Coffs Harbour	2023	Aug	60	5.0	0.283	4.4	5.5
Coffs Harbour	2023	Sep	409	5.9	0.140	5.6	6.1
Coffs Harbour	2023	Oct	18	0.4	0.091	0.2	0.6
Coffs Harbour	2023	Nov	8	5.1	0.871	3.1	7.2
Port Stephens	2022	Oct	155	4.2	0.134	3.9	4.4
Port Stephens	2023	Aug	1	1.6	-	-	-
Port Stephens	2023	Sep	158	2.0	0.169	1.7	2.3
Wollongong	2022	Aug	6	3.7	0.586	2.2	5.2
Wollongong	2022	Sep	74	2.3	0.202	1.9	2.7
Wollongong	2022	Oct	4	1.2	0.481	0.3	2.7
Wollongong	2022	Nov	8	1.4	0.543	0.1	2.7

3.2 Duration of POF cohorts

The results of the Weibel analysis suggest that the spawning dynamics of *S. australasicus* conforms to the conceptual model which Dickerson et al. (1993) developed for *Scomber japonicus* that indicated that POFs remain visible in the ovaries for approximately two days. Ovaries in the very early migratory nucleus stage (MN0) appeared to contain two daily cohorts of POFs (Figure 3.2A). The relative number of POFs of 301.7 (± 70.4) was approximately twice the relative batch fecundity of 139.9 (136.3-143.5) oocytes/gram estimated by Ward et al. (2021). As ovarian development progressed, the older POF cohort appeared to be reabsorbed and only one cohort remained in MN1 ovaries. The relative number of POFs of 117 POFs/gram in MN1 ovaries approximately matches the relative batch fecundity of 139.9 (136.3-143.5) oocytes/gram estimated by Ward et al. (2021).

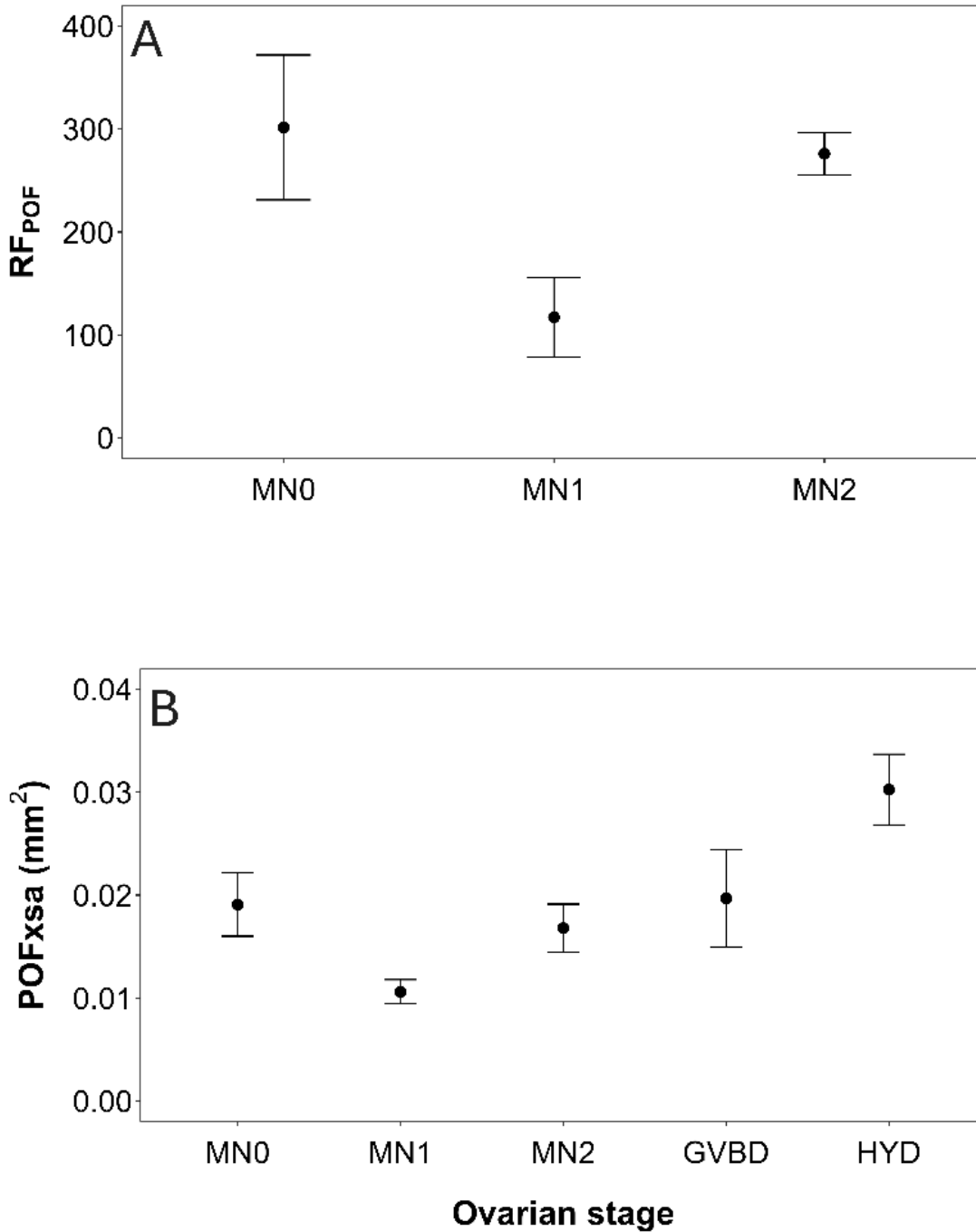
There was also a reduction in POF area of the MN1 stage compared to the MN0 stage (Figure 3.2B). At the MN2 stage, two daily POF cohorts appeared and the mean POF area increased again (Figure 3.2A, B), which suggests that some oocytes from this batch may have already been released. Based on developmental timing, spawning would be expected to begin within 4–6 hours after the appearance of MN2 at the ambient temperature of 20°C (Kurita et al. 2011). The morphological degeneration characteristics suggest that up to two different daily cohorts were present in the ovaries. These results provide evidence that the duration of POFs in *S. australasicus* exceeds 24 hours and does not exceed 48 hours. These findings suggest that the spawning dynamics of *S. australasicus* may conform to the conceptual model which Dickerson et al. (1992) developed for *S. japonicus*.

3.3 Estimates of the spawning fraction

The number of ovaries with Day0, Day1, Day2 and Day2*POFs found at each location sampled in 2022 and 2023 are shown in Table 3.2. Estimates of spawning fraction calculated from POFs that last for 48 hours (i.e., Day0, Day1, Day2 and Day2*POFs) and POFs that last for 33 hours (i.e., Day0, Day1, and Day2 POFs) are shown in Figure 3.3. The two methods provided similar results. For example, the overall estimates of spawning fraction from POFs that last for 48 hours of 0.121 (± 0.043 95%CI) was slightly lower from POFs that last for 33 hours of 0.151 (± 0.057 95%CI). Similar estimates of spawning fraction were obtained using POFs that last for 48 hours in 2022 and 2023 i.e. 0.114 (± 0.075 95% CI) and 0.128 (± 0.063 95% CI), respectively

Estimates of spawning fraction differed among months and locations. However, due to the opportunistic nature of the sampling and limited samples sizes the significance of these differences could not be tested statistically and these differences may not be representative of the patterns of spatial and temporal variation in the population. The two lowest estimates of spawning fraction from POFs that last for 48 hours were 0.035 (± 0.029 95% CI) for Wollongong in 2022 and 0.041 (± 0.025 95% CI) for Tweed Heads in 2023. The two highest estimates of spawning fraction from POFs that last for 48 hours were 0.258 (± 0.139) for Port Stephens in 2022 and 0.151 (± 0.097) for Coffs Harbour in 2023. The overall mean spawning fraction for Coffs Harbour and Port Stephens combined, which are both areas where large numbers of eggs have been collected in previous DEPM surveys (see Figure 2.1), was 0.138 (± 0.055).

Figure 3.2. The mean relative number of postovulatory follicles (RF_{POF}) (A), and the mean measured POF area (POF_{Xsa}) (B) in each ovarian developmental stage. MN0= very early stage of migratory nucleus, MN1= early migratory nucleus stage, MN2= late migratory nucleus stage, GVBD= germinal vesicle breakdown stage, HYD= hydrated stage.



BLUE MACKEREL SPAWNING FRACTION: STAGE 2

Table 3.2. The number of ovaries with POFs assigned in each daily POF cohort (Day0, Day1, Day2 and Day2*), the number of migratory nucleus (MN) oocytes, and the total number of fish analysed are shown for each location.

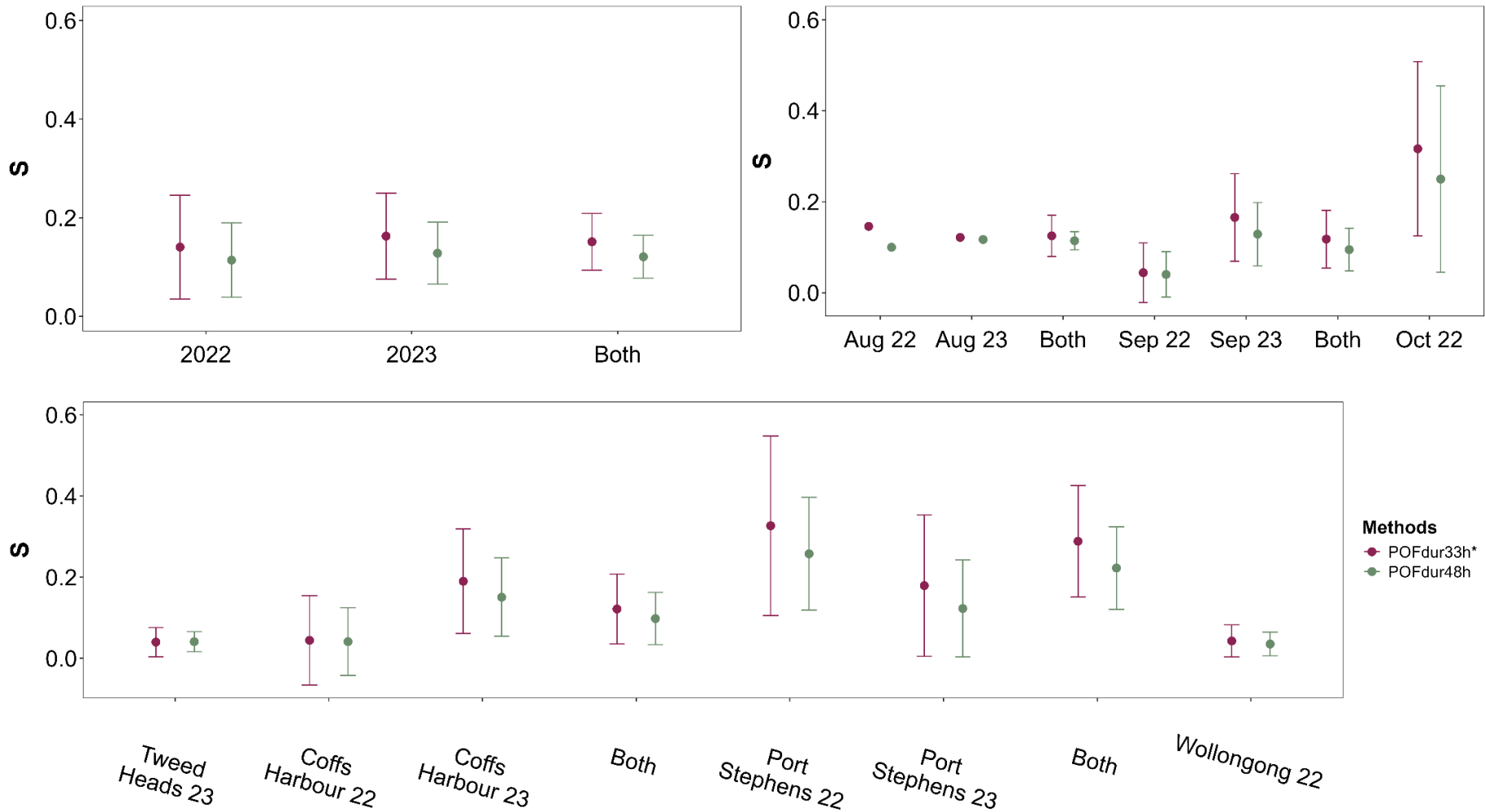
Location	Date	POF Day0	POF Day1	POF Day2	POF Day2*	No.Total POFs	No. Total MN	No. Total Fish
	2022							
Tweed Heads	25/08/2022		1	2		3	10	32
	7/09/2023						3	5
	18/09/2023				1	1	2	16
	30/09/2023	1			1	2	9	20
	Total	1	1	2	2	6	24	73
	2022							
Coffs Harbour	17/09/2022			7	1	8	8	18
	18/09/2022		1	3	1	5	39	69
	19/09/2022						48	69
	19/09/2022			2	2	4	16	25
	20/09/2022		1		1	2	31	49
	Total	0	2	12	5	19	142	230
	2023							
Coffs Harbour	31/08/2023	1	1	3	2	7	14	30
	4/09/2023	3				3	11	30
	6/09/2023	1	4	16	1	22	22	45
	12/09/2023							33
	26/09/2023			30	4	34	27	53
	27/09/2023	8	4	11	6	29	65	124
	Total	13	9	60	13	95	139	315

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

Location	Date	POF Day0	POF Day1	POF Day2	POF Day2*	No.Total POFs	No. Total MN	No. Total Fish
	2022							
Port Stephens	25/08/2022		1			1		1
	4/10/2022	3	2	3	2	10	7	15
	5/10/2022	26	6	14	2	48	54	67
	11/10/2022	2	2	8	5	17	25	43
	12/10/2022	1	1	4	2	8	23	37
	Total	32	12	29	11	84	109	163
	2023							
Port Stephens	26/08/2023							1
	6/09/2023							22
	15/09/2023	12	2			14	9	29
	18/09/2023						1	4
	27/09/2023							1
	Total	12	2	0	0	14	10	57
	2022							
Wollongong	25/08/2022		1			1	1	5
	6/09/2022						2	3
	14/09/2022		1	1	1	3	1	23
	21/09/2022		1			1		12
	28/09/2022		1			1	4	30
	26/10/2022						1	4
	18/11/2022						1	8
	Total	0	4	1	1	6	10	85
	Grand Total	58	30	104	32	224	434	923

BLUE MACKEREL SPAWNING FRACTION: STAGE 1

Figure 3.3. Spawning fraction estimated by two methods, POFdur33h* (red), POFdur48h (green) by year, by year and month, and by year and both years combined (both) in Coffs Harbour and Port Stephens.



4. Discussion

This study resolved several important technical limitations and key knowledge gaps related to the application of the DEPM to Blue Mackerel off eastern Australia. Stage 1 of the study developed effective methods for sampling large spawning Blue Mackerel off eastern Australia and identified several locations where samples could be collected reliably. Stage 2 resolved uncertainties related to the time that POFs persist in the ovaries and as a result provides the most well justified estimates of spawning fraction currently available for Blue Mackerel in Australian waters.

The results of this study show that the assumption of Rogers et al. (2009) and Ward et al. (2009) that POFs persist for two days in the ovaries of Blue Mackerel in Australian waters was appropriate. The estimates of spawning fraction obtained using the all of the samples collected in this study and POFs that last for 48 hours of 0.121 (± 0.043 95%CI) is similar to the estimate of 0.135 (0.102–0.167 95% CI) obtained from southern Australia by Ward et al. (2009), which is the value that has been used in previous applications of the DEPM to Blue Mackerel off eastern Australia (Ward et al. 2009, 2015, 2021a). The overall mean value for the key spawning areas of Coffs Harbour and Port Stephens combined of 0.138 (± 0.055) is even more similar to the estimate of Ward et al. (2009). Applying the method of Ward et al. (2009) to samples collected in the present study provides the same value that we obtained using POFs that last for 48 hours. The method developed in this study that used POFs that last for 48 hours to estimate spawning fraction is effectively the same as the method used by Ward et al. (2009), even though the two studies used different systems to stage POFs. Both methods are underpinned by the premise that POFs persist in the ovaries for two days. This is the first study to demonstrate how long POFs in the persist in the ovaries of *S. australasicus*. This finding and the results obtained this study are major steps forward in the understanding of the reproductive biology of Blue Mackerel off eastern Australia

The only other estimates of spawning fraction available for *S. australasicus* are provided by Sinaga et al. (2021) who followed the methods described by Rogers et al. (2009) and Ward et al. (2009) and assumed that POFs persist in the ovary for two days. Estimates of spawning fraction provided by Sinaga et al. (2021) for north-eastern Taiwan for individual months during the peak spawning season ranged from 0.03 in March 2018 to 0.16 April 2017. Estimates of spawning fraction published for

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

other species of *Scomber* are based on large variety of different methods and are highly variable, i.e. 0.081 to 0.406 for *S. japonicus* and 0.12 to 0.50 for *S. scombrus* (Appendix 1).

In the present study, samples were collected from sites located along a latitudinal gradient during the peak spawning season (i.e. August to October). However, the number of samples collected was relatively small and there were high levels of variability in estimates of spawning fraction both within and among months and locations. The opportunistic nature of the sampling program also meant that potential effects of month and location on spawning fraction were confounded. As a result, the sampling regime did not have the statistical power to detect latitudinal or monthly differences in spawning fraction. However, it is notable that the lowest estimate of spawning fraction (0.035) was obtained from Wollongong, which is near the southern end of the known spawning area where few eggs have been collected in previous DEPM surveys (Figure 2.1). Similarly, the two highest spawning fractions were recorded at Port Stephens (0.258) and Coffs Harbour (0.151) which are locations where large numbers of eggs have been collected previously (Figure 2.1).

The strong similarity of estimates of spawning fraction obtained in this study and by Ward et al. (2009) suggests that spawning fraction of Blue Mackerel in Australian waters may be similar among regions. The similarity of the estimates obtained in 2022 of 0.114 (± 0.075 95% CI) and 2023 of 0.128 (± 0.063 95% CI) suggest that spawning fraction may also be relatively stable among years. As a result, the costs and benefits of conducting large-scale adult sampling programs in future applications of the DEPM to Blue Mackerel in Australian waters should be evaluated (see Ward et al. 2021b). The main argument for undertaking adult surveys in conjunction with each plankton survey is that if spawning fraction varies among years, then this should be measured because it will have a strong influence on the estimate of spawning biomass. The main argument against adult sampling, other than the additional costs, is that, as Ward et al. (2021b) demonstrated for Sardine off southern Australia, it can be difficult to obtain reliable estimate of spawning fraction in individual years. The need for adult sampling to be done in future applications of the DEPM to Blue Mackerel off eastern Australia will be evaluated in the report on the DEPM survey conducted in September 2024 (AFMA 2024/0806) that will present the results from adult sampling done in 2022 and 2023 (i.e. data in this report) and additional adult sampling done in 2024 (see Appendix 2).

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BLUE MACKEREL SPAWNING FRACTION: STAGE 2

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Appendix 1. Estimates of spawning fraction obtained in other studies

Species	Location	Year	Spawning Fraction	Method	Literature	Comments
<i>S. australasicus</i>	South Australia		0.091-0.5		Ward and Rogers 2007	
<i>S. australasicus</i>	South Australia	2002-2006	0.135 (ci=0.102-0.167)	POFDAY0+POFDAY1+POFDAY2 (48h POF duration)	Ward et al 2021	Spawning season
<i>S. australasicus</i>	South Australia	Feb 2002-August 2005	0.167	Hyd+POFDay0	Rogers et al. 2009	
<i>S. australasicus</i>	South Australia	Feb 2002-August 2005	0.143	POFsDay1+	Rogers et al. 2009	
<i>S. australasicus</i>	South Australia	Feb 2002-August 2005	0.125	Hyd+POFDay0+POFsDay1+	Rogers et al. 2009	
<i>S. australasicus</i>	South Australia	Feb 2002-August 2005	0.091-0.5	Hyd+POFDay0+POFsDay1+	Rogers et al. 2009	Spawning frequency Individual season range
<i>S. australasicus</i>	Northeastern Taiwan	2017	0.13	Hyd+POFDay0+POFsDay1+ (1,2 days before sampling)	Sinaga et al. 2021	23-26.6°C; entire season estimate
<i>S. australasicus</i>	Northeastern Taiwan	2018	0.05	Hyd+POFDay0+POFsDay1+ (1,2 days before sampling)	Sinaga et al. 2021	24-27.8°C; entire season estimate
<i>S. australasicus</i>	Northeastern Taiwan	2019	0.09	Hyd+POFDay0+POFsDay1+ (1,2 days before sampling)	Sinaga et al. 2021	24-27.8°C; entire season estimate
<i>S. australasicus</i>	Northeastern Taiwan	2017-2019	0.1	Hyd+POFDay0+POFsDay1+ (1,2 days before sampling)	Sinaga et al. 2021	entire season
<i>S. japonicus</i>	Southern California Bight	April- July 1985	0.081	MN	Dickerson et al. 1992	20oC
<i>S. japonicus</i>	Southern California Bight	April- July 1985	0.092	POFsDay1=10-33h POFs	Dickerson et al. 1992	20oC
<i>S. japonicus</i>	Southern California Bight	April- July 1985	0.087	Mean of POF and MN method	Dickerson et al. 1992	32 females with coexistence of spawning markers, S=0.769
<i>S. japonicus</i>	off Japan		0.091-0.5		Watanabe 1999	
<i>S. japonicus</i>			0.386 (0.090-0.620)		Watanabe & Nishida 2002	
<i>S. japonicus</i>	East China Sea around Goto Islands	June 2000-June 2001	0.169 (0.072-0.250)	4 Independent estimates based on the developmental stage	Shirashi et al. 2009	19oC
<i>S. japonicus</i>	East China Sea around the Goto Islands	June 2000-June 2001	0.189	GVM+POFDay0+POFDay1	Shirashi et al. 2009	
<i>S. japonicus</i>	East China Sea around the Goto Islands	June 2000-June 2001	0.25	GVM+POFDay0	Shirashi et al. 2009	
<i>S. japonicus</i>	East China Sea around the Goto Islands	June 2000-June 2001	0.4	POFDay1	Shirashi et al. 2009	
<i>S. japonicus</i>	East China Sea around the Goto Islands	June 2000-June 2001	0.406	GVM	Shirashi et al. 2009	

BLUE MACKEREL SPAWNING FRACTION: STAGE 2

Species	Location	Year	Spawning Fraction	Method	Literature	Comments
<i>S. japonicus</i>	Pacific subpopulation around the Izu Islands		0.174 (0.076-0.333)	MN+POF0+POF1	Yamada et al. 1998	Spawning season
<i>S. scombrus</i>	Western stock of Atlantic-Northern	March-1989 July	0.182	MN	Priede and Watson 1993	Spawning season
<i>S. scombrus</i>	Western stock of Atlantic-Central	March-1989 July	0.335	MN	Priede and Watson 1993	Spawning season
<i>S. scombrus</i>	Western stock of Atlantic-South	March-1989 July	0.621	MN	Priede and Watson 1993	Spawning season
<i>S. scombrus</i>	Inshore (over the Porcupine Bank to the west of Ireland)	May-Jun 1992	<0.1	MN	Priede et al. 1995	Spawning season
<i>S. scombrus</i>	Offshore (over the Porcupine Bank to the west of Ireland)	May-Jun 1992	~1	MN	Priede et al. 1995	Spawning season
<i>S. scombrus</i>	North Atlantic	March 2019	0.16	POFDAY1+POFDAY2	ICES, 2025	Spawning season
<i>S. scombrus</i>	North Atlantic	April 2019	0.21	POFDAY1+POFDAY2	ICES, 2025	Spawning season
<i>S. scombrus</i>	North Atlantic	2019	0.19	POFDAY1+POFDAY2	ICES, 2025	Spawning season
<i>S. scombrus</i>	North Atlantic	March 2022	0.21	POFDAY1+POFDAY2	ICES, 2025	Spawning season
<i>S. scombrus</i>	North Atlantic	April 2022	0.16	POFDAY1+POFDAY2	ICES, 2025	Spawning season
<i>S. scombrus</i>	North Atlantic	2022	0.18	POFDAY1+POFDAY2	ICES, 2025	Spawning season
<i>S. scombrus</i>	North Sea	2022	0.5	MN	ICES, 2025	Spawning season
<i>S. scombrus</i>	North Sea	2022	0.121	POF method	ICES, 2025	Spawning season

Appendix 2. Summary of samples collected to estimate the spawning fraction of Blue Mackerel off eastern Australia in 2024.

Catch Date	Region	Site	Shot#	Time Start	Depth	Females
10-Sep-24	Ballina	Lennox Head	B1	a.m.	64	9
11-Sep-24	Ballina	Ballina	B2	a.m.	60	30
23-Sep-24	Ballina	Ballina	B3	a.m.	60	34
08-Sep-24	Coffs Harbour	Patch near wave recorder	1	a.m.	70	40
08-Sep-24	Coffs Harbour	Wide 6 NW	2	a.m.	40	34
10-Sep-24	Coffs Harbour	Groper	3	5:45 am	30	20
10-Sep-24	Coffs Harbour	Groper	5	4:30 am	36	41
10-Sep-24	Coffs Harbour	Wide Bait Ground	4	9:00 am	40	16
11-Sep-24	Coffs Harbour	Patch	6	6:30 am	40	24
11-Sep-24	Coffs Harbour	Wave recorder	7	9:00 am	70	5
19-Sep-24	Coffs Harbour	Middle Ground	8	5:30 am	40	52
19-Sep-24	Coffs Harbour	wave recorder	9	9:30 am	70	25
20-Sep-24	Coffs Harbour	Middle Ground	11	a.m.	40	7
20-Sep-24	Coffs Harbour	Wave recorder	10	8:00 am	70	31
21-Sep-24	Coffs Harbour	Groper	12	a.m.	40	40
22-Sep-24	Coffs Harbour	Groper	13	a.m.	40	45
24-Sep-24	Coffs Harbour	Groper	14	a.m.	40	31
22-Sep-24	Port Macquarie	Crowdy Head Shoals	P1	6:00 am	20	32
23-Sep-24	Port Macquarie	Point Plommer	P2	5:30 am	16	38
	Total					554

Appendix 3. Conceptual model of developed of female *Scomber japonicus* developed by Dickerson et al. (1992).

DICKERSON ET AL.: SPAWNING FREQUENCY AND BATCH FECUNDITY OF *S. JAPONICUS*
 CalCOFI Rep., Vol. 33, 1992

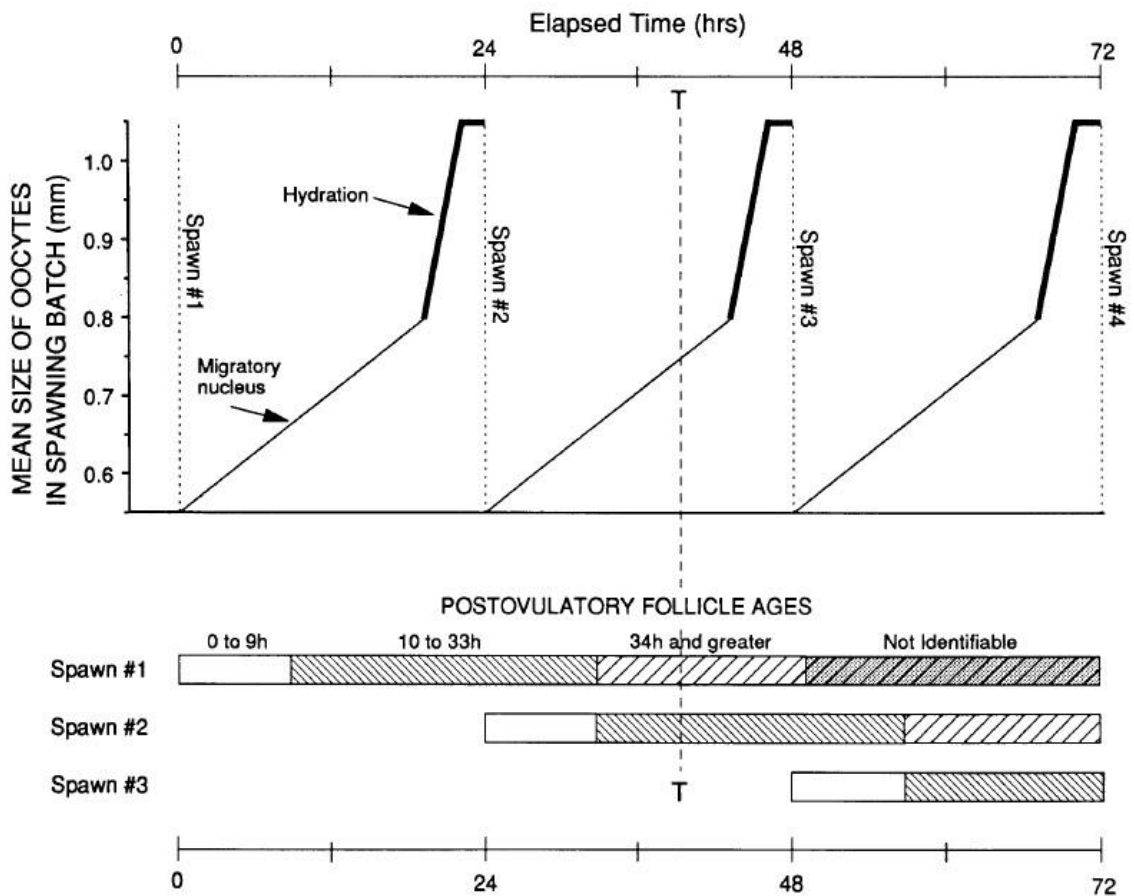


Figure 3. Conceptual diagram showing when various histological stages could be identified in an ovary of an *S. japonicus* female that spawned every day. Stages include migratory nucleus and hydration (0–24 hours before spawning), and postovulatory follicles 0–9 hours old, 10–33 hours old, and 34 hours and older. If a female is collected at time T in the cycle, three spawnings can be identified in the ovary: oocytes with migratory nuclei for spawn #3; 14-hour-old postovulatory follicles from spawn #2; and postovulatory follicles 38 hours old from spawn #1. Stippled area indicates period when postovulatory follicles may be confused with late β atresia.