Developing a fishery independent estimate of biomass for Snapper (*Chrysophrys auratus*)

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Abbreviations

ACRONYM	MEANING
CPUE	Catch Per Unit Effort
DEPM	Daily Egg Production Method
DNA	Deoxyribonucleic Acid
ETOH	Ethanol
FAACC	Formalin Acetic Acid Calcium Chloride
FRDC	Fisheries Research and Development Corporation
FTE	Full Time Employee
GAM	Generalised Additative Model
GIS	Geographical Information System
GSV	Gulf St. Vincent
HRP-DNA	Horseradish Perioxidase
ISH	In Situ Hybridisation
MSF	Marine Scalefish Fishery
NGSV	Northern Gulf St. Vincent
NSG	Northern Spencer Gulf
NSW	New South Wales
PIRSA	Primary Industries and Regions, South Australia
POF	Post Ovulatory Follicles
PPB	Port Phillip Bay
Qld	Queensland
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SA	South Australia
SAFCOL	South Australian Fisherman's Co-Operative Limited
SARDI	South Australian Research and Development Institute
SE	South East
SG	Spencer Gulf
SG/WC	Spencer Gulf/West Coast
SGSV	Southern Gulf St. Vincent
SSG	Southern Spencer Gulf
Vic	Victoria
VNN	Veronoi Natural Neighbour
WA	Western Australia
WC	West Coast
WVS	Western Victorian Stock

Executive Summary

Overview

This study was undertaken by the South Australia Research and Development Institute (SARDI). Through overcoming considerable technical challenges, this study was the first to successfully develop a relatively non-destructive molecular probe that can reliably identify Snapper (*Chrysophrys auratus*) eggs and larvae in mixed ichthyoplankton samples. This highly-specific molecular probe targets Snapper ribosomal (r)RNA and when conjugated with a reactive molecule produces a highly visible blue colour in positive reactions. Snapper eggs are subsequently easily detected using a standard stereo dissecting microscope. This novel use of an established molecular technique has re-invigorated the capability of using the daily egg production method (DEPM) to provide a fishery-independent estimate of spawning biomass for Snapper and has also increased its applicability to other species where egg identification has been problematic. This research has reduced the need to exclusively rely on fishery-dependent catch and effort data to assess Snapper fisheries and has demonstrated that the incorporation of the DEPM into South Australia's existing assessment program is relatively cost-effective and likely to benefit the management and industry. Adding the DEPM will contribute an extra unbiased source of information that can be synthesised with existing fishery-dependent data streams that will lead to more confident assessments of the stock and ensure the long term sustainability of the State's Snapper resource.

Background

The recent change in fishing efficiency combined with the aggregative nature of Snapper and new management regulations have compromised the use of catch per unit effort (CPUE) as a suitable indicator of stock biomass. As such, there is an urgent need to develop a fishery-independent measure of stock status. The DEPM has been suggested to be a feasible, fishery-independent, technique to estimate Snapper biomass (Zeldis and Francis 1998, McGlennon 2003, Jackson et al. 2012) and has been successfully used as an on-going assessment tool for South Australia's Sardine Fishery (Ward et al. 2016). Difficulties identifying Snapper eggs has prevented the development of this method in southern Australia, as the characteristics of Snapper eggs makes them difficult to visually distinguish from other species that also spawn during the same time. Molecular validation of the identification of fish eggs and larvae has become an essential component of some existing DEPM programs (Neira et al. 2014). Given the advancements in molecular ecology, it was suggested that further investigation into developing a DEPM for Snapper was warranted.

Aims/objectives

1. To develop a DEPM for Snapper that provides the most accurate estimate of biomass and integrates with the on-going assessment and management of the resource.
2. To undertake a cost-benefit analysis to explore whether the DEPM can be used as a routine assessment tool to inform the sustainable management of South Australia's Snapper Fishery.

Methodology

Three surveys to estimate DEPM parameters were conducted over three years from SARDI's *RV Ngerin*. Each survey was done in early December to coincide with the peak spawning season. The first survey was carried out in Spencer Gulf from 11-14 December 2013; the second survey was undertaken in Gulf St. Vincent from 11-16 December 2014; and the third and final survey was carried out from 1-12 December 2015 and encompassed both gulfs.

During DEPM surveys the *RV Ngerin* was anchored in known fishing grounds to target adult Snapper using baited hand-lines. Because of the regional variation in growth rates and population size/age structure, adult Snapper were targeted in the northern and southern regions of the survey area. Additional Snapper samples were sourced from the commercial fishery. The size structure, along with key reproductive parameters (i.e. sex ratio, spawning fraction, batch fecundity) were determined from the adult sampling program.

All ichthyoplankton samples collected in 2013 and 2014 were preserved in 95% ethanol and refrigerated at 4°C prior to sorting. Samples collected in 2015 were preserved in 5% buffered seawater formalin, whereas every fifth sample was preserved in 95% ethanol and refrigerated at 4°C prior to sorting and molecular analysis. All samples were pre-sorted into 'possible' and 'unlikely' Snapper eggs on the basis of morphological characteristics. Snapper eggs from the ethanol-preserved samples were validated using the *in situ* hybridisation (ISH) molecular technique developed as part of this project. The validation rates were retrospectively applied to the formalin-preserved samples. Total daily egg production and spawning area was calculated for each survey. This study extended the traditional spawning biomass DEPM estimator to incorporate the inherent size-dependence of batch fecundity and variation in population size frequency, as a function of adult weight.

A cost-benefit analysis was undertaken to assess the feasibility of implementing a DEPM for Snapper as an on-going assessment tool. The existing 'fishery-dependent' program was used as the baseline for comparison with: (1) an exclusively fishery-independent program; (2) an augmented program that substitutes components of the existing assessment with the DEPM; and (3) a fully integrated program that incorporates the DEPM into the existing assessment.

Results

This study successfully designed and validated an *in situ* hybridisation (ISH) approach to identify Snapper eggs (and larvae) from field-collected ichthyoplankton samples using the mitochondrial 16S ribosomal RNA gene as a target for a specific oligonucleotide probe. This overcame the previous egg identification barrier that had inhibited the development of the DEPM as a reliable assessment tool for South Australian Snapper.

Although there were changes in the sampling methodology over the course of the study, the derived estimates of spawning biomass for Snapper generally aligned with the recent status assignment of the South Australian stocks, reflective of the relative levels of commercial catch, and broadly agreed with the

industry's assessment of the resource. Estimates of spawning biomass were consistently higher for the Gulf St. Vincent biological stock in comparison to the Spencer Gulf/West Coast stock.

Applying the DEPM for Snapper is a feasible method to determine a fishery-independent estimate of spawning biomass. A cost-benefit analysis indicated that incorporating the DEPM, either through its full integration into the current assessment program or augmenting it to rely less on fishery-dependent data sources, would benefit the assessment and management of the South Australian Snapper Fishery.

Implications

This project has effectively broadened the capability of using DEPM as a viable fisheries assessment tool for any multiple spawning species that produces pelagic eggs that are difficult to identify and can be sampled effectively.

This research has reduced the need to exclusively rely on fishery-dependent catch and effort data to assess Snapper fisheries. Adding the DEPM into the existing Snapper stock assessment program will contribute an extra unbiased source of information that can be synthesised with the fishery-dependent data streams and lead to more confidence in assessments of the stocks. From this, greater confidence can be placed on the consideration of implementing output controls in the future refinement of the Snapper specific harvest strategy. This level of information can be used to qualitatively assess the effectiveness of a series of spatial 'spawning' closures that were implemented in South Australia in 2013 as part of recent Snapper management strategies.

Finally, this project plays an important role in the global advancement of the DEPM. The overarching challenge in most DEPM programs relates to improving the precision of the biomass estimate. Effort is continually directed at reducing the inherent variance associated with the integrated biological parameters. The new statistical procedures developed as part of this investigation to account for the variation in South Australia's Snapper populations, can be more widely applied and further refined in other DEPM based programs.

Keywords

Snapper, *Chrysophrys auratus*, Spawning Biomass, Daily Egg Production Method, South Australia, In-situ Hybridisation, Egg Identification, Cost-Effective Application.

Introduction

Background

Each Australian mainland State supports a commercial Snapper (*Chrysophrys auratus*) fishery with a combined harvest of approximately 1,200 t (Fowler et al. 2016). Although the stock boundaries of Snapper are not clearly defined, a recent national status assessment was evaluated across four jurisdictions; East Coast (includes Queensland, New South Wales and Eastern Victoria), Western Victoria, South Australia, and Western Australia (Fowler et al. 2016). Management arrangements and assessment processes are inconsistent among jurisdictions and the status of some Snapper stocks could not be determined due to a lack of information. There is a need to align the national assessment for Snapper and develop cross-jurisdictional management strategies to ensure the sustainable harvest of the resource.

A model that integrates fishery-dependent data and population biology metrics to estimate the biomass of a fish stock is a tool for stock assessment. Snapper-specific fishery models have been developed for WA, SA and Qld. The fishery-dependent statistics collected for Snapper are typically complex as they include catch and effort information from a variety of sectors (e.g., commercial, recreational, trawl, and charter) and multiple gear types (e.g., hand-line, long-line, mesh nets, and trawl nets). For some jurisdictions these data are either temporally incompatible, sporadically collected, or absent. Such inconsistencies compromise the reliability of stock assessment models based solely on fishery-dependent data. In these situations, fishery managers commonly adopt a precautionary approach rather than developing prescriptive harvest strategies to ensure long-term sustainability. The integration of fishery-independent estimates of biomass into statistical models can alleviate multi-sectorial and multi-gear issues and provide unbiased measures of stock status that can be used to develop formal harvest strategies.

The Daily Egg Production Method (DEPM)

Estimating biomass from fishery-independent surveys is becoming increasingly more valuable in supporting assessment and management frameworks in modern fisheries. The Daily Egg Production Method (DEPM) has been successfully used to provide an unbiased estimate of spawning biomass for a range of small pelagic species (e.g. Australian Sardine *Sardinops sagax*, Australian Anchovy *Engraulis australis*, Blue Mackerel *Scomber australasicus*) consequently providing a key biological performance indicator that informs species-specific harvest strategies (Stratoudakis et al. 2006, Ward et al. 2011). However, there has been limited extension and application of this method to demersal finfish species. Zeldis and Francis (1998) were the first to apply the DEPM to a Snapper population in New Zealand. Although they indicated wide confidence intervals around the mean estimate of spawning biomass, they identified that the method was a viable assessment tool and “probably” applicable to other demersal finfish with similar life-history characteristics.

This method estimates the spawning biomass of a fish stock by combining measurements of the density of pelagic eggs and estimates of a range of adult parameters obtained from an intensive field sampling program. It relies on the premise that the biomass of spawning adults can be calculated by dividing the mean number of

eggs produced per unit mass of adult fish (Lasker 1985). Total daily egg production is the product of mean daily egg production (P_0) and total spawning area (A). Mean daily fecundity is calculated by dividing the product of mean sex ratio (by weight, R), mean batch fecundity (F), mean spawning fraction (S) by mean female weight (W). Spawning Biomass (SB) is calculated according to the following equation:

$$\boldsymbol{SB = \frac{P_0 \times A}{(R \times F \times S/W)}}. \qquad \text{[Equation 1]}$$

Western Australia is the only jurisdiction that has successfully integrated DEPM into its assessment of Snapper (Jackson and Cheng 2001, Jackson et al. 2012). Difficulties identifying Snapper eggs has prevented the development of this method throughout southern Australia, as the characteristics of Snapper eggs makes them difficult to visually distinguish from other species that also spawn during the same time; e.g. flathead (Platycephalidae). Relying on morphological criteria for such differentiation alone can present problems such as the over-estimation of spawning biomass due to incorrect egg identification (Fox et al. 2005). A recent validation study indicated that Snapper eggs collected in Western Australia were not always correctly identified with visual methods, with rates of misidentification ranging from 0% to 100%, depending on the location from where the plankton sample was taken (Dias et al. 2016)

Molecular validation of the identification of fish eggs and larvae is an essential component of some existing DEPM programs (Neira et al. 2014) and provides an opportunity to extend application of the method to species where egg identification has been problematic. To date, validation methods have relied on destructive sampling, where eggs and larvae are initially identified, ascribed a developmental stage, and their DNA or RNA is chemically extracted for analysis destroying the specimens. This process is typically applied to a sub-set of samples to ensure confidence in morphological identifications (e.g. Ward et al. 2016) or determine a 'correction factor' (Neira et al. 2014). *In situ* hybridisation (ISH) approaches may provide a more streamlined and non-destructive validation alternative (Pradillion et al. 2007). ISH involves the development of a species-specific oligonucleotide probe that targets ribosomal RNA and uses horseradish peroxidase (HRP-DNA) to produce a colour reaction. Coloured eggs and larvae could potentially be identified under a standard stereo microscope, separated from mixed species samples, staged and archived. ISH is a powerful and relatively cost-effective diagnostic technique and has successfully identified a variety of marine taxa, including Bacteria and Archaea (DeLong et al. 1989), diatoms (Scholin et al. 1997), and invertebrate larvae and eggs (Mountford et al. 2007, Pradillion et al. 2007, Thomas et al. 2011). It may also be broadly applicable for identifying fish eggs and larvae, although is yet to be achieved.

A major assumption in the DEPM is that adult parameters used to calculate spawning biomass are constant over the range and duration of the survey (Stratoudakis et al. 2004). This assumption, however, is likely to be violated if the spawning area encompasses numerous sub-populations or spans across different physical environments, where the target species exhibits considerable phenotypic or genotypic diversity. Not accounting for such diversity can potentially bias parameter estimation, and result in imprecise or bias estimates of spawning biomass. In situations where the spatial distribution of adult sampling sites extends throughout the survey area spawning biomass can be calculated using a 'post-stratification' process to account

for spatial differences in spawning rates (Piquelle and Stauffer, 1985). In these circumstances spawning biomass estimates are calculated independently for each stratum.

A recent study used a combination of population-based demographics and physical and chemical characteristics of Snapper otoliths, to partition South Australian Snapper into three distinct stocks: the Spencer Gulf/West Coast Stock (SG/WC); the Gulf St. Vincent Stock (GSV); and the Western Victorian Stock (WVS) (Fowler 2016) (Figure 1). Each of these stocks is considered to be self-sustaining and dependant on a significant primary nursery area (Fowler 2016). The northern gulfs are the nursery areas for the SG/WC and GSV stocks, whereas, the WVS stock extends westward from Port Phillip Bay (PPB), Victoria into the south east region of South Australia (Figure 1). The regional extent of these stocks depends on the emigration of sub-adult and adult fish. There appears to be minimal movement among regional sub-populations. Most recaptures in a tagging study were made within 20 km of the tag site (i.e. residents), relatively few adult Snapper moved distances that would justify them being recognised as 'migrants' (Jones 1981, 1984). This life history model is reflected in the population demography, as there are considerable differences in the size/age structures and growth trajectories among regional sub-populations (Fowler et al. 2013). Given this variation, there is a need to determine if there are similar regional differences in reproductive biology.

The reproductive biology of Snapper has been extensively studied throughout its geographic range (Crossland 1977, Fowler and Jennings 2003, Wakefield 2006, Jackson 2007, Sumpton and Jackson 2010, Saunders et al. 2012). It is a multiple batch spawner with indeterminate fecundity and asynchronous oocyte development (Saunders 2009). Individual fish spawn over consecutive days and ovulation is highly synchronised within an aggregation (Crossland 1977, Scott et al.1993). Snapper aggregate to spawn within a range of 15-22 °C. However, there is strong evidence of regional adaptation of spawning behaviour associated with sea surface temperature (Crossland 1980, McGlennon 2003, Saunders 2009, Pecl et al. 2014). Seasonal spawning typically occurs during the austral winter in tropical latitudes and austral spring/summer in temperate latitudes (Pecl et al. 2014). Investigation into the reproductive biology of South Australian Snapper has, so far, exclusively focused on the Northern Spencer Gulf sub-population (McGlennon 2003, Saunders 2009; Fowler unpublished data).

South Australian Snapper Fishery

There has been a dramatic switch in the spatial structure of South Australia's Snapper Fishery over the past decade. Spencer Gulf (SG) has traditionally yielded the State's highest Snapper catches, however, in recent years it has been replaced by Gulf St. Vincent (GSV) and the South East (SE) (Fowler et al. 2016). This shift has been a consequence of a reduction in the commercial harvest in northern and southern SG coupled with an unprecedented increase in catches from northern GSV. Commercial fishers rapidly responded to this increase by adjusting their fishing behaviour, shifting from conventional hand-line gear to light-weight, long-line technology to maximise their fishing efficiency. Catch per unit effort (CPUE) in this sector increased exponentially and the fishery "boomed" in NGSV raising concerns about the long-term sustainability of the resource. Management responded with a suite of changes including imposing daily trip limits to curtail excessive catches five spatial closures to protect Snapper spawning aggregations in 2013/14. The recent

change in fishing efficiency combined with the aggregative nature of Snapper and new management regulations have compromised the use CPUE as an indicator of stock biomass. As such, there is an urgent need to develop a fishery-independent measure of stock status.

South Australia's Snapper Fishery has been predominantly managed through input controls. Spatial and temporal closures have been implemented to reduce fishing effort and protect known spawning grounds, and gear restrictions have been enforced to curtail excessive catches of Snapper. Despite these arrangements the stocks have not responded as well as expected: the SG/WC stock was recently classified as transitional-depleting; and although the NGSV stock was considered sustainable, the poor status of the adjacent stock provided a warning with respect to the impact of sustained high fishing effort (Fowler et al. 2016). Management subsequently responded to these concerns by placing greater emphasis on output controls, by initially implementing a daily commercial catch limit of 500 kg in 2012, and further reducing it to 350 kg in GSV/SE and 200 kg in SG in 2016. However, it has been acknowledged by industry and management that if output controls are to be effective in managing the sustainable harvest of South Australia's Snapper resource then there needs to be a means of estimating biomass with an improved level of confidence.

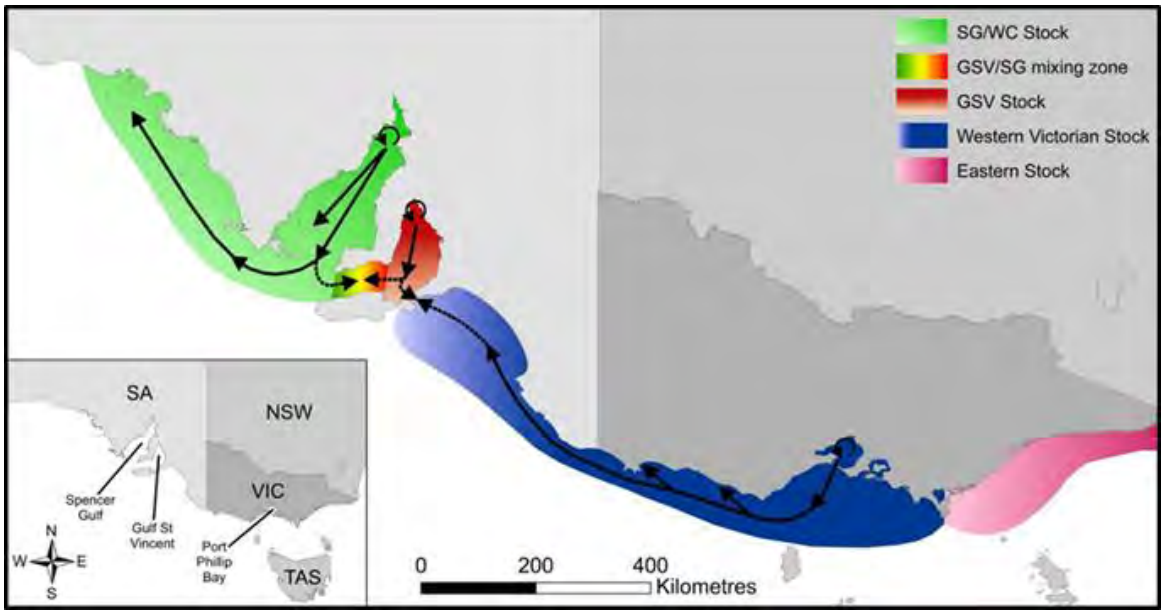

Figure 1. Map of the coast of south eastern Australia, showing the stock structure for Snapper based on fish movement (Fowler 2016). The arrows indicate directions and extent of emigration of fish from three primary nursery areas in Northern Spencer Gulf, Northern Gulf St. Vincent and Port Phillip Bay, Victoria. Inset shows the broader geographic region. SG – Spencer Gulf, GSV – Gulf St. Vincent, WC – west coast of Eyre Peninsula.

Need

Establishing formal harvest strategies for Snapper across Australia's four main jurisdictions (East Coast, Western Victoria, South Australia and Western Australia) was identified as a national priority (at the National Strategic Planning Workshop for Snapper Research in March 2013). Two key outputs were recognised to achieving this outcome: 1) establishing an integrated assessment model for Snapper; and 2) developing fishery-independent estimates of abundance. Each jurisdiction is currently at a different level of advancement in their assessment and management arrangements. A Snapper model is currently used to underpin the assessment of South Australia's Snapper resource, a model is being developed for Western Victoria. There is no model for

the East Coast. South Australia and Western Victoria are at a point where fishery-independent estimates of biomass would considerably enhance their respective stock assessment programs.

The need for a DEPM assessment is urgent in South Australia as recent structural changes in the Snapper fishery have compromised the integrity of the time series of fishery-dependent statistics that have been relied on to assess the resource in the past. Fishery-independent estimates of Snapper biomass are required to feed into the existing stock assessment model to ensure that future assessments and harvest strategies are developed from unbiased information. This research direction has been unanimously supported by stakeholders in South Australia's Snapper Fishery. The Western Victorian fishery is likely to encounter similar issues to South Australia, particularly as their fishery is dominated by the recreational sector where the routine collection of catch and effort data to integrate into the assessment process is often challenging.

Although the principal objective in developing a DEPM is to provide an unbiased, fishery-independent, estimate of Snapper biomass, there are a number of additional benefits. The most useful relates to gaining a greater understanding of the spatial distribution and abundance patterns of Snapper eggs to infer spawning activity. This is particularly relevant given PIRSA's recent implementation of five spatial closures, ranging in area from 200 to 315 km^2, to protect spawning Snapper in 2013/14 as part of their new management strategy. Information gained during the proposed study would be able to quantitatively assess the relative effectiveness of these spawning closures.

Objectives

1. To develop a DEPM for Snapper that provides the most accurate estimate of biomass and integrates with the on-going assessment and management of the resource.
2. To undertake a cost benefit analysis to explore whether the DEPM can be used as a routine assessment tool to inform the sustainable management of South Australia's Snapper Fishery.

Method

Development of a DEPM for Snapper:

Successful application of the DEPM critically relies on the collection of adequate samples of eggs from throughout the spawning area, reliable identification of eggs and concurrent adult sampling to determine their spawning condition.

Study area

Three DEPM surveys were undertaken and refined over three years. Each survey was done in early December to coincide with the peak spawning season (McGlennon 2003, Saunders 2009). These surveys were conducted during the State-wide seasonal closure of the Snapper fishery from midday 1 November to midday 15 December. This ensured that the reproductive behaviour of the Snapper population was relatively undisturbed by fishing activity for approximately one month prior to sampling. The first survey was carried out in Spencer Gulf from 11-14 December 2013 and consisted of 195 stations that encompassed known Snapper spawning areas. Most stations conformed to a 2 x 4 nm grid pattern, however, the four spatial closures were sampled more intensively (2 x 2 nm) (Figure 2). All stations were confined to waters >10 m deep. The second survey was undertaken in Gulf St. Vincent from 11-16 December 2014. This survey consisted of 216 stations that were arranged in a stratified pattern, where sampling intensity decreased from a 2 x 2 nm grind in the northern part of the Gulf, extending to 2 x 4 nm throughout the middle section, and culminating into a 4 x 4 nm pattern in the south (Figure 2). This sampling pattern was adopted because, unlike Spencer Gulf, the key Snapper spawning areas within Gulf St. Vincent are relatively unknown. The third and final survey was carried out from 1-12 December 2015 and encompassed both gulfs. The spatial structure of these surveys were slightly modified in comparison to previous years. An extra 25 stations, arranged in a 4 x 2 nm grid pattern, was added to Spencer Gulf extending its spatial coverage further south. Ten stations were omitted from the western end of Investigator Strait in Gulf St. Vincent due to time and weather limitations (Figure 2).

As Snapper exhibit considerable latitudinal phenotypic variation, with the northern-most sub-populations of the gulfs growing faster and attaining larger sizes than those further south (Fowler et al. 2014), each gulf was partitioned into northern and southern regions (Figure 2). Estimates of spawning biomass were calculated for each of these regions separately. The spatial resolution of the sampling area was refined to a series of zones to accommodate subsequent processing of pooled egg samples.

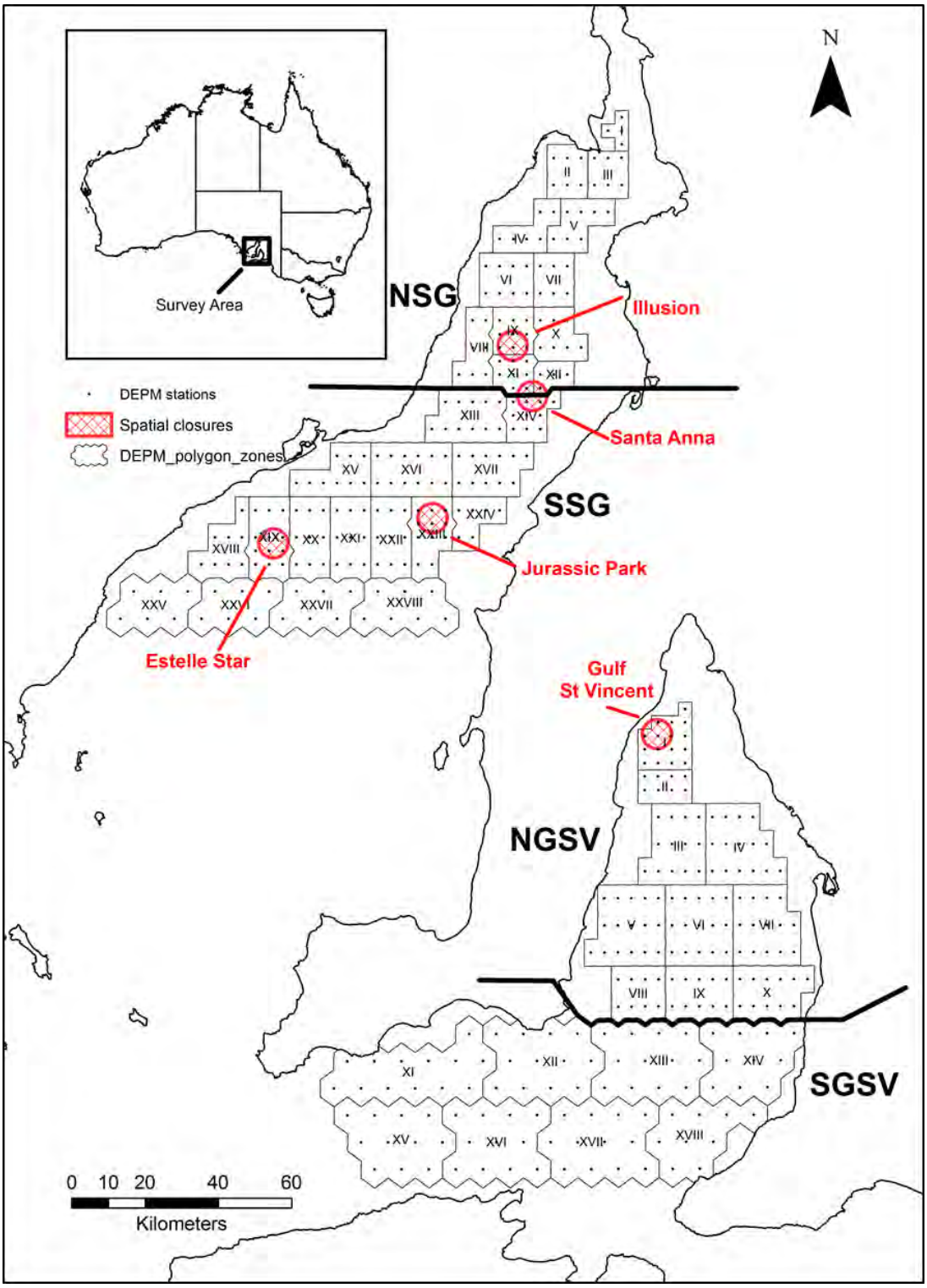

Figure 2. DEPM Survey area. Locations of ichthyoplankton sampling stations (black dots) throughout South Australia's Spencer Gulf (partitioned into northern (NSG) and southern regions (SSG)) and Gulf St. Vincent (northern (NGSV) and southern (SGSV) regions). Zones are represented by roman numerals, and the five spatial spawning closures are identified in red.

Ichthyoplankton surveys

Plankton sampling

The three plankton surveys were undertaken from the *RV Ngerin*. Plankton samples were collected at each station using paired bongo nets. Each net had an internal diameter of 0.57 m, 500 μm mesh and plastic cod-ends. The nets were vertically deployed to within 5 m of the seabed and retrieved at a speed of ~1 $m.s^{-1}$. General Oceanics ™ 2030 flow-metres and factory-calibrated coefficients were used to estimate the distance travelled by the net for each tow. The wire length during each deployment was measured to the nearest metre using a digital counter (General Oceanics). Where there were discrepancies of >500 units between paired flow-metre readings, the relationship between wire length released and flow-metre reading was used to determine the correct value which was substituted for the erroneous reading. Upon retrieval of the nets, they were washed down and the plankton samples were rinsed from the two cod-ends and combined into a 1L sample container. All samples collected in 2013 and 2014 were preserved in 95% ethanol and refrigerated at 4°C prior to sorting. Samples collected in 2015 were preserved in 5% buffered seawater formalin, whereas every fifth sample was preserved in 95% ethanol and refrigerated at 4°C prior to sorting and molecular analysis. The preservation method was amended in 2015 as unforeseen technical challenges delayed the development of the ISH probe, placing an increased reliance on the visual identification of Snapper eggs in the event that the ISH probe was unsuccessful. This decision was made as morphological characteristics are easier to distinguish in formalin-preserved samples compared to those stored in ethanol (Figure 3.). It was anticipated that the validated Snapper eggs from the ethanol-preserved samples would be used to 'correct' the Snapper egg counts derived from the formalin-preserved samples if the ISH technique was reliable.

Identifying Snapper eggs

Snapper egg reference samples were obtained from broodstock maintained at the South Australian Aquatic Sciences Centre, SARDI Aquatic Sciences. Spawning was induced via the implantation of a slow release cholesterol/cellulose LHRHa pellet (see Ham and Hutchinson 2003) and the resultant eggs were incubated at ambient temperature (≈18-21°C) in an on-site flow through system. Egg samples (n > 5,000) were collected throughout the embryonic developmental period, with approximately half preserved in 96% ethanol and refrigerated at 4°C, and the remaining half preserved in 5% buffered formalin. These samples covered all stages of egg development from 30 minutes after spawning to hatching (≈36 hrs). Digital images were taken of live, ethanol- and formalin-preserved eggs throughout the developmental series. Images were captured under both transmitted and reflected light at 2.5x magnification on a stereo microscope (Leica MS5). The maximum diameter of the egg and oil globule and width of the perivitelline space were measured (to the nearest 0.01 μm) for up to 30 replicate eggs at each developmental stage and for each preservation method. Embryonic descriptions of Snapper by Norriss and Jackson (2002), McGlennon (2003) and Cassie (1956) were used to inform stage descriptions. This study delineated nine egg stages of Snapper eggs (Table 1). The stages were based on distinguishable developmental features and aimed to cover similar time periods (Figure 4: Stages I to IX).

Plankton samples were sorted using either a Ward Counting Wheel or a modified Sedgwick-Rafter sorting tray under a stereo dissecting microscope. All teleost eggs were removed from each plankton sample and separated into two categories: 'possible' and 'unlikely' Snapper eggs. The main diagnostic features used to classify 'possible' Snapper eggs were established from the reference material: eggs were spherical ranging from 0.7 to 1.0 mm in diameter; had a smooth chorion; small perivitelline space (0.01 to 0.15 mm); prominent, unsegmented yolk; a single oil globule ranging from 0.15 to 0.30 mm in diameter; pigmentation spots on the oil globule that appeared during the mid to later stages of embryonic development; and pigment pattern (as described in Cassie (1956), Norriss and Jackson (2002), McGlennon (2003)) (Figure 3 and Figure 4). All remaining eggs were separated as 'unlikely' Snapper eggs.

All eggs were retained in fresh 95% ethanol and refrigerated at 4°C, prior to species validation through molecular analysis. The formalin-preserved eggs collected during the 2015 surveys were retained for morphological identification.

Snapper Egg Validation

Snapper eggs were identified from the 'possible' and 'unlikely' ethanol-preserved samples using an *in situ* hybridisation molecular technique. This technique utilises an oligonucleotide probe that binds with Snapper-specific DNA that can be colour-labelled (blue) through oxidisation (Figure 4). This technique requires mechanically piercing the chorion of each egg to expose the internal embryonic tissue to the molecular probe. Although the structural integrity of the eggs was compromised by this technique they could still be assigned a developmental stage.

All 'possible' Snapper egg samples were analysed separately, whereas the 'unlikely' egg samples were pooled into their respective spatial zones. This two stage validation process was undertaken to determine the success rate of identifying Snapper eggs based entirely on 'possible' morphological diagnostic features and the proportion of Snapper eggs that failed to be detected from within the 'unlikely' Snapper egg samples. These metrics provided a 'correction-factor' which was then applied to the formalin-preserved samples collected in 2015. Each zone was 'corrected' independently.

Egg ageing

Each Snapper egg was assigned an average age (in hours) using the temperature dependent embryonic developmental key developed by McGlennon (2003). This key is described by the following equation:

$$y_{i,t} = 36.158 \, . \, e^{(-0.12t)} \, i^{0.827} \qquad \text{[Equation 2]}$$

Where $y_{i,t}$ is the average age of the ith stage at temperature t°C. The parameters were solved for Snapper through a structured egg incubation experiment described in McGlennon (2003). Egg stages were standardised to this study's nine stage scheme by calculating the mean of McGlennon's (2003) equivalent developmental stages (Table 1).

Table 1. The description of the nine (IX) stages of Snapper embryo development used in this study. Numbers in parentheses indicate the equivalent developmental stages ascribed by McGlennon (2003).

STAGE	*	DESCRIPTION
I	*(1, 2)*	< 64 Cells. Individual cells are discernible in live eggs, but have a rough 'bubbled' appearance when preserved in ethanol and formalin.
II	*(3, 4)*	Blastoderm covers less than half of the yolk.
III	*(5, 6)*	Blastoderm covers more than half of the yolk, becoming hemispherical. The blastopore is not yet formed.
IV	*(7, 8)*	Blastopore apparent, the embryonic axis forms, and the head becomes distinct.
V	*(9)*	Blastopore is closed, optic vesicles visible, first myomeres visible, sparse pigment spots on the dorsal and ventral surfaces of the embryo.
VI	*(10, 11)*	Embryo extends 50% around the yolk. Tail becomes bulbous and begins to separate from the yolk. Pigment appears on oil globule (as stellate melanophores) and become more prominent on the embryo and yolk sac.
VII	*(12, 13)*	Embryo extends 66% around yolk. The tail lifts from the yolk and extends to the oil globule. Caudal finfold begins to develop. Melanophores appear more prominent on the anterior end of the embryo.
VIII	*(14, 15)*	Embryo extends 75% around the yolk. Head structure and caudal finfold are well developed. The tail extends beyond the oil globule.
IX	*(16)*	Embryo is almost fully developed, tail long and twisted off embryo axis. Oil globule is posteriorly located near the anus.

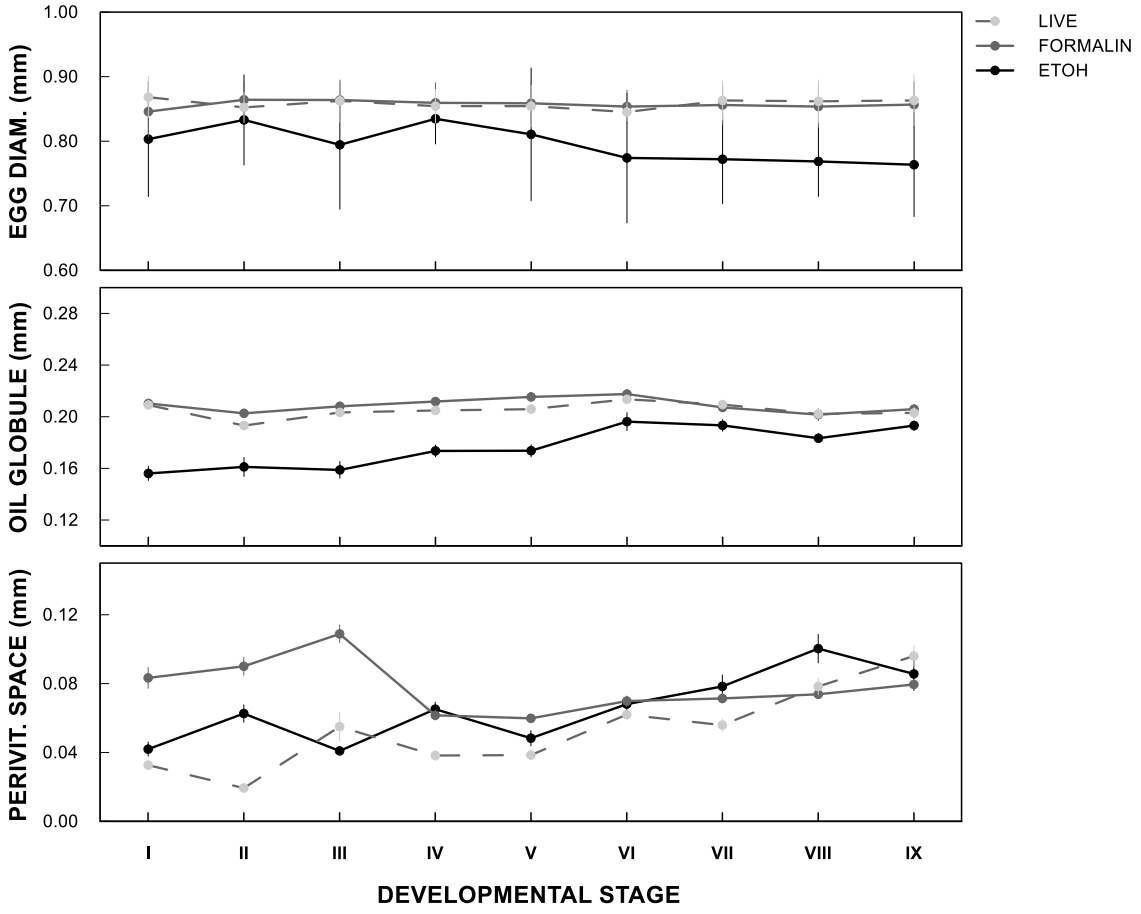

Figure 3. Stage specific Snapper egg dimensions, including the mean (mm ± standard error) maximum egg diameter, oil globule diameter, and maximum perivitelline space for live eggs and those preserved in 5% formalin and 96% ethanol (ETOH).

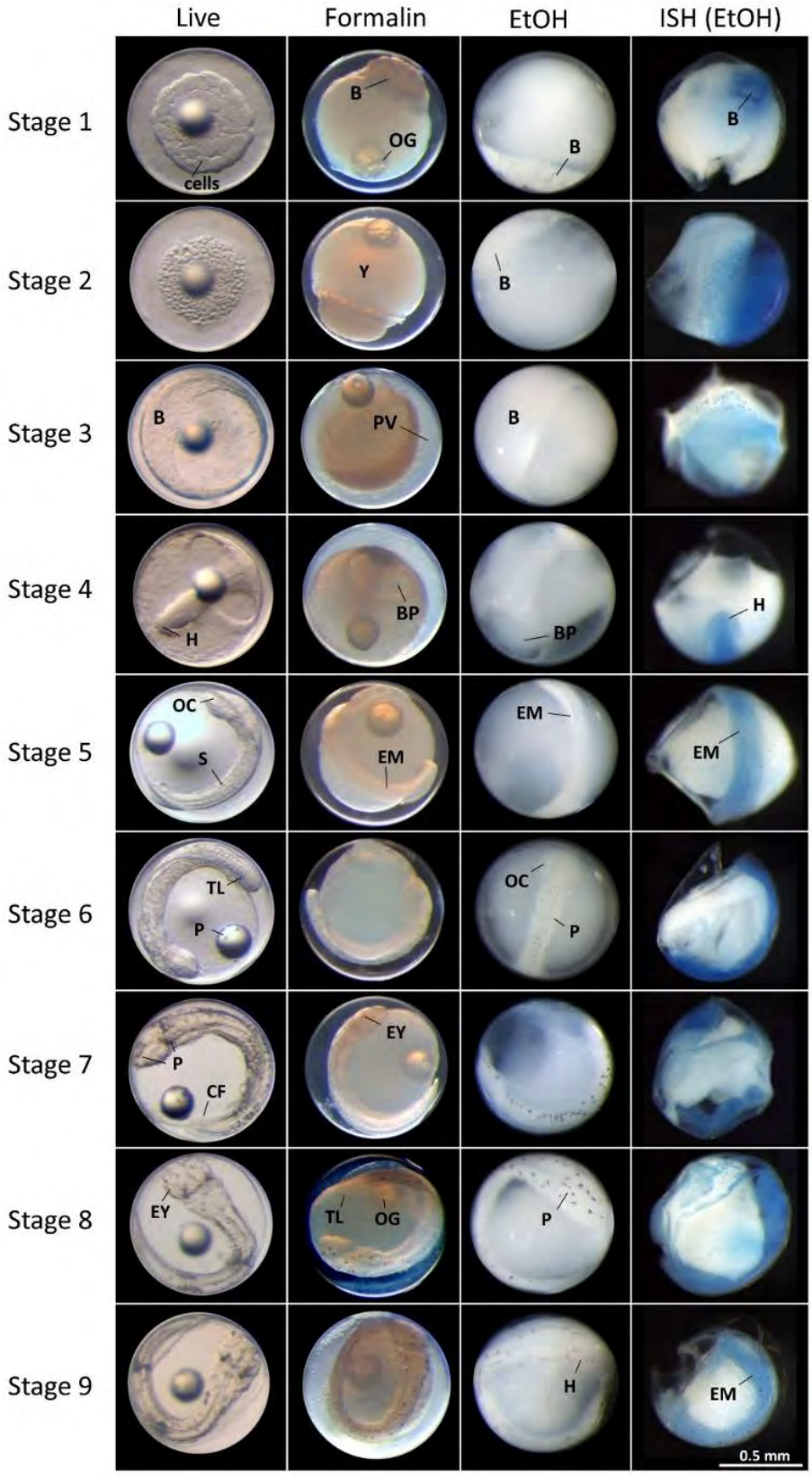

Figure 4. The nine stages of Snapper egg development in live eggs (Live) and after various treatments: preservation in 5% formalin (Formalin); preservation in 96% EtOH (EtOH); and after application of the *In Situ* Hybridisation (ISH) technique (ISH (EtOH)). The stages follow the characteristics of embryonic development described by Norriss and Jackson (2002), McGlennon (2003) and Cassie (1956). See document text for specific descriptions. B: blastoderm; BP: blastopore; CF: caudal fold; EM: embryo; EY: eye; H: head; OG: oil globule; OC: optic cup; P: pigment; PV: perivitelline space; S: somite; TL: tail; Y: yolk.

Egg density ($Eggs^{surv}$)

Egg density under one square metre of water ($Eggs^{surv}$) was estimated at each station according to:

$$Eggs^{surv} = \frac{C \times D}{V} \qquad \text{[Equation 3]}$$

where C is the number of eggs in each sample, V is the volume of water filtered (m^3) estimated using the flowmeters and D is the maximum depth (m) to which the net was deployed.

Estimates of spawning area (A^{sp})

The Veronoi natural neighbour (VNN) method (Watson 1981) was applied using the geographic information system package 'ArcGIS' to generate a polygon around each sampling station with the boundary as the midpoint equidistant between each station. The area represented by each station (km^2) was then determined and defined the overall sampling area for each of the annual surveys. Geostatistical kriging refined the extent of the spawning area (A^{sp}) within the sampled area. This method interpolated the georeferenced point data ($eggs.m^{-2}$ at each sampling station) to predict the intermediate values through a Gaussian process governed by prior covariances. A minimum egg density of 0.1 $eggs.m^{-2}$ was used to define the outer boundary of spawning activity for each survey and determine the extent of A^{sp}.

Daily egg production (D_0)

The timing of egg release for Snapper occurs throughout the day, making the traditional approach of estimating mean density at time of peak spawning (P_0) inappropriate for Snapper (McGlennon 2003; Jackson 2007). A new method was developed to estimate daily egg production (D_0) for Snapper, which accounts for the continuous spawning pattern (see Appendix 1). This method accounts for loss of eggs through natural mortality (Z) from the time of spawning to the time of sampling for each observed egg stage in each of the plankton tows, rather than for the overall population through a least squares regression (Lo 1991) or from a prior assumed Z determined from a generalised linear model (GLM) (Bernal et al. 2011). Mean daily egg production was estimated using assumed instantaneous mortality rates, ranging from 0.2 to 0.6 day^{-1}.

Adult Reproductive Parameters

Sampling methods

During DEPM surveys the *RV Ngerin* was anchored in known fishing grounds to target adult Snapper using baited hand-lines. Because of the regional variation in growth rates and population size/age structure (Fowler et al. 2013), adult Snapper were targeted in the northern and southern regions of the survey area. Time of capture was recorded and fish were measured (caudal fork length to the nearest cm) and weighed using a marine balance (0.01 kg). Fish were eviscerated, sexed and macroscopically assigned a maturity stage according to Saunders et al. (2012). The gonads were dissected and weighed (1 g). All advanced ovaries (≥ Stage III) were subjected to detailed histological and batch fecundity analyses. For each of these ovaries a small tissue section was dissected from the centre of one ovarian lobe, placed in a histological cassette and preserved in FAACC for further microscopic analysis. For hydrated (Stage IV) ovaries a sub-section of approximately 10% by weight was dissected from the remaining lobe and weighed (1 g). This section was cut

longitudinally and the oocytes hose-washed from the connective lumen, collected in a 500 μm sieve, transferred to a 1L container and preserved in 5% sea water buffered formalin for later batch fecundity analysis.

Additional adult samples were sourced from the commercial fishing sector via the centralised SAFCOL market upon the reopening of the fishery (December 15th of each year). This sampling program occurred over two stages: the first involved targeting commercial catches from regions of interest and measuring the size of each Snapper within the catch. The second stage involved processing a sub-sample of the catch to obtain further biological information according to the procedure outlined above. The landing details, such as the capture date and location, of these market-sourced fish were cross-referenced from the fisher's compulsory catch return logs.

Female weight (N_w^{fem})

Traditionally the mean female body weight is used as a standard parameter to estimate spawning biomass. For species, such as adult Sardines and other small pelagic fish (California northern anchovy, Parker 1980; blue mackerel, Ward et al. 2009) where female body size is not multi-modal, the population mean is an appropriate measure. The size structure of Snapper, however, is typically variable, due to the sporadic temporal variation in recruitment and spatial variation in growth rates (McGarvey and Feenstra 2004, Fowler and McGlennon 2011). Ignoring this variation would reduce the accuracy of the spawning biomass estimates. To account for this variation this study divided the Snapper population into 26 weight classes, ranging from 0.5 to 13.5 kg, grouped into 0.5 kg bins and this size-dependence was incorporated into the estimate of spawning biomass (Appendices 3 and 4).

Batch Fecundity (F^{batch})

Formalin-preserved oocyte samples stripped from stage IV ovaries were rinsed in a 150 μm sieve to remove the preservative. Rinsed oocytes were transferred into a glass beaker and filled with water to a standard 1L volume. This 1L sample was thoroughly mixed to ensure the oocytes were evenly distributed throughout the solution. Ten to 15 1 mL sub-samples were pipetted from the mixture and examined using a Sedgwick Rafter tray under a stereo dissecting scope using transmitted light. For each sub-sample, hydrated oocytes (>700 μm) were counted. The average number of hydrated oocytes per mL was calculated. The final estimate of batch fecundity (F^{batch}) was calculated for each fish according to Equation 4:

$$\boldsymbol{F^{batch} = \left[\frac{(\bar{E}_{sub} \times 1{,}000)}{W_{sect}}\right] \times W_o} \qquad \text{[Equation 4]}$$

Where, $\bar{E}_{sub}$ is the mean count of hydrated eggs per mL, W_{sect} is the weight of the sub-section of ovary and W_o is the whole weight of the paired ovaries.

The relationship between female weight (W) and batch fecundity was determined by linear regression and used to estimate the batch fecundities of mature females in all samples. The allometric function for F^{batch} against weight was taken as a continuous variable.

$$\hat{F}^{batch}(W) = \alpha \cdot W^{\beta}. \qquad \text{[Equation 5]}$$

A maximum likelihood estimator that accounted for heteroscedasticity in the spread of the residuals was used in the model fit to estimate the parameters α and β (see Appendix 2). For comparative purposes, derived estimates of batch fecundity for Snapper were compiled from the literature (Fowler 2000; Jackson 2007; Saunders 2009). The weight-dependent batch fecundity parameter estimates, along with estimated error structure, were incorporated into the size-dependent estimation of spawning biomass (Appendices 3 and 4).

Sex ratio (R)

Quantities of mature males and females in each sample were used to estimate the sex ratio ($\bar{R}_i$) according to Equation 6:

$$\bar{R}_i = \frac{F_i}{(F_i + M_i)}. \qquad \text{[Equation 6]}$$

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

The population mean sex ratio (R) was weighted by sample size according to Equation 7:

$$R = \sum \left[\bar{R}_i \times \frac{n_i}{N} \right]. \qquad \text{[Equation 7]}$$

Where, $\bar{R}_i$ is the mean sex ratio of each sample, *n* is the number of fish in sample *i* and *N* is the total number of fish collected in all samples. Standard errors were determined using a mean ratio estimator (Rice 1995).

Spawning fraction (S)

The estimates of spawning fraction (S) were calculated as the mean proportion of females that were in spawning condition during the survey period. Histological sections were prepared from the FAACC preserved ovarian tissue samples for microscopic analysis. Tissue was sectioned at 6 μm and stained with haemotoxylin and eosin. Several sections from each ovary were examined to determine the presence/absence of post-ovulatory follicles (POFs). Females were determined to be in spawning condition if ovaries contained hydrated oocytes and/or POFs (Figure 5). Given Snapper are known to spawn daily (Scott et al. 1993; Wakefield 2010), it was assumed that the presence of POFs indicated that spawning had occurred within 24 hrs of capture. The mean spawning fraction of the population was calculated from the average of the sample means weighted by the proportional sample size according to Equation 8.

$$S = \sum \left[\bar{S}_i \times \frac{n_i}{N} \right]. \qquad \text{[Equation 8]}$$

Where, $\bar{S}_i$ is the mean spawning fraction of each sample, *n* is the number of fish in sample *i* and *N* is the total number of fish collected in all samples. Standard errors were determined using a mean ratio estimator (Rice 1995).

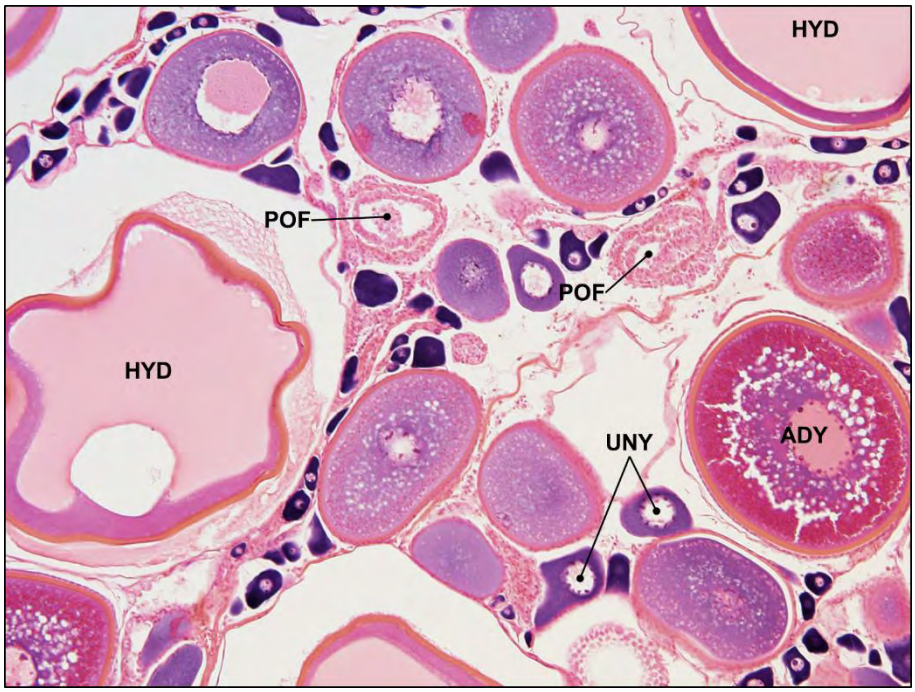

Figure 5. A histological section of an ovary collected from a female Snapper in spawning condition. Note the co-occurrence of un-yolked (UNY), advanced yolked (ADY) and hydrated (HYD) oocytes, along with post-ovulatory follicles (POF) which is indicative of asynchronous oocyte development and high frequency spawning (i.e. daily).

Spawning Biomass (B^{sp})

This study extended the traditional spawning biomass DEPM estimator (Equation 1) to incorporate the inherent size-dependence of batch fecundity and variation in population size frequency, as a function of adult weight. Adult Snapper market samples were partitioned into 26 weight classes. In three of four regions, these showed an approximately bimodal distribution that reflects sporadic recruitment of Snapper year classes typical for this species, but would be poorly approximated by a single mean weight. Similarly, because adult females span such a wide range of body sizes, estimates of egg production are made substantially more precise by incorporating the dependence of batch fecundity on female body weight. The full derivation of the spawning biomass estimation formula below (Equation 9) incorporating size dependence of adults is given in Appendix 3:

$$\hat{B}^{sp} = \frac{D_0 \cdot A^{sp}}{S \cdot R} \cdot \left(\frac{\sum_w P_w^N \cdot \bar{w}_w}{\sum_w F_w^{batch} \cdot P_w^N} \right). \qquad \text{[Equation 9]}$$

To compute confidence intervals on this size-dependent estimate of spawning biomass, the delta approximation method, first applied by Parker (1980) to the classic DEPM biomass estimate, was applied. The derivation of this method is given in Appendix 4.

Cost-Benefit Analysis

Given the urgent need to provide a fishery-independent assessment of biomass for Snapper and the potential for the on-going use of the DEPM as a means of addressing this need, there is a clear requirement to determine the feasibility of this assessment tool through a cost/benefit analysis.

The staff and operating costs associated with substituting or augmenting the existing assessment program with DEPM estimates of spawning biomass were compared. Costs shared by the two methods and potential savings were identified. The salary and operating costs in the budget for the current Snapper Fishery assessment program in the 'Service Level Agreement' developed with PIRSA and industry were identified. The estimated costs of a DEPM program were determined from the staffing requirements and operating expenses associated with the field and laboratory components of this project.

The existing 'fishery-dependent' program was used as the baseline for comparison with: (1) an exclusively fishery-independent program; (2) an augmented program that substitutes components of the existing assessment with the DEPM; and (3) a fully integrated program that incorporates the DEPM into the existing assessment. The cost of each program is presented as a percentage change against the baseline assessment.

The flow-on benefits of the proposed assessment programs in assigning stock status and management advice for Snapper to industry are complex. Consequently, it is difficult to ascribe a meaningful monetary value that can be directly compared against their known costs. Instead, the benefit of each of the program's outputs were categorically scored across three key areas to provide a qualitative analysis of their relative value. These are: (1) assessing fishery performance; (2) the level of biological information provided; and (3) achieving the objectives of management. A series of key performance indicators, derived from the objectives of the current Snapper harvest strategy outlined the MSF Management Plan (PIRSA 2013), were identified within each of these three areas. Scores ranged from 0 (low) to 5 (high). The average score for each benefit was calculated from six separated assessments, which included three fisheries scientist, a fishery modeler, and two fishery managers.

Results

DEPM

Snapper Egg Validation

Of the 416 'possible' eggs collected from the 2013 Spencer Gulf survey, 81 were confirmed to be Snapper through ISH hybridisation, representing a 19.5% identification success rate when relying on coarse morphological characteristics. This success rate increased to 80.2% in samples collected from Gulf St. Vincent in 2014, where 73 Snapper eggs were positively identified from 91 'possible' eggs. A parallel molecular investigation indicated that non-hybridised eggs from the possible Snapper egg samples represented a range of different fish species, including species of the families Carangidae (trevally), Callionymidae (stinkfish), Cynoglossidae (sole), Gerreidae (silverbelly), Labridae (wrasse), Mullidae (mullet), Neosebastidae (scorpionfish), Pinguipedidae (wavy grubfish), Platycephalidae (flathead), Sillaginidae (whiting) and Tetrapontidae (striped perch). Twenty Snapper eggs were identified from 31,493 'unlikely' eggs sampled from Spencer Gulf in 2013, representing 0.06% of the entire sample. Similarly, 130 Snapper eggs were identified from 12,334 eggs from the corresponding sample collected in Gulf St. Vincent in 2014, representing 1.1%. These samples also included large quantities of conspicuous Anchovy (*Engraulis australis*) eggs consequently inflating overall counts.

Every fifth plankton sample collected during the 2015 DEPM surveys was preserved in ethanol for ISH molecular analysis. This resulted in a total of 40 (of 202) samples in GSV and 43 (of 212) samples in SG. Consequently, the quantities of eggs collected were considerably lower than the previous surveys. Of the 3,937 ethanol-preserved eggs collected from SG in 2015, eight were pre-sorted as 'possible' Snapper eggs; however, none were confirmed through ISH analysis. Of the remaining 'unlikely' eggs, 18 were positively identified as Snapper, representing 0.46% of the entire sample. In total 3,285 fish eggs were collected from GSV in 2015 and preserved in ethanol. Seven were considered 'possible' Snapper eggs of which two were confirmed, representing a 28.6% identification success rate. Twenty of the remaining 3,278 'unlikely' eggs were positively identified as Snapper, representing 0.61% of the sample.

The relative proportions of validated Snapper eggs in both the 'possible' and 'unlikely' ethanol-preserved samples collected during the 2015 surveys were used to adjust the formalin-preserved egg counts for each of the sampling zones (see Figure 2).

Egg Density (P_t)

The overall distribution pattern of Snapper eggs within Spencer Gulf in 2013 and 2015 was similar. Relatively high density patches of eggs up to 10 eggs.m^2 were observed around Point Lowly, north of the gulf (Zone III), close to the 'Illusion' spawning closure (Zone IX) and southwest of the 'Jurassic Park' spawning closure (Zone XXIII) (Figure 6). Egg densities, however, were highest during 2013, particularly through the central corridor of the gulf where they peaked at 28.0 eggs.m^2 (Zone IX) (Figure 6). Low quantities (<5 eggs.m^2) of Snapper eggs were found throughout the southern boundary of the extended survey area in 2015.

A clear spawning 'hot-spot' was identified off Edithburgh in Gulf St. Vincent in 2014, where egg densities peaked at 97.4 eggs.m^2 (Zone VIII) (Figure 6). Egg densities exceeded 10 eggs.m^2 at each sampling station within this central western zone (VIII). Despite a few small patches of eggs that exceeded 4 eggs.m^2 within the northern half of the gulf, the distribution of eggs throughout this region was relatively uniform. Low level spawning activity was observed throughout most of the southern gulf with a relatively large isolated patches of eggs >1.0 eggs.m^2 located in the south-eastern corner (Zone XVIII) (Figure 6). The distribution of eggs in 2015 was patchier, particularly throughout the southern gulf. Similar to the previous survey, low level spawning activity was observed in the south-eastern corner of the gulf. The extent of spawning in the northern gulf was also reduced, with the bulk of eggs aggregated at densities of 1-5 eggs.m^2 within a narrow band located in the centre of the gulf. Egg density peaked at 6.2 eggs.m^2 within the centre of this band (Zone VI) (Figure 6).

Determining the proportion of eggs encompassed by the closed areas provides some indication as to their relative effectiveness in protecting spawning Snapper. The 'Illusion' closure encompassed the greatest proportion of eggs during the 2013 survey, with approximately 9% of all eggs surveyed within Spencer Gulf located within the boundaries of this closed area (Table 2). The remaining three closures contained a further 0.86% of the eggs, ranging from 0.09% for 'Estelle Star' to 0.4% for 'Jurassic Park'. This level of 'protection' was not maintained in 2015, where the total proportion of eggs located within the four closed areas was approximately 1.3% (Table 2). With the exception of 'Jurassic Park' which was devoid of eggs during the 2015 survey, the closures afforded similar levels of protection, each accounting for approximately 0.5% of the total eggs surveyed within the gulf. The single closed area in Gulf St. Vincent accounted for 0.15% of eggs sampled in 2014, and no Snapper eggs were detected within it in 2015 (Table 2).

Table 2. The relative proportion (%) of Snapper eggs encompassed by the five spatial spawning closures that were implemented in 2013 for each of the DEPM surveys. * includes formalin-corrected samples.

YEAR	GULF	CLOSURE	NO. EGGS (CLOSED)	TOTAL EGGS	% EGGS
2013	SPENCER GULF	ESTELLE STAR	2.63E+06	3.02E+09	0.09%
		ILLUSION	2.82E+08	3.02E+09	9.34%
		JURASSIC PARK	1.22E+07	3.02E+09	0.40%
		SANTA ANNA	1.12E+07	3.02E+09	0.37%
		TOTAL	***3.08E+08***	***3.02E+09***	***10.20%***
2015	SPENCER GULF*	ESTELLE STAR	7.05E+06	2.05E+09	0.34%
		ILLUSION	9.25E+06	2.05E+09	0.45%
		JURASSIC PARK	0.00E+00	2.05E+09	0.00%
		SANTA ANNA	9.63E+06	2.05E+09	0.47%
		TOTAL	***2.59E+07***	***2.05E+09***	***1.26%***
2014	GULF ST. VINCENT	GSV	1.79E+07	1.16E+10	0.15%
		TOTAL	***1.79E+07***	***1.16E+10***	***0.15%***
2015	GULF ST. VINCENT*	GSV	0.00E+00	2.76E+09	0.00%
		TOTAL	***0.00E+00***	***2.76E+09***	***0.00%***

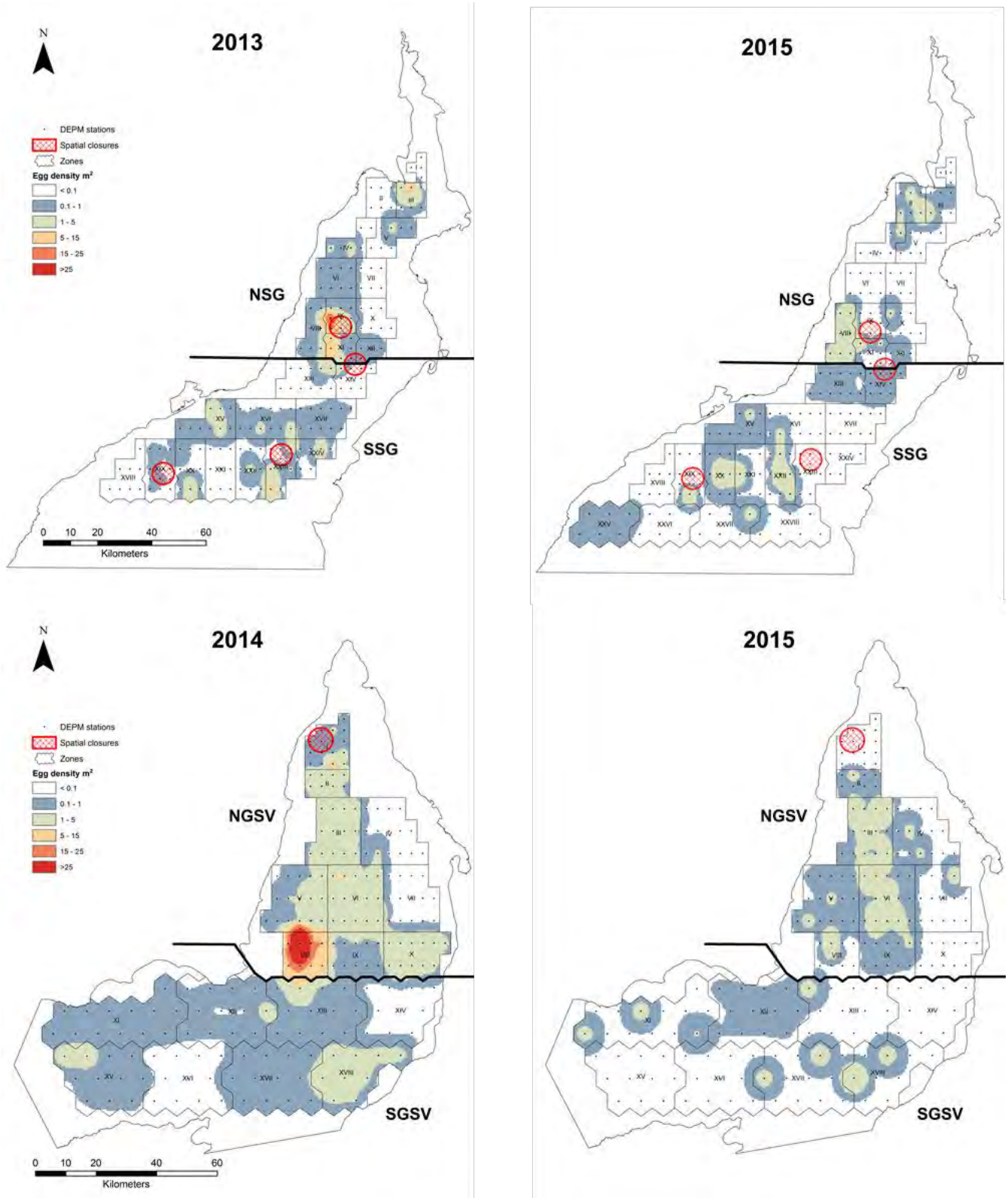

Figure 6. Snapper egg densities (eggs.m^{-2}) for each of the four DEPM surveys. A minimum egg density of 0.1 eggs.m^{-2} was calculated using interpolated GIS software (ArcGIS) used to define the outer boundary of spawning activity for each survey.

Spawning Area (*A*)

The extent of the area surveyed within each gulf was modified over the course of the study. A greater area was surveyed in Gulf St. Vincent compared with Spencer Gulf on both occasions (Table 3). The relative proportion of spawning area was consistently greater in the northern gulfs. Approximately 58% of the survey area contained Snapper eggs of densities >0.1 eggs.m^{-2} in Spencer Gulf in 2013 (Table 3). This was reduced to 41% in 2015. Similarly, 80.2% of the survey area in GSV 2014 displayed evidence of spawning activity. This was subsequently reduced to approximately 36% in 2015 (Table 3). It is important to note, however, that the 2015 estimates included formalin-corrected egg samples, and are likely to be less accurate than the previous (2013, 2014) samples.

Table 3. Snapper surveys areas, spawning areas, and the proportion of spawning area, by survey and year, computed from GIS contour mapping of the egg survey estimates, defined as interpolated egg densities > 0.1 eggs.m^{-2}. * includes formalin-corrected samples.

YEAR	GULF	REGION	SURVEY AREA (km^2)	SPAWNING AREA (*A*)	AREA WITH EGGS (%)
2013	SPENCER GULF	NSG	1837.83	1188.28	64.66
		SSG	2968.22	1611.71	54.30
		TOTAL	***4806.05***	***2799.98***	***58.26***
2015	SPENCER GULF*	NSG	1623.73	800.62	49.31
		SSG	4310.22	1631.74	37.86
		TOTAL	***5933.95***	***2432.36***	***40.99***
2014	GULF ST. VINCENT	NGSV	3161.73	2563.17	81.07
		SGSV	4860.04	3871.30	79.66
		TOTAL	***8021.77***	***6434.48***	***80.21***
2015	GULF ST. VINCENT*	NGSV	3161.73	1651.79	52.24
		SGSV	4476.43	1094.13	24.44
		TOTAL	***7638.16***	***2745.92***	***35.95***

Daily Egg Production (D_0)

Individual spawning time for each egg was estimated by subtracting its age from the time of day it was sampled. Combining these data across all surveys indicated that Snapper spawn throughout the day, with spawning activity increasing from late morning through to 2300 hours (Figure 7). With the exception of an irregular peak in spawning activity identified at 1100 hours, which was inflated by a single sample containing a high number of Snapper eggs, spawning activity appeared to steadily increase from midday and peak during the evening (1700 to 2100 hours) (Figure 7). There was evidence of low level spawning during the other times of the day. Given this continuous spawning pattern, a 'peak' spawning time could not be determined.

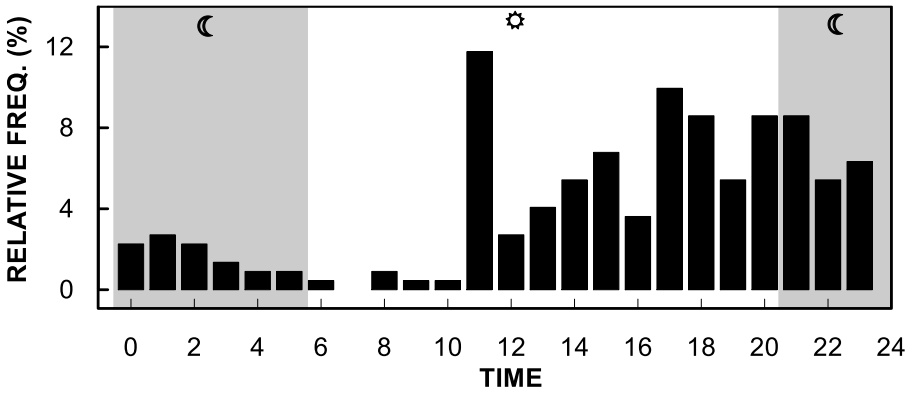

Figure 7. Daily cycle of spawning of South Australian Snapper. Spawning times were derived for each egg by subtracting the estimated age (in hours) from the time it was sampled. All data were combined across all DEPM surveys. The daylight hours are indicated by the sun icon.

The distribution of estimates of mean daily egg production (D_0) for each sample tow were over-dispersed for the 2013 and 2014 DEPM surveys, where the majority of sampling stations yielded zero-egg counts (Figure 8). This was accentuated in GSV in 2014 where approximately 60% of the stations surveys within the defined spawning area had estimated daily egg production rates that were less than 1 eggs.day^{-1}.m^{-2}. These estimates were less frequent in SG in 2013 accounting for approximately 35% of the samples. In both surveys the northern gulfs contained more stations with relatively high egg densities that exceeded production rates of 7 eggs.day^{-1}.m^{-2} compared with the southern gulfs. A similar dataset was not calculated for the 2015 DEPM surveys as the proportions of validated Snapper eggs in both the 'possible' and 'unlikely' samples were used to adjust the formalin-preserved egg counts.

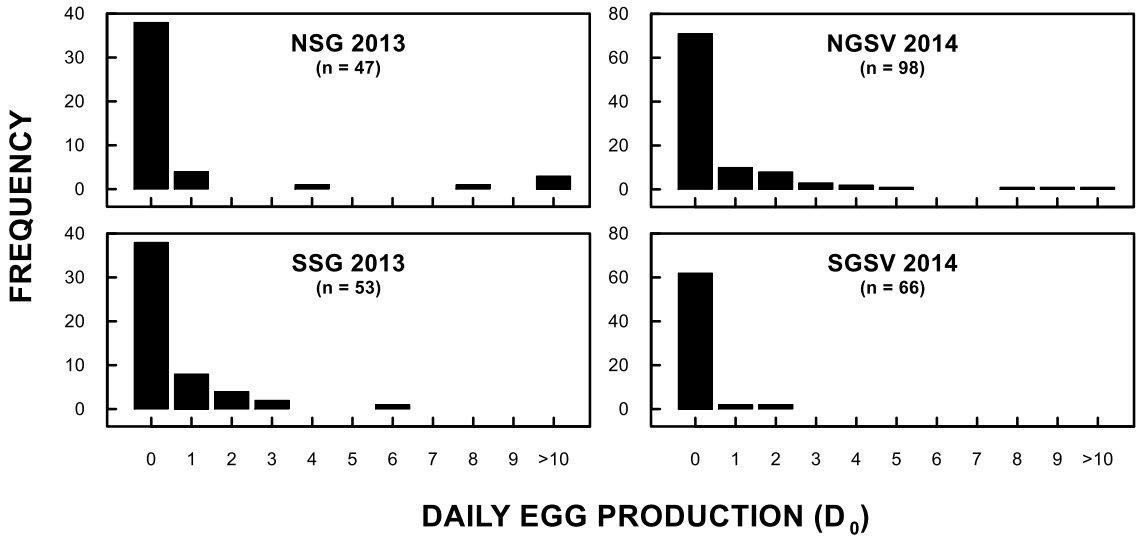

Figure 8. The frequency of the estimates of daily egg production (D_0) for each sample tow undertaken in the 2013 and 2014 DEPM surveys. All tows that fell outside the spawning area (A_{sp}) were excluded from the analysis.

At an assumed instantaneous mortality rate of 0.4 day^{-1} mean daily egg production (D_0) ranged from 1.54 to 20.67 eggs.day^{-1}.m^{-2} throughout the study (Table 4). Egg production was consistently higher in the northern

gulfs, with the difference ranging from 2.0 to 2.1 times greater in Spencer Gulf and 4.4 to 10.2 times greater in Gulf St. Vincent. Gulf St. Vincent was up to 6.4 times more productive than Spencer Gulf. The influence of increasing mortality rates from 0.2 to 0.6 day^{-1} had a variable influence on mean egg production, increasing estimates of D_0 by 19.2% in NSG in 2013 up to 34.4% in NSG in 2015 (Table 4).

Table 4. Estimates of D_0, the mean egg density at time of spawning for each survey region and year. Values of D_0 are given for five *a priori* values of egg mortality Z, with the middle value (Z = 0.4 day^{-1}) used to estimate of spawning biomass in this study. * includes formalin-corrected samples.

YEAR	GULF	REGION	MORTALITY (Z)				
			0.2	*0.3*	*0.4*	*0.5*	*0.6*
2013	SPENCER GULF	NSG	2.97	3.09	**3.23**	3.38	3.53
		SSG	1.38	1.46	**1.54**	1.64	1.74
2015	SPENCER GULF*	NSG	6.71	7.22	**7.78**	8.37	9.02
		SSG	3.45	3.64	**3.84**	4.05	4.28
2014	GULF ST. VINCENT	NGSV	18.36	19.48	**20.67**	21.95	23.31
		SGSV	4.14	4.42	**4.73**	5.06	5.42
2015	GULF ST. VINCENT*	NGSV	11.39	12.07	**12.80**	13.57	14.40
		SGSV	1.11	1.18	**1.25**	1.33	1.41

Female Weight (*W*)

Weight frequency histograms for the northern and southern regions of each gulf were reconstructed from length data using an allometric length-weight relationship. As previously noted by Fowler et al. (2013), the size/weight composition of the regional populations were different in each gulf. Greater proportions of large Snapper, exceeding 6 kgs, were evident in the northern gulfs, whilst the southern gulfs were dominated by < 2 kg fish. These regional differences were emphasised in Gulf St. Vincent, where there was an absence of Snapper > 8 kg in the southern gulf and individuals weighing up to 14 kg in the northern gulf (Figure 9). The large range in Snapper weights suggests that substantial improvement in biomass estimates would be achieved by explicitly accounting for the size structure of South Australian Snapper compared with the standard DEPM approach of using a mean weight. This more refined approach was adopted in this study (Appendix 2).

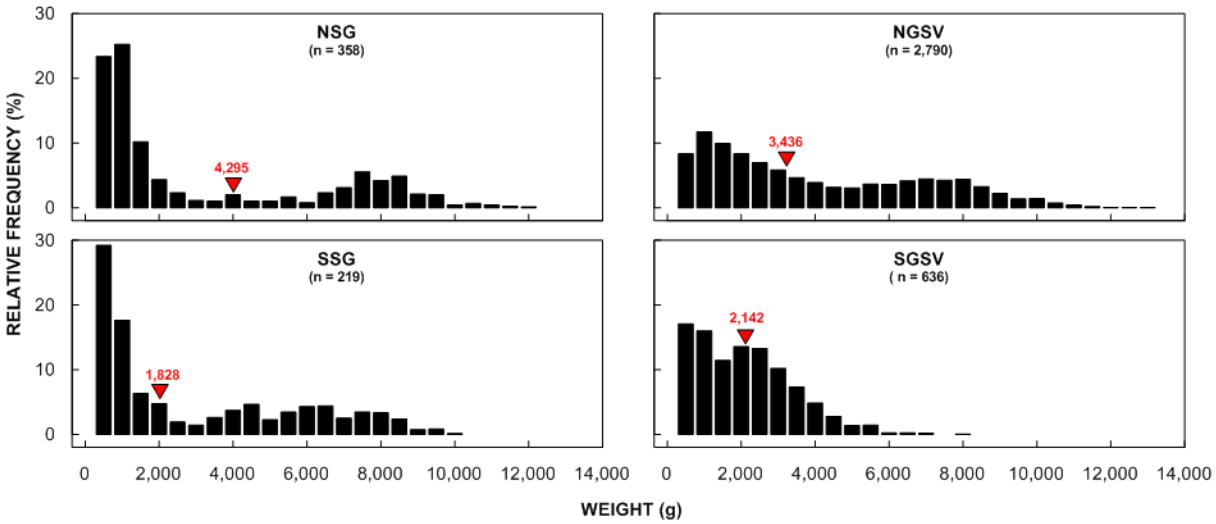

Figure 9. Weight frequencies of South Australian Snapper combined from the fishery-dependent market sampling program and fishery-independent adult sampling for each of the four regions. Mean Snapper weights are indicated by red arrows.

Sex Ratio (*R*)

Female sex ratios were neither spatially nor temporally consistent. With the exception of the 2015 Gulf St. Vincent survey, the Snapper population was biased towards females in the northern gulfs, accounting for 57% of the population by weight in GSV 2014 to 63% in SG 2013 (Table 5). The sex ratios of the southern gulfs were relatively equal (approximately 50%). The lowest proportion of females was observed in Northern GSV in 2015, where they accounted for 37% of the population by weight. Alternatively, in the southern gulf, they accounted for 69% of the population, although, not many Snapper (n = 14) were sampled from this region. Gulf-wide sex ratios by weight varied by < 30%.

Table 5. Population sex ratio (*R*) by weight (± standard error) for each survey region and year. * indicates the combination of all gulf specific samples due to insufficient regional samples.

YEAR	GULF	REGION	*n*	SEX RATIO (*R*)	*SE*
2013	SPENCER GULF	NSG	116	0.63	0.15
		SSG	103	0.45	0.11
2015	SPENCER GULF	NSG	47	0.54	0.24
		SSG	59	0.49	0.17
2014	GULF ST. VINCENT	NGSV	94	0.57	0.17
		SGSV	18	0.45	0.16*
2015	GULF ST. VINCENT	NGSV	100	0.37	0.05
		SGSV	14	0.69	0.05*

Batch Fecundity (*F*)

The relationship between batch fecundity (*F*) and total female weight (*W*) was best described by allometric linear regression. No statistical differences were detected between the relative slopes (analysis of covariance, year*weight interaction: $F_{2, 109} = 0.07$, p = 0.94) nor intercepts (year: $F_{2, 109} = 0.23$, p = 0.53) of the linear relationships between years. Consequently all data were combined into a single analysis and fitted using maximum likelihood (Figure 10; see Appendix 2). This overall relationship was similar to previous studies that examined reproduction and spawning dynamics of Snapper in South Australia over the past 16 years (Figure 10).

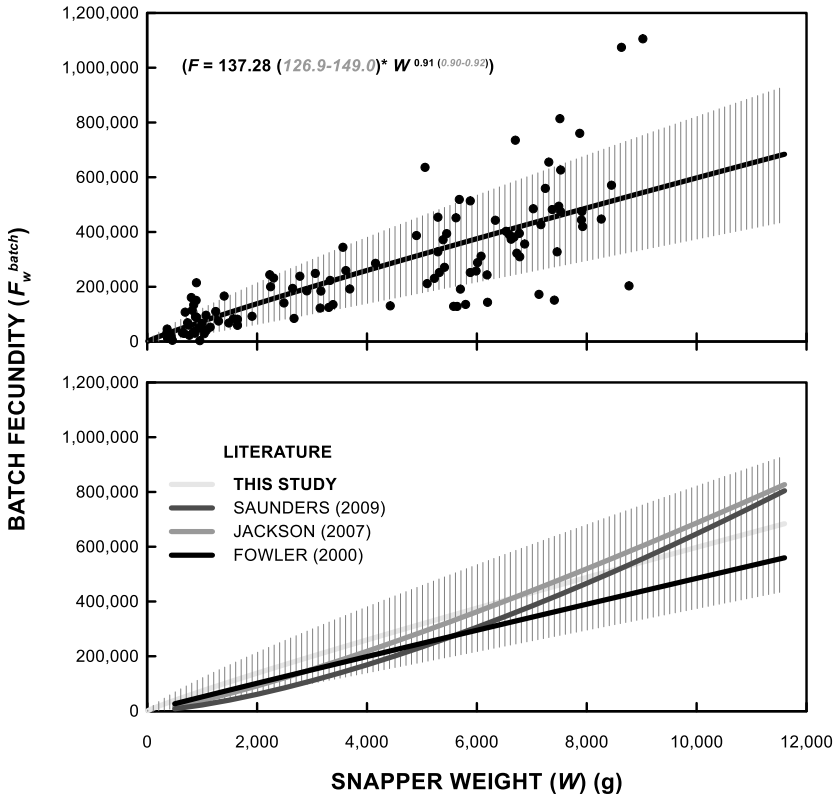

Figure 10. Batch fecundity versus body weight for South Australian Snapper. *Upper panel*: Fit of measured batch fecundity using maximum likelihood. The error bars indicate estimated 95% confidence intervals (shown as grey error bars in both panels). *Lower panel*: Comparison of the batch fecundity by weight relationship for Snapper derived in this study with previous published studies.

Spawning Fraction (*S*)

All three surveys were carried out when either half, or the majority, of the females within the population were in spawning condition, with spawning fractions ranging from 50% in NGSV in 2015 to 82% in NSG in 2015 (Table 6). At the time of these surveys, the spawning fractions were consistently higher in NSG in both 2013 and 2015, with >72% of the females contributing to the spawning population. This was up to 28% higher than SSG where an estimated 55% of the females exhibited physical evidence of spawning activity. This marked regional difference was not evident in Gulf St. Vincent, where spawning fractions differed by < 7% between

the northern and southern regions in both years. Spawning fractions within this gulf exceeded 75% in 2014 and declined to approximately 50% in 2015 (Table 6).

Table 6. Population spawning fraction (*S*) by weight (± standard error) for each survey region and year. * indicates the combination of all gulf specific samples due to insufficient regional samples.

YEAR	GULF	REGION	*n*	SPAWNING FRACTION (*S*)	*SE*
2013	SPENCER GULF	NSG	54	0.72	0.29
		SSG	43	0.58	0.23
2015	SPENCER GULF	NSG	28	0.82	0.23
		SSG	28	0.54	0.18
2014	GULF ST. VINCENT	NGSV	54	0.76	0.12
		SGSV	7	0.71	0.11*
2015	GULF ST. VINCENT	NGSV	34	0.50	0.12
		SGSV	7	0.57	0.08*

Spawning Biomass (*SB*)

Given the changes in the 2015 survey methodology, where estimates of egg densities for the formalin-preserved samples were coarsely adjusted at the 'zonal' level, the associated estimates of spawning biomass cannot be directly compared with the previous surveys (SG 2013 and GSV 2014). However, considering this incompatibility, it was generally observed that estimates of spawning biomass were consistently lower in SG compared with GSV (Table 7). Furthermore, the regional estimates of spawning biomass displayed the same trend within each gulf over their respective surveys, where they were consistently higher in southern SG and northern GSV (Table 7).

All Snapper eggs collected during the 2013 SG and 2014 GSV surveys were validated using the ISH molecular technique, consequently there is greater confidence in the estimates of spawning biomass obtained from these surveys, compared with the 2015 surveys that applied a 'zonal' egg correction factor. At an assumed egg mortality rate of 0.4 day^{-1} the estimate of Snapper spawning biomass in SG in 2013 was 280 t with a standard error of 55% (Table 7). Snapper spawning biomass in GSV 2014 was an order of magnitude higher at an estimated 2,780 t with a standard error of 52%. The Snapper population was estimated to consist of 45,194 females in SG 2013 and 421,619 females in GSV 2014 (Table 7). The commercial catch of Snapper in the corresponding years was 50.1 t in NSG 2013 and 430 t in GSV 2014, representing approximately 18% and 15% of the estimated spawning biomass, respectively (Figure 11).

The 2015 estimates of spawning biomass, which included the 'zonal' egg correction to account for the formalin-preserved egg samples, exhibited the same trend where the spawning biomass was higher in GSV compared with SG. Snapper spawning biomass in GSV was estimated at 1,856 t (± 684 t) consisting of 172,155

females, which was approximately three times greater than SG which had an estimated spawning biomass of 592 t (± 355 t), and consisted of 91,566 females (Table 7). The corresponding commercial catch of Snapper was 47 t in NSG 2015 and 380 t in GSV 2015, representing approximately 7.9% and 21.0% of the estimated spawning biomass, respectively (Figure 11).

Table 7. Snapper spawning biomass estimates (t ± standard error), and the estimated total number of female Snapper in the spawning population, by region and year. * includes formalin-corrected egg samples.

YEAR	GULF	REGION	NUMBER OF FEMALES	SPAWNING BIOMASS (*SB*) t (± SE)	% SE
2013	SPENCER GULF	NSG	24,466	132 (54 - 210)	59%
		SSG	20,728	148 (74 - 222)	50%
		TOTAL	***45,194***	***280 (127 - 433)***	***55%***
2015	SPENCER GULF*	NSG	34,882	220 (61 - 379)	72%
		SSG	56,684	371 (175 - 567)	53%
		TOTAL	***91,566***	***592 (237 - 946)***	***60%***
2014	GULF ST. VINCENT	NGSV	259,008	1,933 (912 - 2,954)	53%
		SGSV	162,611	847 (424 - 1,270)	50%
		TOTAL	***421,619***	***2,780 (1,336 - 4,224)***	***52%***
2015	GULF ST. VINCENT*	NGSV	156,959	1,804 (1,143 - 2,466)	37%
		SGSV	15,196	52 (29 - 74)	44%
		TOTAL	***172,155***	***1,856 (1,171 - 2,540)***	***37%***

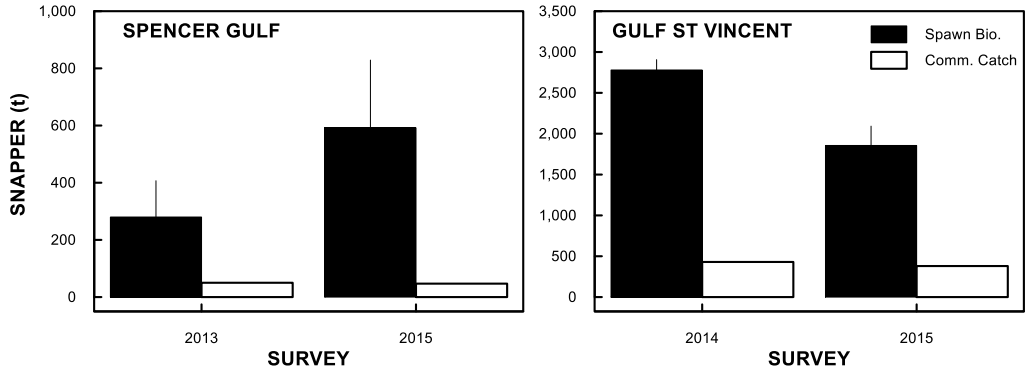

Figure 11. Comparison of the Snapper spawning biomass estimates (t ± standard error) with the commercial catch of Snapper for Spencer Gulf 2013 and 2015; and Gulf St. Vincent 2014 and 2015.

Sensitivity Analysis

Like the method of Bernal et al. (2011), the modified DEPM estimation method derived in this study assumed that individual surveys cannot yield usable estimates of egg mortality rate (*Z*). This is because mortality would need to be measured as a variation of egg density over time, and DEPM surveys measure variation over space. To counter this, prior values of *Z* are often assumed, and used as input quantities. There were marginal differences in the estimates of spawning biomass when various assumed mortality rates ranging from 0.2 to

0.6 day^{-1} were modelled (Figure 12). This was relatively consistent for all eight DEPM surveys, where all spawning biomass estimates fell within the 95% confidence intervals of each other, regardless of the assumed *Z* value. The greatest divergence was evident in the NGSV 2014 survey for which the estimates spanned approximately 463 t. Conversely, there was less than 25 t difference in spawning biomass estimates across the ranges of *Z* values for the NSG 2013 survey (Figure 12).

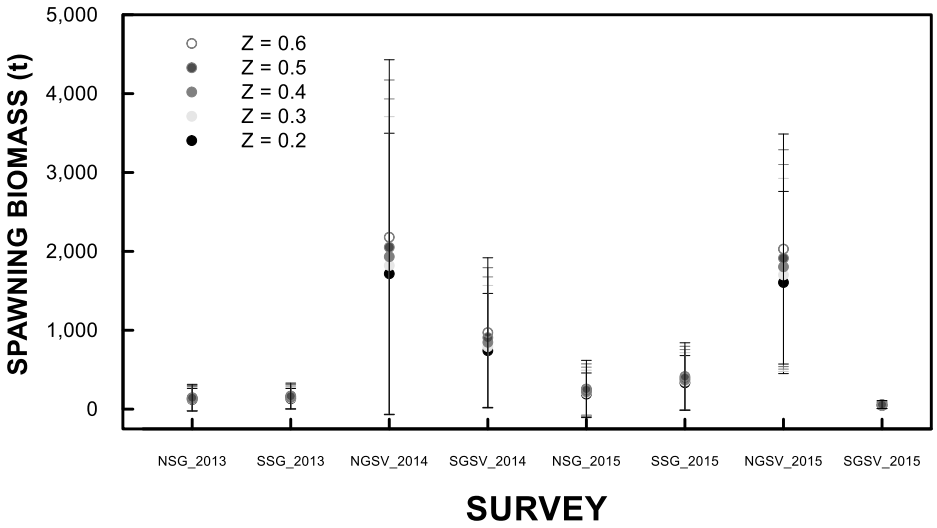

Figure 12. Snapper spawning biomass estimates from the eight DEPM surveys, computed assuming five different values for egg mortality rate (*Z*). 95% confidence intervals are shown for each spawning biomass estimate.

Cost-Benefit Analysis

The cost differential between the exclusively fishery-independent program and the fully integrated assessment compared to the existing 'baseline' fishery-dependent program ranged from -42.3% to +26.5%, respectively (Figure 13). The augmented program, which omitted the fishery modelling component of the assessment and placed greater reliance on the estimate of spawning biomass derived from the DEPM, represented a 7.5% increase in cost.

Although there are cost savings of an exclusively fishery-independent program, the estimated overall benefit is less (-0.2 points) than the current fishery-dependent program (Table 8). The strength in the current program relates to improving biological knowledge of Snapper spawning dynamics and supporting environmental processes, but trades off against the level of information required to assess the performance of the fishery. Furthermore, without reference to any fishery-dependent metrics this program scores poorly against the essential management objectives in ascribing the status of the stock (-0.8 points) and determining the allocation of shares amongst the other sectors (-2.6 points). Despite these short-comings in achieving these specific management objective, there is considerable benefit in undertaking this program to assess and optimise the location of spawning closures (+3.8 points) (Table 8).

The integrated program which encompasses all available sources of information currently available is 26.5% more expensive than the current program (Figure 13). This increase in cost translates to a 31.5 point improvement in overall benefit, representing a 74.3% increase. A 1% increase in cost for this program

subsequently converts to a 2.8% increase in benefit. The greatest benefits were associated with the level of biological information the program was expected to provide, scoring 21.5 points, which was more than double the relative amount provided by the baseline program. The program was also considered to provide greater benefit in terms of the associated estimates of fishable (spawning) biomass and harvest fractions, and also improved level of information to support the assignment of 'stock status'.

The augmented program, which substituted the fishery modelling component with the DEPM represented a 7.5% increase in cost. The overall benefit of this program was 25.7 points greater than the baseline program, representing an increase of 60.6% (Table 8). This translated to an 8.1% increase in benefit for every 1% increase in cost, which was the most cost-effective of the three proposed alternative programs. Excluding the fishery modelling component clearly impacted on the relative value of the model-derived fishery performance indicators (i.e. fishable biomass, harvest fraction, egg production and recruitment), scoring 2.8 points less than the fully integrated program. The provision of biological information, however, scored the same as the integrated program, whereas the relative benefits in achieving management objectives was 2.3 points less (Table 8). This reduction was mostly evident in 'ensuring the long-term sustainable harvest' (-1.0 points) and determining the 'stock status' (-0.7 points) of the resource.

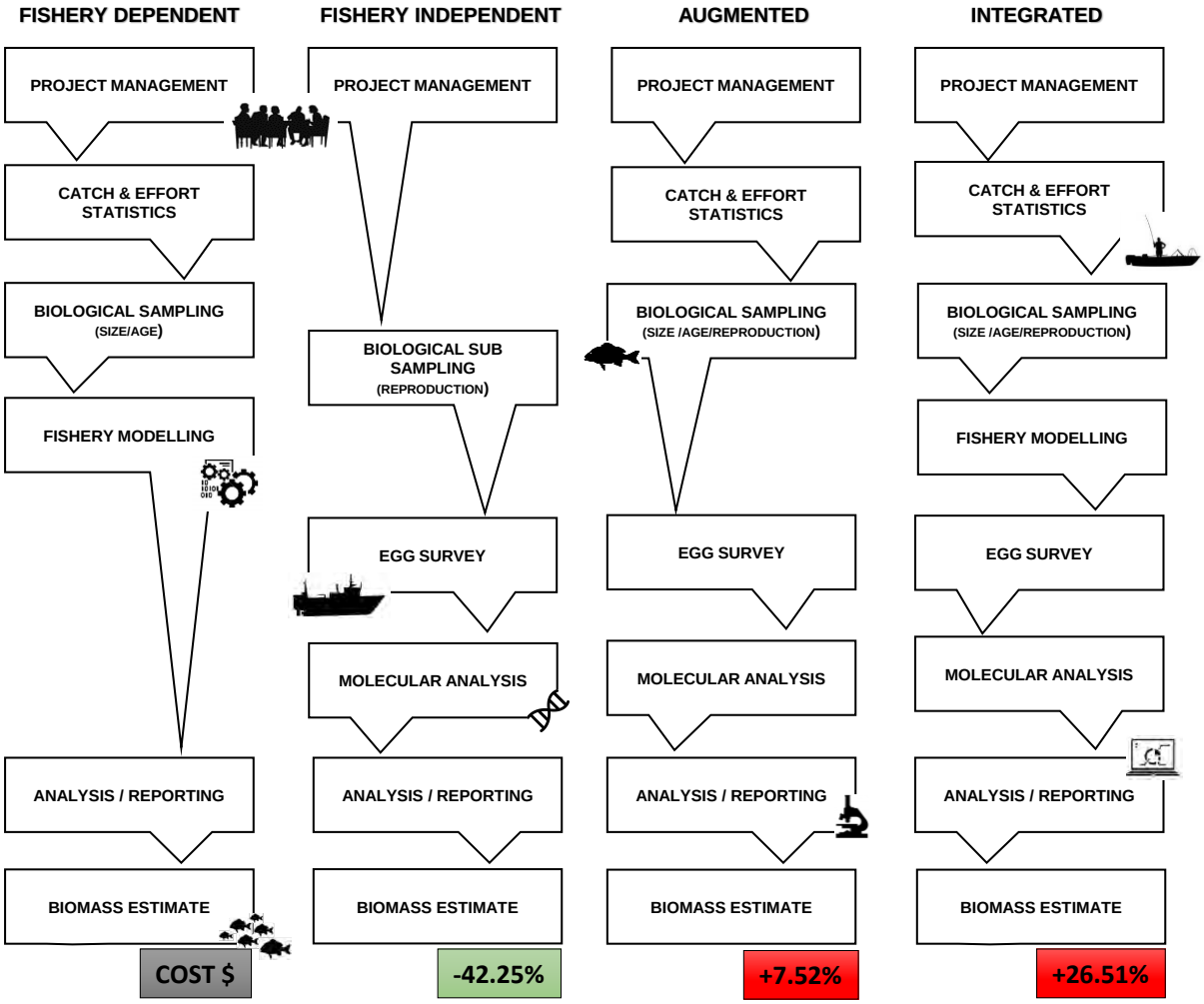

Figure 13. A schematic representation of the 'fishery-dependent' program that is current used to assess South Australia's Snapper Fishery and the relative cost differential (as % change) with three proposed alternative programs; 'Fishery-independent', 'Augmented' and 'Integrated'.

Table 8. The results of a qualitative 'score-based' assessment of the potential benefits of the 'fishery-dependent' program that is currently used to assess South Australia's Snapper Fishery in comparison with three proposed alternative programs; 'Fishery-independent', 'Augmented' and 'Integrated'. Scores are averaged across six independent assessments undertaken by two fisheries managers, three fisheries scientists and a fisheries modeller.

BENEFITS	FISHERY-DEPENDENT	FISHERY-INDEPENDENT	AUGMENTED	INTEGRATED
1. ASSESSING FISHERY PERFORMANCE				
TRENDS IN CATCH & EFFORT	4.7	0.0	4.8	4.8
FISHABLE (SPAWNING) BIOMASS	1.8	3.2	3.3	3.8
HARVEST FRACTION	1.8	1.7	3.2	3.8
EGG PRODUCTION	1.7	1.5	2.3	3.5
RECRUITMENT	2.7	1.5	3.0	3.5
ALLOCATION	2.8	0.7	3.2	3.2
SCORE (/30)	*15.3*	*8.5*	*19.8*	*22.7*
2.LEVEL OF BIOLOGICAL INFORMATION				
AGE/SIZE STRUCTURE	4.5	0.8	4.5	4.5
REPRODUCTION	2.8	3.0	4.3	4.3
EARLY LIFE HISTORY	0.3	2.3	2.3	2.3
PATTERNS OF SPAWNING	1.2	3.3	4.0	4.0
PLANKTON ASSEMBLAGE (OTHER SPECIES)	0.0	3.0	3.2	3.2
ENVIRONMENTAL PARAMETERS (TEMP, SALINITY)	0.2	3.0	3.2	3.2
SCORE (/30)	*9.0*	*15.5*	*21.5*	*21.5*
3. ACHIEVING MANAGEMENT OBJECTIVES				
STOCK STATUS	3.3	2.5	3.8	4.5
ENSURE LONG-TERM SUSTAINABLE HARVEST	2.5	3.0	3.3	4.3
MAINTAIN CATCHES WITHIN ALLOCATION	3.8	1.2	3.8	3.8
MINIMISE IMPACT ON ECOSYSTEM	1.2	1.2	1.8	1.8
TAKE ACCOUNT OBJECTIVES OF OTHER SECTORS (REC)	2.3	1.7	2.7	3.0
OPTIMISE SPAWNING CLOSURES*	0.7	4.5	4.5	4.8
SCORE (/30)	*13.8*	*14.0*	*20.0*	*22.3*
TOTAL SCORE (%)	***42.4***	***42.2***	***68.1***	***73.9***

Discussion

Feasibility of using the DEPM to assess Snapper Stocks

This study successfully designed and validated an *in situ* hybridisation (ISH) approach to identify Snapper eggs (and larvae) from field-collected ichthyoplankton samples using the mitochondrial 16S ribosomal RNA gene as a target for a specific oligonucleotide probe (see Oxley et al. unpublished). This highly-specific molecular probe targets Snapper ribosomal RNA and when conjugated with a reactive molecule produces a visible colour in positive reactions. Snapper eggs are subsequently coloured blue and easily detected under a standard stereo dissecting microscope (Figure 14). The relatively non-destructive nature of this probe represents a significant advancement in its application in DEPM programs as the developmental stage and subsequent age of each egg can be accurately determined and archived. The 100% efficacy of the probe was validated through a series of structured tests, including: identifying Snapper eggs in mixed plankton samples spiked with a known amount of reference material; in samples that contained closely related species (i.e. Black Bream, *Acanthopagrus butcheri*); across the entire embryonic developmental sequence (i.e. from < 4hrs post fertilisation to hatching); and from field-collected ichthyoplankton samples (Oxley et al. unpublished).

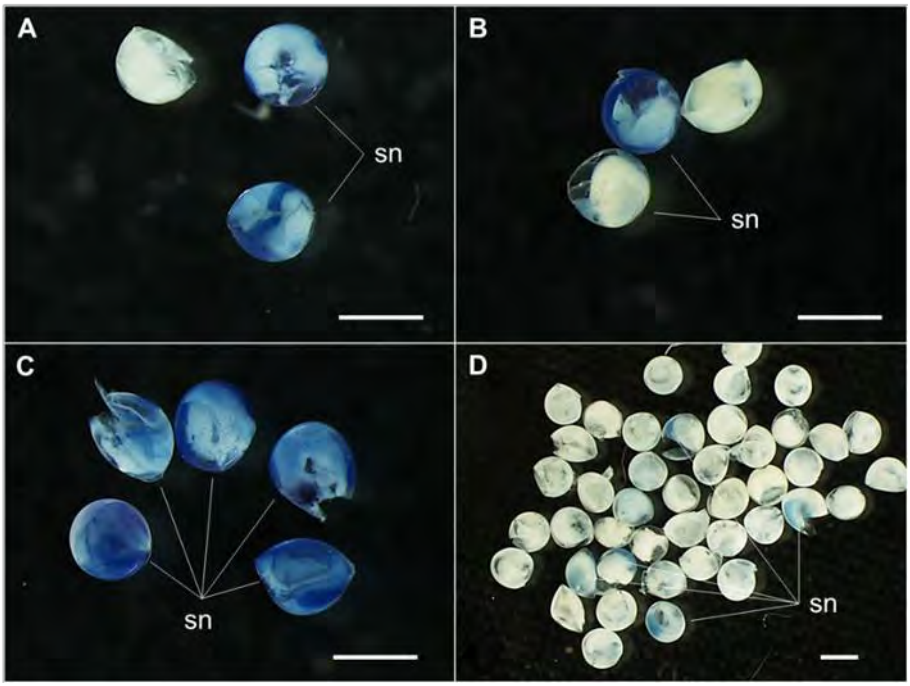

Figure 14. Examples of successful hybridisation of the molecular Snapper probe. Snapper eggs are stained blue clearly differentiating them from other fish eggs collected from the DEPM ichthyoplankton surveys.

Applying the molecular probe to egg samples that were visually pre-sorted to contain 'possible' Snapper eggs which were separated from 'unlikely' Snapper eggs on the basis of gross morphological characteristics such as egg size, oil globule dimensions, and embryo morphology, indicated that approximately 19% to 80% were misidentified depending on the origin of the sample. Conversely, a small proportion (< 2%) of fish eggs that

were ‘unlikely’ to be from Snapper were confirmed as Snapper. Further genetic analysis indicated that those eggs that were misidentified represented a range of different fish species, including species from the families Carangidae (Mackerel), Callionymidae (Stinkfish), Cynoglossidae (Sole), Gerreidae (Silverbelly), Labridae (Wrasse), Mullidae (Mullet), Neosebastidae (Scorpionfish), Pinguipedidae (Wavy Grubfish), Platycephalidae (Flathead), Sillaginidae (Whiting) and Tetrapontidae (Striped Perch) (Oxley et al. unpublished). Similar visual misidentification rates were reported for Snapper in ichthyoplankton samples collected from Western Australia, with misidentified eggs including Common Jack Mackerel (*Trachurus declivis*), Rusty Flathead (*Inegocia japonica*) and Longhead Flathead (*Leviprora inops*) (Dias et al. 2016). These results highlight the importance of using molecular-based techniques to validate morphological identification of eggs in application of the DEPM (Neira et al. 2014; Dias et al. 2016). The ISH method developed in this study can be extended to other species where egg identification has been problematic, such as King George Whiting (*Sillagnodes punctatus*) (Fowler 2000), and some carangids (i.e. Yellow Scad, *Trachurus novaezelandiae*, and Silver Trevally, *Pseudocaranx dentex*) (Keane and Lyle 2015, Ward and Grammer 2016).

Level of Confidence in the Estimate of Biomass

Currently, the determination of stock status of South Australia’s Snapper Fishery is assisted through assessment of fishery performance indicators that are compared against trigger reference points (Fowler et al. 2016). There are two sets of fishery performance indicators, ‘general’ and ‘biological’ (PIRSA 2013). The general performance indicators are based entirely on commercial fishery statistics, whereas the biological performance indicators are based on output from a computer-based model, The model, ‘SnapEst’, integrates all fishery and biological data available on population structure and outputs a time series of four parameters: fishable biomass; harvest fraction; egg production; and recruitment (McGarvey and Feenstra 2004). The most recent stock assessment identified considerable divergence between trends in the biomass of Snapper derived from the empirical data and output from the fishery model. Clear declining trends in fishery catch, effort and catch rates (CPUE) coupled with evidence of poor recruitment derived from an absence of early year classes in the population age structure suggested declining biomass in the SG/WC stock leading to it being assigned a status of ‘transitional-depleting’. Conversely, the SnapEst model interpreted broad age structures which included the presence of old (>10 years) fish and reduced levels of fishing effort as low exploitation, and subsequently inferred an increasing fishable biomass (Fowler et al. 2016). Greater emphasis was placed upon the empirical data in the assessment as it was acknowledged that the model does not have the capacity to resolve the inherent complexities within the fishery that relate to: dynamic fisher behaviour; advancing fishing technologies; reactive management arrangements; and Snapper habitat use and migration. However, it was understood that these complexities also introduced a level of subjectivity in the interpretation of fishery-dependent catch and effort data. Fishery management and industry were aware of these issues and initiated the development of the current project to explore whether a fishery-independent estimate of biomass for Snapper was achievable.

Fishery-independent estimates of spawning biomass for Snapper, derived from the DEPM in this study, aligned with the recent status assignment of the SG/WC and GSV stocks, reflected the relative levels of commercial

catch, and broadly agreed with the industry's assessment of the resource, where biomass was an order of magnitude higher in GSV compared with SG. It must be recognised, however, that a primary focus of this study was to determine the feasibility of using the DEPM as an assessment tool for Snapper. Changes in the egg sampling methodology were employed during the 2015 surveys as a contingency measure in the event the egg validation component of the study was unsuccessful. As such, the associated estimates of spawning biomass for SG and GSV in 2015 should be interpreted cautiously and greater emphasis placed on the biomass estimates derived for SG in 2013 and GSV in 2014. In these two years, fishery-independent estimates of spawning biomass were 280 t in SG (2013) and 2,780 t in GSV (2014). The corresponding estimates of fishable biomass derived from the fishery-dependent SnapEst model were approximately 7,000 t and 14,500 t, respectively (Fowler et al. 2016); suggesting that the model estimates were unrealistically inflated.

The DEPM provides unbiased estimates of spawning biomass for the survey period (Parker 1980) and, in most cases, is assumed to be the absolute estimate of abundance for a given year (Stratoudakis et al. 2006). However, estimates are typically imprecise with coefficients of variation generally exceeding 30% (Alheit 1993; Stratoudakis et al. 2006). Error variances in this study bounded the mean by approximately 50%, and were comparable to previous studies (Zeldis and Francis 1998; Jackson et al. 2012). There are numerous sources of biological variation that contribute to the imprecision of the biomass estimates, specifically relating to the size structure of spawning population, relative spawning fraction, batch fecundity, population sex ratio and egg density estimates (Alheit 1993). This study adopted new statistical approaches to account for the wide variation in the population size structure typical of Snapper, body-weight specific dependence on batch fecundity, and variation in the timing of spawning to refine estimates of daily egg production (Appendices 3 and 4). These approaches extended the traditional use of DEPM which have been largely confined to small pelagic fish that do not exhibit the same level of variation in population demography and spawning dynamics evident for Snapper, and are likely to have improved the accuracy of the biomass estimate. Although accounting for these sources of variation was a significant improvement, it is recognised that the uncertainty in estimates of mean daily egg production remains a key source of error. This is not unique to Snapper, but is rather a ubiquitous issue with the DEPM that is strongly influenced by the spatial variability in egg abundance, associated estimates of daily egg mortality rates, and the relative effectiveness of using vertical tows to sample disparate patches of eggs (Dickey-Collas et al. 2012). This issue is typically evidenced by over dispersed datasets that are characterised by a high proportion of zero egg counts (Pepin and Helbig 2012, Figure 8) and in many cases precludes the calculation of biologically meaningful egg mortality rates (Z) (Somarakis et al. 2002). Daily egg mortality rate, in this study, was assumed to be 0.4 day^{-1}, which was well within the published range for Snapper (-1.59 to 1.4 day^{-1}, Zeldis and Francis (1988); McGlennon (2003); Jackson et al. (2012)). Altering these rates from 0.2 to 0.6 day^{-1} had little effect on the overall estimates of spawning biomass implying that other sources of uncertainty are more influential than the assumed value of Z. It is likely that altering the egg sampling methodology to ensure that sampling size was more consistent with egg patches and involved sampling a greater volume of water would increase the probability of encountering eggs in a patchy environment and improve the precision of estimates of mean egg density at each site (McGarvey, unpublished).

Despite the inherent variance within DEPM estimates of biomass, it is important to consider its relative value in relation to the fishery-dependent alternative. Strong inconsistencies among the data sources that integrate into the current SnapEst model introduces considerable uncertainty in its output. Furthermore, the model struggles to reconcile the persistence of old fish in a population that is undergoing 'transitional-depletion', because, theoretically, low levels of fishing effort coupled with the presence of old fish is indicative of a sustainable resource. This interpretation, however, ignores the transfer of fishing effort towards other species as the Snapper resource becomes economically unviable to fish, or potential behavioural differences of older fish that may reduce their relative catchability (Fowler et al. 2016). The DEPM overcomes these deficiencies, as it provides an estimate of the absolute abundance of the stock.

Application in the Assessment and Management of Snapper

Relying exclusively on fishery-independent sources would not adequately support the appropriate future management of South Australia's Snapper Fishery. Regular DEPM surveys of each of the main Snapper stocks (i.e. SG/WC and GSV), would provide suitable information to assess stock status and develop management strategies (i.e. output controls) that would improve long-term sustainability. However, there would be insufficient information to meet other important management objectives that relate to maintaining catch allocations and access between the fishing sectors (i.e. commercial, recreational and traditional). Similarly, there would be a lack of important biological information obtained from fishery-dependent catch sampling programs to track trends in the demography of the population, particularly the appearance and relative strength of recruiting cohorts.

The integration of DEPM into existing fishery-dependent programs is relatively common, and in many cases the unbiased estimates of spawning biomass are used to 'tune' indirect assessment methods like catch-at-age analysis or biomass-based models (Jacobson et al. 1994; Deriso et al. 1996; Murua et al. 2010; Ward et al. 2015). A simplified cost benefit analysis indicated that a fully integrated assessment approach would provide a 2.8% rate of return on investment in the improvement of the assessment and management of Snapper. This program would include tuning the existing SnapEst model with the DEPM output, however, it remains unknown as to whether this would improve the overall confidence of the resulting biological performance indicators, or whether the strong inconsistencies in the input fishery-dependent data would continue to compromise model outputs. Substituting the SnapEst modelling component with the DEPM in an augmented assessment program appears to be the most-cost effective approach, yielding a return on investment of approximately 8%. The costing of these programs fits within the current triennial assessment schedule, where a full scale assessment is undertaken for Snapper every three years with limited interrogation of commercial catch and effort data in the intervening years. The regularity of the DEPM surveys, either as part of a fully integrated or augmented program, would depend on the specific objectives of the Snapper harvest strategy, which is scheduled for review in 2018, and associated budget considerations.

The DEPM provides a suite of extra information that may add 'value' to the assessment and management of the resource. Essential fish habitats, realised spawning areas and spatial variability in spawning behaviour can all be derived from DEPM surveys (Dickey-Collas et al. 2012). Furthermore, information on regional

hydrodynamics, ichthyoplankton distributions, relative productivity and environmental conditions are routinely collected, which may be of use in the assessment of broader ecosystem function. The patterns of egg density identified in this study provided an important insight into the relative effectiveness of the five Snapper spawning spatial closures that were implemented in 2013. All of the closures were strategically placed to encompass either wrecks or benthic structure that were known to support large quantities of Snapper. Those located in Spencer Gulf were the most effective, particularly the 'Illusion' closure which encompassed 9.3% of the eggs surveyed in 2013, offering clear protection to spawning Snapper during the time of the survey. Collectively, the four SG closures encompassed 10.2% of the eggs in 2013. Conversely, the 'GSV' closure in GSV offered little protection (<1%) as most of the spawning activity occurred further south. Nevertheless, the information derived from these surveys can be used to optimise the location of these Snapper spawning closures in the future and the methodology will also be relied upon in refining the recently implemented spatial closure for King George Whiting in South Australia's southern gulfs (Steer et al. 2016 - FRDC project No. 2016-003).

Refining the Method for Snapper

Throughout the course of this investigation three key areas of improvement were identified to enhance the application of the DEPM in the assessment of South Australian Snapper stocks. They specifically related to: maximising the effectiveness of the egg sampling methodology; streamlining the identification of Snapper eggs from mixed ichthyoplankton samples; and rationalising the level of data processing.

An important consideration in ecological field studies is to adopt a survey design that maximises the precision of absolute population density estimates in spatially clustered populations. McGarvey et al. (unpublished) demonstrated that long, narrow transects were consistently more precise than square quadrats in even moderately clustered populations. This finding could improve ichthyoplankton surveys and associated DEPM spawning biomass estimates. Snapper eggs are positively buoyant immediately post-fertilization and tend to become more neutrally buoyant during development (Kitajima et al. 1993; Nahas et al. 2003). Consequently, their relative abundance is likely to be higher at the sea surface (Parsons et al. 2014). Furthermore, the aggregative nature of adult Snapper along with their ability to spawn throughout the day contributes to the patchiness in the distribution of eggs (Jackson 2007). So far, Snapper eggs have been sampled using either short vertical or oblique net tows with insufficient replication to achieve optimal precision (Zeldis 1993; McGlennon 2003; Jackson 2007). SARDI has recently purchased a high speed plankton sampler ('Gulf VII' or 'Nackthai' net) that is designed to undertake controlled oblique tows throughout the water column over longer distances. This new sampler could improve sampling efficiency and contribute to the optimisation of the precision estimates of mean daily egg production. However, this will require validation by comparing its relative performance against traditional samplers (Ward et al. 2017 – FRDC 2017-027).

The Snapper egg identification and validation component of this study was extremely challenging. The robust nature of the eggs, particularly their tough protective extracellular casing (or 'chorion'), was highly resistant to chemical dissolution (see Oxley et al. unpublished). Consequently, the specific molecular probe was unable to penetrate the chorion and hybridise with the embryonic ribosomal RNA. To counter this, each egg was

manually perforated to expose the embryonic tissue, allowing positive hybridisation in all Snapper eggs tested (see Oxley et al. unpublished). It was anticipated that throughout the course of this three-year study the molecular validation method would improve our understanding of Snapper embryo taxonomy and inform future visual egg identification methods, potentially alleviating the need to perforate each egg. Despite storing eggs in formalin during the 2015 surveys, which precludes molecular validation but clearly preserves the morphological characteristics of the egg and developing embryo (Steedman, 1976; Keane 2015), the visual identification of Snapper eggs was not 100% conclusive (see also Dias et al. 2016). In order to streamline the egg identification component of the DEPM, it is suggested that future surveys rely exclusively on the unbiased molecular validation technique, alleviating the source of error associated with visual identification.

Considerable data computation was required to derive an estimate of spawning biomass and its associated error variance for Snapper over the eight DEPM surveys. Unlike traditional DEPM programs, this project extended the statistical analysis to account for the inherent size-dependence in Snapper reproductive output and population structure (Appendix 3). Furthermore, it derived a new method to estimate egg density at time of spawning, where instead of accounting for loss of eggs through mortality for the overall population, it accounted for each observed egg in each individual sample. This was necessary to overcome the challenge of ascribing a specific time of spawning to Snapper as they spawn at different times throughout the day (Appendix 1). The formula required to integrate the error variance associated with each set of DEPM inputs, for the eight spatially distinct egg surveys, and across five assumed values of mortality (i.e. $Z = 0.2$ to 0.6 eggs.day^{-1}) was onerous (Appendix 4). For fisheries to be effectively managed, particularly those that require information to set subsequent harvest strategies (i.e. quota setting, scheduling seasonal closures), it is important that the information is delivered in an appropriate time frame. Future development of an 'R' based statistical package to specifically deal with the complex dataset generated from a standardised Snapper DEPM program would be highly beneficial and contribute to the timely delivery of biomass estimates to fisheries management and industry.

Conclusion

The work carried out in the study is a considerable improvement on the previous attempts to apply the DEPM to assess South Australia's Snapper Fishery. The ability to positively identify Snapper eggs through the novel use of an established molecular technique has re-invigorated the capability of using the DEPM to provide a fishery-independent estimate of spawning biomass for Snapper and has also increased its applicability to other species where egg identification has been problematic. Furthermore, the extension of the traditional statistical approaches to account for the inherent variation in Snapper population dynamics has contemporised the method to provide more precise, unbiased biomass estimates. Despite the acknowledged level of imprecision associated with estimates of biomass in this study, a characteristic ubiquitously observed in DEPM based assessments, the overall trends aligned with the recent status assignment of the South Australian Snapper stocks and broadly agreed with the industry's assessment of the resource. A relatively simple cost benefit exercise indicated that the incorporation of the DEPM would benefit the assessment and management of South

Australia's Snapper resource, either through its full integration into the current assessment program or augmenting it to rely less on compromised fishery-dependent data sources. It is likely that the incorporation of the proposed methodological refinements, which include improving the sampling techniques; streamlining laboratory processing; and rationalising data collection and analysis, would improve the DEPM's overall efficiency and increase the precision of the future assessments.

Implications

The flow-on implications of this study are diverse. Firstly, the successful development of the relatively non-destructive molecular probe to positively detect Snapper eggs in mixed ichthyoplankton samples is a major advancement for DEPM programs. This project has effectively broadened the capability of using DEPM as a viable fisheries assessment tool for any multiple spawning species that produces pelagic eggs that are difficult to identify and can be sampled effectively. Furthermore, the technique is equally capable of identifying larval fish, or any other biogenic material of interest, from mixed plankton samples.

Secondly, this research has reduced the need to exclusively rely on fishery-dependent catch and effort data to assess Snapper fisheries. The Snapper-specific molecular probe can be shared with other agencies that are required to assess the status of their local Snapper stock (i.e. WA Fisheries; NSW Department of Primary Industries; Fisheries Victoria; Queensland Department of Agriculture, Fisheries and Forestry; NIWA New Zealand). Its application in assessing the Western Victorian Snapper stock (WVS) would be of particular value, as the majority of the spawning population occurs within Port Phillip Bay which is a relatively small enclosed body of water that can conveniently accommodate a DEPM survey (Hamer and Conron 2016). Recent studies have indicated that at times of exceptionally high spawning success in Port Phillip Bay, movement of resultant offspring into south-east South Australian coastal waters can be extensive (Fowler 2016).

Thirdly, the relative cost-effectiveness of incorporating the DEPM in South Australia's current fishery assessment program for Snapper, either through its full integration or via an augmented program, provides options that are likely to benefit management and industry. Adding the DEPM will contribute an extra unbiased source of information that can be synthesised with the existing fishery-dependent data streams that will lead to more confidence assessments of the stocks. From this, greater confidence can placed on the consideration of implementing output controls in the future refinement of the Snapper specific harvest strategy. South Australia's Sardine Fishery provides a clear example of how the DEPM derived estimate of spawning biomass is the key biological performance indicator that underpins a tiered harvest strategy (Ward et al. 2015).

Fourthly, the intensive DEPM surveys have provided considerable information relating to Snapper early life history, particularly the spatial distribution and abundance of Snapper eggs that identify areas of high spawning activity. This level of information can be used to qualitatively assess the effectiveness of a series of spatial 'spawning' closures that were implemented in South Australia in 2013 as part of the recent Snapper management strategies. The relative value of this information has been broadly acknowledged by management and industry as the technique is currently being adopted to assess key spawning grounds for King George Whiting in South Australia's southern gulfs to inform future management of the fishery (Steer et al. 2016 - FRDC project No. 2016-003).

Finally, this project plays an important role in the global advancement of the DEPM. The overarching challenge in most DEPM programs relates to improving the precision of the biomass estimate. Effort is continually directed at reducing the inherent variance associated with the integrated biological parameters.

The new statistical procedures developed to account for the variation in South Australia's Snapper populations, can be more widely applied and further refined in other DEPM based programs.

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Recommendations

It is recommended that the results of this study be broadly disseminated to PIRSA Fisheries and Aquaculture (managers); the Commercial, Recreational and Charter Boat sectors of the South Australian Marine Scalefish Fishery (MSF); DPI Vic; DAFF Qld; NSW DPI; national and international fisheries scientists; general public.

Extension and Adoption

It is expected that there will be considerable engagement with PIRSA and industry regarding the integration of the DEPM into the on-going assessment of South Australia's Snapper stocks and associated harvest strategy development. This may serve as an example to other jurisdictions and lead to an alignment of the national assessment capabilities for Snapper and develop cross-jurisdictional management strategies to ensure the sustainable harvest of the resource.

Project coverage

If applicable report on any media, industry or government article on the project.

2015

The Lead: http://www.theleadsouthaustralia.com.au/industries/primary-industries/turning-eggs-blue-will-aid-in-snapper-tally/

18 March 2015: Food Navigator Asia: http://www.foodnavigator-asia.com/Policy/Turning-eggs-blue-will-transform-snapper-fisheries

2017

11 January 2017: ABC Country Hour SA: http://www.abc.net.au/news/rural/programs/sa-country-hour/2017-01-11/sa-country-hour-11-january-2017/8175740

13 January 2017: ABC online http://www.abc.net.au/news/2017-01-12/snapper-eggs-turned-blue-to-help-fish-management/8177772

13 January 2017: Sport Fishing magazine: http://www.sportfishingmag.com/red-snapper-eggs-turned-blue

13 January 2017: WAFIC: http://www.wafic.org.au/news/turning-snapper-eggs-blue-help-fisheries-management/

March 2017: Channel 10 Scope TV featuring snapper blue eggs (Not aired yet)

Appendices

APPENDIX 1: Derivation of a new stage-tow-specific egg density (D_0) estimator

Introduction

In this Appendix we describe and mathematically detail a new method to estimate D_0, the DEPM survey measured mean egg density at time of spawning (also known as P_0). We adopted the approach recommended by McGarvey and Kinloch (2001) and Bernal et al. (2011a; 2011b) where mortality rate from any given survey was not attempted.

Estimating mortality, a change over time, from DEPM survey samples is not generally feasible. Vertical tow egg samples vary only in space, and afford no repeated sampling over time at any single location or patch of spawned eggs. So information on a temporal change in egg density is minimal or absent. Moreover, the sample variation in space of egg counts for South Australian Snapper, and for many pelagic fish stocks is large, often immense. The distribution is typically highly skewed with many zeros and a few very high counts, so estimates of mean egg density averaged over space are imprecise. To extract a temporal signal for morality from such highly variable data would be challenging even if there were repetition over time. To counter this, we assume a set of plausible values for instantaneous egg mortality rate (Z in days^{-1}) based on previous literature and compute the D_0 estimate for each assumed Z.

Overview of method

The principal innovation of this new method for estimating D_0 is to account for mortality loss (from the time of spawning to the time of observed sample measurement) for each observed egg stage in each individual vertical sample tow, rather than for the overall population through a least squares linear regression (Lo 1985) or from a prior assumed Z in a GAM (Bernal et al. 2011b). This individual egg density at time of spawning $D_{0,i,s}$ is computed from the observed egg density by stage and tow $D_{i,s}^{obs}$, where stage is subscripted by s and tows vary over subscript i. Several advantages accrue by accounting for egg mortality and converting to a time rate for each stage-tow sample of eggs individually.

One important challenge in applying DEPM to South Australian Snapper is that the timing of egg release is not well approximated by a single daily instantaneous time of spawning, such as midnight, in each 24-hour day. In this project, Snapper were found to spawn over approximately 16 hours from 11:00 to 2:00, with some spawning evident in every hour of the day (Figure 7) Therefore, the standard DEPM method of assigning egg age based on (1) an assumed fixed instantaneous spawning hour in each day, (2) the time of day each vertical tow sample was taken, and (3) an adjudicated allocation of stage/tow samples into day 1 or day 2 is not employable with South Australian Snapper.

The stage-tow-mortality-specific method we present below for estimating D_0 does not require allocating each stage/tow sample to either day 1 or day 2. Since the methods for making this binomial allocation involve additional assumptions, this removes one source of DEPM estimate uncertainty. The assumption of a single instantaneous spawning time each day also carries additional error in egg ageing since, in reality, spawning times vary. Moreover, this variation in spawning time can also confound the allocation of samples to day 1 or day 2 in the standard DEPM treatment of stage/tow egg samples.

In place of a breakdown of samples into day 1 and day 2, we require only the stage- and temperature-dependent egg ageing relationship used in all DEPM analysis. From that, the average lifespan of eggs is computed. To obtain a daily egg production rate from the measured egg density per unit area, we divide by this average time eggs exist, and so can be observed in the water column. Specifically, for each stage s and sample i, we divide $D_{i,s}^{obs}$ after accounting for expected mortality from time of spawning, which is a survey measure of egg density per unit area, by the expected hatching time ($H(T_i)$ in days), to obtain $D_{0,i,s}$, which is a rate of eggs spawned per day and also per m^2.

Estimator derivation

Each measured egg density from each combination of stage and survey tow is a data point in this estimator. We treat survey tows as independent as in most DEPM estimators. By dividing each vertical tow into day 1 and day 2, most DEPM estimators also effectively treat days 1 and 2 in each tow as independent. Here, we instead sum the measured egg density at spawning from all observed stages in each tow, to get one sample measure of eggs spawned per unit area per unit time from each vertical tow. Summing across stages is possible by this new method because each $D_{0,i,s}$ is individually adjusted for mortality back to spawning time, and also converted to a rate per day by dividing by the expected egg lifespan for each stage individually prior to summing. In other words, each $D_{0,i,s}$ estimates the same quantity of eggs released at time of spawning per unit area per unit time. Because the observed measure of eggs per m^2 for each stage is individually converted to a rate per day, we must sum over all stages in each tow to get a sample measure of total eggs spawned per day per m^2. We have confirmed this D_0 model estimation logic, devised under this project, using basic simulations in Excel that are summarised briefly below.

As in previous DEPM estimators, we assume an exponential survival of eggs over the time (age) from spawning to the time of vertical tow sample measurement. Using prior South Australian Snapper egg development experiments in the laboratory by McGlennon (2003), we applied the McGlennon formula for the average age of an egg, given the stage and temperature (T):

$$a(s_M, T) = 36.158 \cdot \exp(-0.12 \cdot T) \cdot s_M^{0.827}. \qquad \text{(A2.1)}$$

The conversion from the 16 stage categories (s_M) that McGlennon (2003) defined to the 9 stages used to stage Snapper eggs in this study (s_S) (Table A2.1).

Table A2.1. Conversion mapping of stages applied in the McGlennon staging formula, from 1 to 16, to the 9 egg stage categories applied in the current study.

Egg stages defined by McGlennon (2003) (s_M)	Egg stages used in this study (s_S)
1.5	1
3.5	2
5.5	3
7.5	4
9	5
10.5	6
12.5	7
14.5	8
16	9

From the staging formula we can derive an estimated egg duration (hatching age), as a function of *T*. The hatching time ($H(T)$) is taken as the age of the last stage prior to hatching (McGlennon (2003) stage s_M =16) plus half the difference in age between the last two defined stages:

$$H(T) = a(16,T) + \frac{a(16,T) - a(15,T)}{2} \qquad \text{(A2.2)}$$

From the observed temperature $T[i]$ in each tow i, the expected hatching time $H(T[i])$ is computed.

Based on the logic given in the preceding section, $D_{0,i,s}$ is computed as follows:

$$D_{0,i,s} = D_{i,s}^{obs} \cdot \exp\left(Z \cdot a(s_M[s_S], T[i])\right) \Big/ H(T[i]). \qquad \text{(A2.3)}$$

The egg density at time of spawning, per day per m^2, for each vertical tow *i* is the sum over all stages of observed egg densities:

$$D_{0,i} = \sum_s D_{0,i,s} \ . \qquad \text{(A2.4)}$$

Finally, the estimate of mean egg density D_0, for each survey, is obtained by averaging over all n_i tows:

$$D_0 = \frac{1}{n_i} \cdot \sum_i D_{0,i} \,. \qquad \text{(A2.5)}$$

For the surveys undertaken in this project, where some eggs were identified as Snapper within survey zones of approximately 5-12 tows in addition to the egg samples where Snapper eggs were identified by individual tow, the final egg density was computed by taking a weighted average over zones, weighting by zonal spawning area, A_{zone}:

$$D_0 = \frac{1}{\sum_{zone} A_{zone}} \left\{ \sum_{zone} A_{zone} \cdot \left(D_0^{zone} + \frac{1}{n_i^{zone}} \cdot \sum_{i \text{ in } zone} D_{0,i} \right) \right\}. \qquad \text{(A2.6)}$$

Simulation testing of the new stage-tow specific D_0 estimator

To test the accuracy of the new stage-tow-specific D_0 estimator, we constructed a basic data simulator in Excel with two purposes. The first purpose was to test that (1) dividing by hatching time and inverting mortality losses for each stage and tow (Eq. 2.3), and then summing over stages (Eq. 2.4) and averaging over tows (Eq. 2.5), recovered the correct (simulation assumed) population egg density at time of spawning. Second, we sought to test the impact on estimate reliability of variations in the actual spawning times over 24 hour days.

Simulation Method

This egg spawning simulation accounts for no variation in space. Only hourly spawning times are represented. A user-specified number of eggs (per m^2) are spawned in each hourly time step of the simulation. 7 days of spawning were simulated, with spawning proportion by hour permitted to vary. The proportion of eggs spawned in each hour of the 24-hour day, was repeated for each of the 7 days.

Mortality of eggs, and so the declining egg numbers with stage of each cohort, were computed assuming a negative exponential decline. Average ages of each hourly cohort are computed directly from the McGlennon formula, given temperature. A single temperature, constant in time, was assumed, though this could vary if desired. Given mortality rate, age, and the number of eggs spawned in each hour, the egg population numbers of all 9 stages of each hourly cohort are computed for each daily hour over 7 days.

The times of spawning and subsequently of persistence for each hourly cohort are explicit for purposes of specifying times of sampling. Adding stage age to the spawning time of each hourly cohort gives the average hour and day in which each stage and hourly cohort existed, and so was observable in the water column. Taking the midpoints between each average age, the beginning and end times of each stage for each hourly cohort were computed. For each given time when egg density was sampled (hourly time, in decimal days), the density of eggs present and so measured (for these two simulation purposes, without error) by a vertical tow was computed.

From sampled egg densities (per m^2) by stage sampled, we apply the stage-tow specific D_0 estimator to obtain each single vertical tow measure of D_0.

Stage-Tow-Specific D_0 Estimator Simulation Test Results

To achieve the first purpose, we imposed a scenario that assumes an identical density of 100 eggs spawned per m^2 in all 24 hours of each day. This gives a daily egg production of 2400 eggs m^{-2}. Applying the stage-tow specific estimator yielded an estimate of 2417.3 eggs m^{-2}, or 0.721% above the true value of 2400.

This difference of 0.721% is sufficiently small so as to confirm the accuracy of the stage-tow-specific D_0 estimator and could possibly be explained by a number of approximations, for example, the hourly discreteness of simulation time steps.

For the second purpose, using the observed spawning proportions, by hour, that were back-computed from the data averaged over all regions/surveys (Figure 7), with a scenario of repeatedly sampling for egg density once hourly over a 24 hour period, the average of 24 hourly tows gave 1.0072 compared to a true value (for this simulation) of 1.

This difference of 0.721% is identical (to at least 7 significant digits) to the error obtained with the entirely different scenario of the first purpose described above. Such close (effectively identical) agreement suggests that the difference may lie in the approximations made to construct the simulation.

Thus accepting that the 0.721% difference obtained for the two scenarios tested is negligibly small and is plausibly due to the simulation approximations used to test it rather than to the estimation method we here sought to test, these simulations confirm the accuracy of the stage-tow-specific D_0 estimator.

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APPENDIX 2. Estimating parameters for an allometric relationship of Snapper batch fecundity to body weight

Introduction

To model the relationship of eggs released per female per day (batch fecundity) versus Snapper body weight, an allometric relationship was assumed. Other models were tested using least squares fits and an allometric was found to be the best fitting model. The allometric function for batch fecundity (F^{batch}) versus weight taken as a continuous variable (W) is

$$\hat{F}^{batch}(W) = \alpha \cdot W^{\beta} . \qquad \text{(A3.1)}$$

Examining the scatterplot of observations in this relationship (Figure 10), there is a non-uniform spread of residuals, that is, the residuals increase with body mass along x. To account for the increasing spread of observations about the model predicted mean, the fit to these data was formulated as a maximum likelihood estimate.

In this Appendix, we describe the maximum likelihood estimator including the error structure which specifies the assumed dependence of the residuals on body weight, write out the likelihood function, and present the final weight-dependent batch fecundity parameter estimates used in the DEPM spawning biomass estimation (Appendix 4).

Method

A normal likelihood was used. The standard deviation of the likelihood which quantifies the spread of residuals about the curve of Equation 3.1 was (like the mean itself) written as an allometric function of body weight:

$$\sigma(W) = \sigma_0 \cdot W^{\sigma_1} \qquad \text{(A3.2)}$$

The normal negative log likelihood is written:

$$-\ln L = \sum_{i=1}^{n_F} 0.5 \cdot \ln\left(2\pi \cdot \sigma(W_i)^2\right) + \frac{\left[\hat{F}^{batch}(W_i) - F_i^{batch}\right]}{2 \cdot \sigma(W_i)^2}, \qquad \text{(A3.3)}$$

where the set of n_F observations of female Snapper weight and batch fecundity is denoted by $\left\{W_i, F_i^{batch}; i = 1, n_F\right\}$, and where $\hat{F}^{batch}(W_i)$ is the model (3.1) evaluated at each observed body weight, W_i.

The four model parameters $\left\{\alpha, \beta, \sigma_0, \sigma_1\right\}$ were estimated by numerically minimising the negative log likelihood, giving the values of Table A3.1.

Table A3.1. Batch fecundity symbols, their estimated parameter values, and 95% confidence bounds (CB) on each parameter.

Symbol	Parameter estimated value	95% CBs (lower, upper)

α	137.3	(126.9, 149.0)
β	0.910	(0.900, 0.920)
σ_0	11.70	(10.31, 13.43)
σ_1	0.741	(0.731, 0.753)

The resulting allometric model fit curve and associated modelled spread of observations about this curve are plotted in Figure 10.

APPENDIX 3. An extended DEPM estimator of spawning biomass, accounting for body-weight specific dependence of batch fecundity and population structure

Introduction

Snapper vary over a much wider range of body sizes than adult sardines and other small pelagic species, and therefore size-dependence of adult population parameters is stronger. Snapper batch fecundity (eggs released per female per day) has an allometric dependence on body mass (Appendix 3) that varies over about an order of magnitude among South Australian Snapper. In addition, the size-frequency data for Snapper are far from normal (Figure 9). Due to highly sporadic temporal variation in recruitment, South Australian Snapper show a widely bi-model size-frequency structure. This renders inaccurate the approximation of a single mean female body weight made in nearly all prior DEPM studies.

To improve accuracy, one objective is to develop a DEPM estimator of spawning biomass that incorporates explicit size-dependence of adults. This Appendix will derive and present new formulas for an extended DEPM adult spawning biomass estimation method.

Output estimates of the size-dependence extended DEPM method will be twofold: the total number of spawning females, and the total spawning biomass.

Model derivation

In order to incorporate size-dependence of batch fecundity and population size frequency, both as functions of adult weight class, we derive the spawning biomass estimate from first principles, incorporating the same or similar logic that previous DEPM estimators presumably did (though Parker's seminal paper (1980) does not present this derivation, instead citing previous work). The population is divided into 26 weight classes, ranging from 0.5 to 13.5 kg, grouped into 0.5 kg bins. The derivation is achieved by forming two equations for average total daily egg production of the population under study, and equating them.

First, an expression for total eggs produced per day can be derived from adult reproductive parameters (Table 1), including the total number of females in the adult population to be estimated.

In any given weight class, subscripted by *w*, the eggs produced equals the batch fecundity of an average actively spawning female of that weight (F_w^{batch}), times the proportion of female Snapper actively spawning during the DEPM survey (the spawning fraction S , assumed constant with size), times the total number of adult females in the spawning area (N_w^{fem}).

Summing over all weight classes, the following expression is obtained for the total number of eggs spawned per day in the spawning area:

$$Eggs^{adult} = \sum_w F_w^{batch} \cdot N_w^{fem} \cdot S \,. \qquad \text{(A4.1)}$$

SAFCOL market samples provide the proportions of female Snapper in the population by weight class, assuming that the market sampling of the catch reflects the size structure of the spawning population, and further assuming sexual dimorphism for Snapper, which is supported by biological studies and widely assumed for most Snapper assessments. Female Snapper numbers by weight class can be written as a product of the total number of Snapper females ($N^{fem,Tot}$, to be estimated) times the Snapper number proportion by weight class (P_w^N):

$$N_w^{fem} = N^{fem,Tot} \cdot P_w^N . \tag{A4.2}$$

Substituting (2) into (1) gives the first expression for total population egg production:

$$Eggs^{adult} = N^{fem,Tot} \cdot S \cdot \sum_w F_w^{batch} \cdot P_w^N . \tag{A4.3}$$

A second expression for total egg production can be obtained from the DEPM survey. The eggs released by all spawning females per day equals the survey-measured egg density, the mean number of eggs spawned per 24-hour day per m^2 (D_0), times the total spawning area (A^{sp} in m^2):

$$Eggs^{surv} = D_0 \cdot A^{sp} . \tag{A4.4}$$

By equating the two expressions (3) and (4) for the total eggs produced per day in the spawning area and solving, an estimate is obtained for the total number of females in the spawning area:

$$N^{fem,Tot} = \frac{D_0 \cdot A^{sp}}{S \cdot \sum_w F_w^{batch} \cdot P_w^N} . \tag{A4.5}$$

To obtain an estimate of total spawning biomass (B^{sp}), including males, we multiply the estimated number of females in each weight class (Equation 2) by the mean weight ($\bar{w}_w$, taking the midpoint of each weight class), and divide by the sex ratio given as a proportion by weight of females (R, assumed constant):

$$B^{sp} = \frac{N^{fem,Tot}}{R} \cdot \sum_w P_w^N \cdot \bar{w}_w . \tag{A4.6}$$

Finally, inserting (5) into (6), we obtain a closed form solution for total spawning biomass in which size dependence of batch fecundity and of population structure are explicit:

$$B^{sp} = \left(\frac{D_0 \cdot A^{sp}}{S \cdot R \cdot \sum_w F_w^{batch} \cdot P_w^N} \right) \cdot \sum_{w'} P_{w'}^N \cdot \bar{w}_{w'} \tag{A4.7}$$

Table A4.1. Variables used in the derivation, and their definitions.

Variable	Description
w	Weight class subscript
D_0	Survey measured total Snapper egg production, per day, in given region and year
F_w^{batch}	Batch fecundity, the number of eggs produced per day by an average female of weight *w* on the days when she is actively spawning
P_w^N	Population number proportions by weight class, *w*, from market catch samples
$\bar{w}_w$	Mean weight in each weight class (taken as the midpoint)
S	Spawning fraction, proportion of female Snapper actively spawning in days of egg survey
A^{sp}	Area of active Snapper spawning in given region and year, see Figure 6
R	Sex ratio, the proportion of the population, in biomass, that is female
N_w^{fem}	Estimated number of females in the spawning population, by weight class, *w*
$N^{fem,Tot}$	Estimated number of females in the spawning population, total
B^{sp}	Estimated spawning biomass of the population, both sexes included
$Eggs^{adult}$	Total eggs produced by females inside the spawning area for a given region and year, estimated from adult reproductive parameters
$Eggs^{surv}$	Total eggs produced by females inside the spawning area for a given region and year, estimated from DEPM survey-measured egg density.

APPENDIX 4. Computing standard errors for the DEPM spawning biomass estimate

Introduction

In this Appendix, we describe the methods used to compute confidence intervals for spawning biomass estimates from the eight surveys.

The estimation of confidence intervals applied is the delta approximation method. This is a standard approach to estimating approximate confidence intervals for an estimator that is written as function of a set of variables (see e.g. Cassella and Berger 2002). The DEPM spawning biomass estimator (Appendices 2 and 4), is written as a function of measured egg density and spawning area, and adult spawning parameters, whose variances (the standard errors squared) were estimated individually. The delta method applies a first-order Taylor approximation assuming the uncertainty in inputs are not large compared with the input estimate values themselves.

A delta function estimate for adult-weight-frequency DEPM spawning biomass

For spawning biomass (B^{sp}), the input quantities are the six inputs to Eq. A4.7, namely D_0 , S, R , $\{P_w^N\}$, and $\{F_w^{batch}\}$ defined in Appendix 4. Since the mean weights ($\bar{w}_w$) are taken as the midpoints of each weight bin, the mean weights ($\bar{w}_w$) have no uncertainty.

The spawning biomass estimation formula (from Appendix 4) is:

$$B^{sp} = \left(\frac{D_0 \cdot A^{sp}}{S \cdot R \cdot \sum_w F_w^{batch} \cdot P_w^N} \right) \cdot \sum_{w'} P_{w'}^N \cdot \bar{w}_{w'} . \qquad \text{(A4.7)}$$

The delta approximation formula for the variance of spawning biomass, given in terms of the variances of the input quantities (two of which, $\{P_w^N\}$, and $\{F_w^{batch}\}$, are vectors over the 26 weight bins) is written following Casella and Berger (2002):

$$V\left(\hat{B}^{sp}\right) = \left(\frac{\partial B^{sp}}{\partial D_0}\right)^2 \cdot V(D_0) + \left(\frac{\partial B^{sp}}{\partial A_{sp}}\right)^2 \cdot V(A_{sp}) + \left(\frac{\partial B^{sp}}{\partial S}\right)^2 \cdot V(S) + \left(\frac{\partial B^{sp}}{\partial R}\right)^2 \cdot V(R) + \sum_w \left(\frac{\partial B^{sp}}{\partial P_w^N}\right)^2 \cdot V(P_w^N) + \sum_w \left(\frac{\partial B^{sp}}{\partial F_w^{batch}}\right)^2 \cdot V(F_w^{batch}) \qquad \text{(A5.1.)}$$

Here the partial derivatives ($\frac{\partial B^{sp}}{\partial D_0}$, etc.) are all evaluated at the mean (point) estimate values of the independent variables $\left\{D_0, A_{sp}, S, R, \{P_w^N\}, \{F_w^{batch}\}\right\}$. The variances of the inputs to the DEPM spawning biomass estimate, $\left\{V(D_0), V(A_{sp}), V(S), V(R), \{V(P_w^N)\}, \{V(F_w^{batch})\}\right\}$, must be supplied as prior inputs, generated from the prior estimation of these independent variables, i.e. from the standard errors squared.

Computing prior input variances

The prior variance for D_0 ($V(D_0)$) was estimated by the textbook (s^2/n) variances of a sample mean, the square of the standard error. In future estimates, this will be computed from the sum of square differences (s^2) among all vertical tows in each survey. In these surveys, where the D_0 estimates were computed from egg density measurements aggregated into zones that included multiple vertical tows, we used a standard weighted square difference sum among zones of each survey to compute s^2, where $n_{surv,zone}$ is the number of zones in each survey. The weighting by zone ($W_{Surv,Zone}$) is simply the area proportion covered by each zone in each survey region:

$$W_{Surv,iZone} = \frac{A_{Surv,iZone}}{\sum_{iZone=1}^{n_{Surv,Zone}} A_{Surv,iZone}}. \qquad \text{(A5.2)}$$

The weighted mean over zones for D_0 in each survey were computed as

$$D_{0,Surv} = \frac{1}{n_{Surv,Zone}} \sum_{iZone=1}^{n_{Surv,Zone}} W_{Surv,iZone} \cdot D_{0,Surv,iZone} \,. \qquad \text{(A5.3)}$$

The variance for this estimate of a weighted mean is computed as

$$V(D_{0,Surv}) = \frac{1}{n_{Surv,Zone}} \sum_{iZone=1}^{n_{Surv,Zone}} W_{Surv,iZone} \cdot \left(D_{0,Surv,iZone} - D_{0,Surv}\right)^2 . \qquad \text{(A5.4)}$$

Variances for the spawning areas (A^{sp}) were difficult to quantify and uncertainty in spawning area was omitted (setting all $V(A_{sp})$=0) in these confidence interval estimates.

Like D_0, the prior variances for spawning fractions S ($V(S)$) were also computed as the variance of a weighted mean using the method described for $V(D_0)$ in Eqs. A5.2-A5.4. For $V(S)$, the variation is computed as the weighted sum of square differences among all four locations where adult Snapper were sampled (by rod and line fishing), undertaken for each of the eight surveys. Weightings were by proportional numbers of adult female Snapper sampled in each adult sample location.

The variance for the mean of female sex ratio ($V(R)$) was statistically estimated as a ratio estimator. Inputs to the estimate of spawning fraction ratio $R = \bar{N}_{fem}/\bar{N}_{tot}$ in each survey (from n repeated samples of adult Snapper at different locations) were numbers of females (N_{fem}) and total adults (N_{tot}) sampled at these locations. The variance of a ratio estimate (see e.g. Rice 1995) is estimated by standard Taylor expansion (delta approximation) as

$$V(R) = \frac{1}{n \cdot \bar{N}_{tot}^{\,2}} \cdot \left\{ \left(\frac{\bar{N}_{fem}}{\bar{N}_{tot}} \right)^2 \cdot V\left(N_{tot}\right) + V\left(N_{fem}\right) - 2 \cdot \left(\frac{\bar{N}_{fem}}{\bar{N}_{tot}} \right) \cdot \mathrm{cov}\left(N_{fem}, N_{tot}\right) \right\}. \qquad \text{(A5.5)}$$

To compute the input variances for the weight-frequency proportions ($\{V(P_w^N)\}$), standard multinomial proportion sampling errors were assumed. The formula for the variance of these multinomial weight proportions is given by

$$V(P_w^N) = \frac{P_w^N \cdot (1 - P_w^N)}{n} \qquad \text{(A5.6)}$$

for all n = 26 weight bins w (each 0.5 kg). Because multiple years were needed to provide sufficient Snapper weight-bin proportion sample sizes, and to cover all survey years given that South Australian Snapper size-frequency market samples are taken in only two of every three years, one set of $\{ P_w^N \}$, and so one set of $\{ V(P_w^N) \}$ were computed for each of the four regions, and used for both years of survey in each region.

The variances for the batch fecundities $\{ F_w^{batch} \}$ were computed from the computed error bars of the fit (Figure 10), reflecting the spread of residuals. These fit errors are estimated in the maximum likelihood fit of batch fecundity versus body weight from the two parameters defining the spread of observations about the normal likelihood (Eq. A3.2 of Appendix 3). The estimated variances of batch fecundity for each weight bin w are:

$$V(F_w^{batch}) = \sigma^2(\bar{w}_w) = \left[\sigma_0 \cdot \bar{w}_w^{\,\sigma_1} \right]^2. \qquad \text{(A5.7)}$$

Discussion

Given that $\{P_w^N\}$ and $\{F_w^{batch}\}$ are vectors each of 26 elements (26 weight bins) the total number of independent variables in this Taylor expansion formula is 56. Since each derivative is itself a relatively lengthy mathematical expression, deriving the explicit formula for Eq. A5.1 is not practically feasible by ordinary pen and paper algebra. For this reason, we derived an explicit variance estimation formula by coding Eq. A5.1 in Mathematica symbolic manipulation language and performed the derivation using machine calculus and algebra. This produced a formula 17 pages long that was incorporated into a single Mathematica function. This was applied to the eight survey datasets (consisting of the point estimates for each of the 56 DEPM inputs, at which the derivatives were evaluated, and their 56 prior variances $\left\{V(D_0), V(A_{sp}), V(S), V(R), \{V(P_w^N)\}, \{V(F_w^{batch})\}\right\}$. Combining these permitted numerical evaluation of Eq. A5.1 for each survey, providing the estimate of variance, and so also standard error (the square root of the variance) for the estimate of spawning biomass of each DEPM survey.

Because multiple prior values for egg mortality Z of {0.2, 0.3, 0.4, 0.5, 0.6} were assumed, for each Z, a full set of 56 point estimates for D_0, S, etc. (in R, Appendices 2 and 4) and their prior input variances (in R or Excel) for each survey were generated. Running the delta estimator over Z values produced independent estimates of variance and so also confidence interval for each Z and survey.

The uncertainties for survey egg density (D_0) were highly skewed. With zero as a lower bound for egg density by tow, negative residuals (for D_0 's by tow below the mean) were also bounded. A few very large values of D_0 produced much larger positive residuals above the mean of D_0. To more accurately characterise confidence intervals for D_0, a simple method was sought to summarise this asymmetry in the confidence intervals above and below the estimated mean which serves as the point estimate of D_0. The most simple method is to simply compute different variances, and so also standard errors using only the negative and positive residuals for two separate sums of squares, giving two estimate of variance, rather than one, denote them $V_{-}(D_0)$ and $V_{+}(D_0)$.

There is additional uncertainty in the reported spawning biomass estimates from this project due to the breakdown in the sampled eggs stored in formalin or ethanol. Only the latter could be genetically identified as Snapper, so additional stages of the estimation were implemented to apply the binomial proportions of Snapper in the ethanol samples to scale up Snapper egg density from the total egg densities (all species) in the formalin samples. Uncertainty in this additional stage was omitted in these estimation methods developed under this project for future stock assessment applications since future samples will all be stored in ethanol for genetic identification using the genetic egg species identification techniques developed in this project that was found to be highly effective. The statistical methods developed here, and in Appendices 1 and 3, assume the application of genetic egg species identification.

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